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Biogeographic distribution of the eel *Anguilla luzonensis*: dependence upon larval duration and oceanic currents

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ABSTRACT: *Anguilla luzonensis*, a recently identified tropical *Anguilla* species, was categorized as 'Near Threatened' in the International Union for Conservation of Nature (IUCN) Red List in 2014. However, its biogeographic distribution and dispersal mechanism remain unconfirmed. This study aimed to clarify the biotic and abiotic factors that may shape the eel's dispersal range, which could help to establish suitable conservation strategies. Glass eel distribution was investigated in Taiwan, Luzon, Mindanao, and Sulawesi. *A. marmorata* was used as a reference eel species for comparison. Although both species were found year-round, *A. marmorata* was found in all locations and was abundant at most sites. However, *A. luzonensis* was concentrated only on Luzon Island, mainly between June and October. It was rarely observed in Taiwan and Mindanao, and was not found in Sulawesi. The spawning site of *A. luzonensis* is probably located more northward in the North Equatorial Current (NEC), whereby the eel larvae avoid entering the Mindanao Current (MC). In addition, *A. luzonensis* typically spawns between February and May, and is preferentially transported into the Kuroshio from May to August, when the NEC bifurcation latitude reaches its southernmost position and the Kuroshio transport volume peaks. Furthermore, *A. luzonensis* has a shorter/narrower range of larval duration than *A. marmorata*, and its dispersal distance is therefore restricted. The life history traits of *A. luzonensis*, together with the oceanic current regime, may act together to concentrate its biogeographic distribution on Luzon Island.

KEY WORDS: *Anguilla luzonensis* · *Anguilla marmorata* · Biogeography · Fish larvae · Oceanic current · Spawning ground

INTRODUCTION

Anguillid eels (genus *Anguilla*) are catadromous freshwater fish with a unique life cycle (Tesch 2003). A total of 19 species and subspecies of the genus *Anguilla* are identified worldwide (Tesch 2003, Aoyama 2009). They spawn in the tropical and subtropical open oceans with a leptocephalus larval stage, and drift along ocean currents from their spawning area to continental shelves. After metamorphosing into glass eels, they grow in rivers and estuaries for many years before returning to their birthplace to spawn and die

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(Kuroki et al. 2008, Aoyama 2009, Pous et al. 2010, Han et al. 2012a). A total of 13 *Anguilla* species are tropical eels with nearly year-round recruitment because of protracted spawning seasons (Arai et al. 2001, Sugeha et al. 2001, Aoyama 2009, Kuroki et al. 2009, Han et al. 2012b). Recently, a new anguillid eel species, *A. luzonensis*, was discovered on Luzon Island in the northern Philippines (Watanabe et al. 2009). It was categorized as a 'Near Threatened' fish species in the International Union for Conservation of Nature (IUCN) Red List in 2014 (Jacoby & Gollock 2014). Kuroki et al. (2012) identified leptocephali of this species offshore in the western North Pacific (13 to 17.5° N, 125 to 141° E), and showed that they were up to 103 to 138 d old, a larval duration close to that of *A. marmorata*. The discovery of glass eels (Aoyama et al. 2015) and adults (Watanabe et al. 2009) on Luzon Island, and the offshore presence of leptocephali of *A. luzonensis* in the NEC, suggested that this species spawns in the NEC. Its early life history characteristics and distribution range, however, are still poorly understood.

Some eel species have overlapping spawning areas and use the same oceanic currents for larval transport. For example, *A. japonica*, *A. marmorata*, and *A. bicolor pacifica* larvae, with presumed spawning sites in waters west of Mariana Island, are transported by the North Equatorial Current (NEC) of the northwest Pacific Ocean (Kuroki et al. 2006a, 2009). *A. bicolor*, *A. nebulosa labiata*, *A. mossambica*, and *A. marmorata*, which spawn in waters in the western Indian Ocean, are transported by the South Equatorial Current to East African habitats (Pous et al. 2010). Moreover, *A. reinhardtii*, *A. dieffenbachii*, and *A. australis*, 3 eel species distributed in Australia and New Zealand, are transported by the South Equatorial Current of the southwest Pacific Ocean (Shiao et al. 2002, Kuroki et al. 2008, Jellyman & Bowen 2009). In theory, organisms with similar traits and life history will share similar niche requirements, and will thus be sorted into similar environments (Wolf & Weissing 2012). However, each fish species possesses certain unique traits, which may affect its geographic range size. For tropical reef fish, adult traits like body size, schooling behavior, and nocturnal activity, as well as larval traits like pelagic larval duration, have important effects on their geographic distribution (Luiz et al. 2013). In freshwater fish, environmental parameters, such as mean precipitation, temperature, altitude range, and river slope and length, could explain their current spatial patterns (Kang et al. 2013). The temperate eel *A. japonica* and the tropical eel *A. marmorata* spawn in sympatric waters in the Northwest Pacific (Tsukamoto 1992, 2006, Kuroki et al. 2006a, 2009, Minegishi et al. 2008, Chow et al. 2009). The larvae of both eel species drift along the NEC and arrive at the eastern coast of the Philippines, where it bifurcates into the northward-flowing Kuroshio and the southward-flowing Mindanao Current (MC) (Hu & Cui 1991, Tsukamoto 2006, Kuroki et al. 2009). However, *A. marmorata* distributes mainly in the Philippines, while *A. japonica* distributes mainly in East Asia in areas of Taiwan, China, Japan, and Korea (Han et al. 2012a,b). Differences in the length of larval duration (Leander et al. 2013) and recruitment temperature preferences of glass eels (Han et al. 2012b) may explain the limited overlap in their geographic distribution ranges. Tropical waters around the Philippines and Indonesia, the center of marine biodiversity worldwide (Miller & Tsukamoto 2006), have more than 10 eel species (Tesch 2003). The biotic and abiotic factors responsible for shaping their biogeographic range sizes, however, are still little known.

The latitudinal shift of spawning locations can significantly affect the recruitment success for *A. japonica* (Kimura et al. 2001, Zenimoto et al. 2009). If the Japanese eel larvae can enter the Kuroshio, they may have a much greater probability of successful recruitment (Kim et al. 2007). In addition, analyses based on historical hydrographic data, assimilation model outputs, and satellite altimeter measurements revealed that the bifurcation latitude of the NEC reaches its northernmost position in November and December, and its southernmost position in May and June (Qiu & Lukas 1996, Qu & Lukas, 2003, Yaremchuk & Qu 2004, Wang & Hu 2006). The genetic variability and structuring of 2 reef rabbitfish populations along the east Philippines coasts may be affected by the NEC bifurcation (Magsino & Juinio-Meñez 2008). The seasonal changes in the NEC bifurcation, however, may also affect the eel dynamics along the Philippine coast as well as in the western Pacific Ocean. Understanding the causes and consequences of current biogeography for each eel species is important for ecology, evolution, and conservation issues. Thus, the present study aimed to (1) investigate the detailed biogeographic distribution size of *A. luzonensis*, and (2) clarify the possible biotic and environmental factors responsible for its dispersal mechanism.

MATERIALS AND METHODS

Anguillid glass eel sample collection

Anguillid glass eel sampling was performed at 7 areas in Taiwan, the Philippines, and Indonesia (Fig. 1).

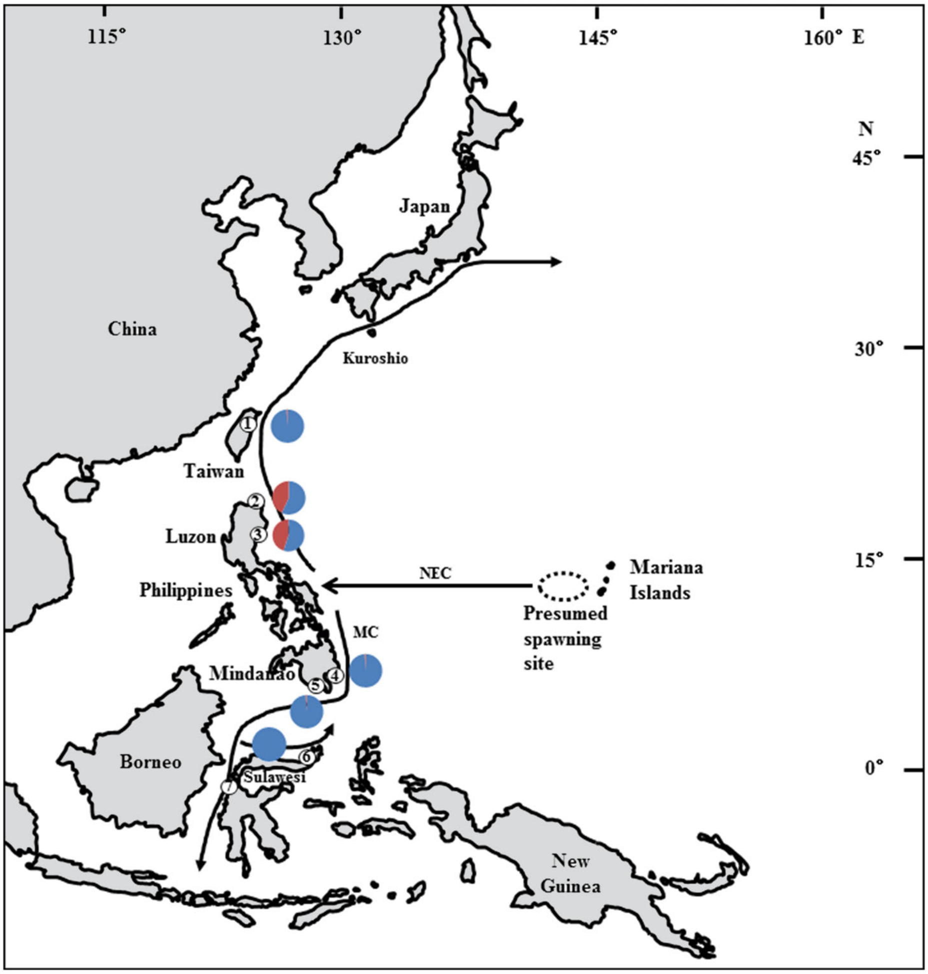

Fig. 1. Presumed spawning locations (ellipse), oceanic currents, sampling sites, and overall relative abundances of *Anguilla marmorata* (blue) and *A. luzonensis* (red) in East and Southeast Asia. The proportional abundances of both eel species in the Dumoga and Palu Rivers were combined. 1: Taiwan (Yilan and Siouguluan Rivers); 2: Cagayan River; 3: Baler; 4: Mati; 5: Gen. San.; 6: Dumoga River; 7: Palu River

Glass eels from Taiwan were caught and expressed as catch per unit effort (CPUE) using 1 fyke net (10 m wide at the opening) for 2 h before high tide at night, 1 to 2 times monthly between October 2010 and October 2015 from the Yilan River estuary (24.7162° N, 121.8352° E) (Fig. 1). Glass eels from Luzon Island were purchased from local fishermen, who collected them using a fyke net once a month in the Cagayan River estuary (18.3590° N, 121.6298° E) of north Luzon Island from May 2008 to December 2013, and from the Baler area (16.7915° N, 121.5548° E) of East Luzon Island in September and October 2013, and between April and September 2015 (Fig. 1). Glass eels in areas around the Celebes Sea were purchased once a month from local fishermen, who collected them using hand nets (2 m wide) from the Buayan River (6.0972° N, 125.2375° E) of General Santos City (Gen. San.) in southern Mindanao from January 2009 to December 2013, from the Dumoga River (0.9220° N, 124.0903° E) of Sulawesi in May and October 2011, and from the Palu River (0.8875° S, 119.8596° E) of Sulawesi between April 2012 and December 2013 (Fig. 1). Glass eels from the Mayo Bay estuary (6.9596° N, 126.3090° E) of Mati City in southeastern Mindanao were caught daily between May 2014 and May 2015 for 2 h before high tide at night using 1 hand net (2 m wide) and expressed as CPUE. These sampling sites all had commercial glass eel catching activity. Data from partial samples from Taiwan, the Philippines, and Indonesia have been published previously (Han et al. 2012b, Aoyama et al. 2015). After collection, the glass eels were stored in 95 % alcohol for subsequent analysis. The methods used to capture the glass eels in Taiwan were approved by the Fisheries Agency of Executive Yuan, Taiwan. Glass eel collection in the Philippines and Indonesia does not require permission. Moreover, the experimental procedures all complied with the experimental animal ethics criteria and were approved by the Institutional Animal Care and Use Committee (IACUC) of the National Taiwan University.

Species identification based on morphometry

To clarify the eel species composition at each location, glass eel samples were first identified to the species level using morphological methods, followed by DNA sequencing if necessary. Firstly, the total length (TL), pre-anal length (AL), and predorsal length (DL) of each glass eel were measured to the nearest 0.1 mm with a stereomicroscope to classify whether the eel was long-finned or short-finned by the following equation: fin difference = (AL − DL)/TL × 100 %, where (AL − DL) is the vertical distance between the origin of the dorsal fin and the anus (Tesch 2003). Secondly, the morphology of caudal cutaneous pigmentation was determined based on Tesch (2003). According to the morphological criteria for anguillid eels (Tesch 2003), *Anguilla bicolor pacifica* was the only short-finned eel species, and *A. japonica* was the only eel species without caudal pigmentation in the sampling area. These 2 species were easily validated without further molecular identification. Then, samples with a fin difference >13 % were all identified as *A. marmorata*, based on molecular identification by Lo (2013). Finally, because *A. luzonensis* has a fin difference between 8 % and 13 % (Lo 2013), specimens (except *A. bicolor pacifica*) with a fin difference <13 % from all sampling sites were all subsequently identified by molecular methods.

Species identification based on molecular tools

Genomic DNA was extracted from a small piece of muscle (about 10 mg) by a commercial DNA purification kit (EasyPure Genomic DNA spin kit; Bioman Scientific). The universal *Anguilla* primer set for cytochrome *b* (forward: 5′-GAT GCC CTA GTG GAT CTA CC-3′; reverse: 5′-TAT GGG TGT TCT ACT GGT AT-3′) was used for polymerase chain reaction (PCR) amplification. The procedure followed a previously described method (Han et al. 2012b). The PCR product was DNA sequenced and then species identified by the National Center for Biotechnology Information (NCBI) GenBank database (http://www.ncbi.nlm.nih.gov/nuccore).

Otolith daily increment counts of glass eels

Samples of *A. marmorata* and *A. luzonensis* for age determination were collected from Siouguluan River (23.4675° N, 121.5018° E, south of Yilan; n = 28), Cagayan River (n = 28), Baler (n = 30) and Gen. San. (n = 28). The daily growth increments of otoliths were analyzed using a scanning electron microscope, according to a previously described method (Han 2011). The images were photographed at 2000× magnification for the daily increment analysis. Presumed daily increments in the otoliths were counted from the first ring outside the core to the otolith edge (McCleave 2008, Han 2011). The presumed 9 d of the preleptocephalus stage, in which no otolith ring is formed (Shinoda & Tsukamoto 2009), were added to the total age. The spawning dates for both eel species at each location can be calculated according to the back-calculated numbers of otolith increments of glass eels.

Numerical model description

The study used 20-yr current velocity data from 1990 to 2009 from the North Pacific Ocean (NPO) model (Hsin et al. 2008, Hsin et al. 2012) based on the Princeton Ocean Model (POM) (Blumberg & Mellor 1987). The NPO model is highly consistent with Archiving, Validation and Interpretation of Satellite Oceanographic (AVISO) data and observation data (e.g. shipboard acoustic Doppler current profilers, Sb-ADCP) (Hsin et al. 2013). Based on hydrostatic approximate analysis, this model solves 3-dimensional primitive equations for momentum, salinity, and heat, and estimates turbulence by the Mellor–Yamada level 2.5 scheme (Hsin et al. 2008). The NPO model covers the domain of the northern Pacific Ocean from 30° S to 65° N and 99° E to 71° W, with a horizontal resolution of 0.25 × 0.25° and 26 sigma levels in the vertical. The model is driven by the monthly 2.5 × 2.5° NCEP-DOE 10 m wind climatology derived from averaging all historical data month by month (http://www.esrl.noaa.gov/psd/data). In addition, the monthly climatology of the Simple Ocean Data Assimilation (SODA) reanalysis products, similarly derived from averaging over all available years with 0.5° resolution (http://apdrc.soest.hawaii.edu/), provides lateral boundary conditions for the NPO model. After spinning up, the NPO model is subsequently forced by 6-hourly NCEP-DOE reanalysis surface winds from 1948 to 2008. Furthermore, the monthly SODA data provide the open boundary conditions for the NPO model (Hsin et al. 2012, He et al. 2014).

Tracer experiments

The NPO model is a credible model for tracer experiments (Hsin et al. 2012, He et al. 2014), and was used here to understand the presumed passive behavior of eel larvae. Because the spawning area of *A. japonica* was located latitudinally between 12 and 15° N within the constant westward flow of the NEC, and longitudinally along the western side of the West Mariana Ridge (Aoyama et al. 2014), the larvae dispersal patterns of *A. luzonensis* and *A. marmorata* were thus simulated at 100 m depth, starting from 12 and 13° N, respectively. The float particles were released at 139.5, 140, and 140.5°E monthly each year for a total of 19 yr between 1990 and 2008. Thus, each simulation cast 57 float particles (3 particles × 19 yr). The percentage of the particles that successfully passed the dashed boxes of the Kuroshio (18–19° N and 122–125° E) and MC (6–7° N and 126–129° E) was regarded as the rate of successful recruitment.

Data analysis

SPSS Statistics (ver. 12) software was used for statistical analysis. Differences in the mean ages between species or among sampling sites were tested by 1-way analysis of variance (ANOVA) with Tukey's honestly significant difference (HSD) multiple-comparison test. Differences were regarded as significant when $p < 0.05$.

RESULTS

Monthly glass eel population composition by location

The monthly population composition of glass eels by location is shown in Table 1. A total of 5 *Anguilla* species was identified from Taiwan and Luzon Island: *A. marmorata* (n = 9901), *A. japonica* (n = 4488), *A. luzonensis* (n = 4139), *A. bicolor pacifica* (n = 901), and *A. celebesensis* (n = 2). A total of 6 eel species was identified from Mindanao Island: *A. marmorata* (n = 7456), *A. bicolor pacifica* (n = 601), *A. luzonensis* (n = 82), *A. celebesensis* (n = 57), *A. interioris* (n = 41), and *A. borneensis* (n = 3). A total of 4 species was found in the Dumoga and Palu Rivers of Sulawesi, Indonesia: *A. marmorata* (n = 260), *A. celebesensis* (n = 165), *A. bicolor pacifica* (n = 68), and *A. interioris* (n = 16). In this study, all eel species other than *A. marmorata* and *A. luzonensis* were excluded from analysis.

In Taiwan, glass eels of *A. marmorata* were found throughout the year, and were far more predominant (>99%) than those of *A. luzonensis*, which were found scattered mainly between July and November (Table 1). *A. marmorata* was abundant (with high CPUE) between March and September in Taiwan (Table 1). On Luzon Island, *A. marmorata* was abundant between February and August, especially between February and May, based on interviews with glass eel fishermen and eel traders in Taiwan and the Philippines. *A. luzonensis*, however, oc-

Table 1. Monthly catch numbers of glass eel samples of *Anguilla marmorata* (AM) and *A. luzonensis* (AL) collected from Taiwan (Yilan), Luzon (Cagayan and Baler), Mindanao (Mati and Gen. San.), and Sulawesi (Dumoga and Palu). Data from Taiwan and Mati are also expressed as catch per unit effort (CPUE) (ind. net^{-1} h^{-1}). Blank spaces indicate no sampling

Site	Taiwan		Cagayan		Baler		Mati		Gen. San.		Sulawesi	
Month	AM (CPUE)	AL (CPUE)	AM	AL	AM	AL	AM (CPUE)	AL (CPUE)	AM	AL	AM	AL
Jan	90 (0.7)	0 (0.0)	629	57			190 (4.3)	0 (0.0)	195	0		
Feb	303 (4.9)	0 (0.0)	789	19			231 (5.0)	0 (0.0)	302	0		
Mar	1122 (32.0)	0 (0.0)	564	13			66 (1.4)	0 (0.0)	236	0		
Apr	545 (27.0)	0 (0.0)	328	18	200	0	42 (1.1)	0 (0.0)	300	0	62	0
May	552 (33.1)	0 (0.0)	976	59	70	0	372 (8.5)	2 (0.1)	475	0	59	0
Jun	453 (30.5)	0 (0.0)	379	12	69	54	1076 (48.9)	33 (1.5)	556	0	25	0
Jul	384 (24.1)	1 (0.1)	258	1022	14	51	959 (53.3)	1 (0.1)	293	4	0	0
Aug	176 (11.9)	0 (0.0)	312	1057	8	43	192 (10.7)	12 (0.7)	238	2		
Sep	643 (50.1)	23 (1.6)	202	1161	46	186	107 (10.7)	1 (0.1)	324	27		
Oct	39 (2.4)	0 (0.0)	73	28	12	11	100 (5.6)	0 (0.0)	470	6	104	0
Nov	138 (2.4)	4 (0.1)	314	26			194 (10.8)	0 (0.0)	518	1		
Dec	25 (0.5)	0 (0.0)	169	120			95 (9.5)	0 (0.0)	364	4	10	0
Total	4470	28	5012	3766	419	345	3185	38	4271	44	260	0

curred mainly from June to October on Luzon Island, but was rare in the Taiwan and Mindanao areas (Table 1). For *A. marmorata* and *A. luzonensis* between June and October, *A. luzonensis* comprised 69.8, 72.8, and 1.4% along the Kuroshio pathway in Baler, Cagayan, and Taiwan, respectively. However, in the same period, *A. luzonensis* comprised 1.9, 2.0, and 0.0% along the MC pathway in Mati, Gen. San., and Sulawesi, respectively. In contrast to the wide distribution of *A. marmorata* in Southeast Asia, *A. luzonensis* is mainly distributed on Luzon Island in summer, but is rare in Taiwan and Mindanao, and not found at all in Sulawesi (Fig. 1, Table 1).

Leptocephalus duration and hatching date

The larval durations of glass eels of *A. marmorata* and *A. luzonensis* in the Baler and Cagayan River of Luzon Island, the Siouguluan River in Taiwan, and the Gen. San. in Mindanao Island are shown in Table 2. Glass eels from Baler had the smallest mean age among the sites, irrespective of species ($p < 0.05$ for both) (Table 2). No significant differences in the mean age of glass eels from Cagayan, Taiwan, and Gen. San. were observed for either species ($p > 0.05$ for both). For *A. marmorata* and *A. luzonensis* collected in the same locations, no significant differences in mean age were observed ($p > 0.05$) except in the Cagayan River ($p < 0.05$).

Based on the back-calculations from the numbers of otolith increments of the *A. luzonensis* glass eels (Table 2), the estimated eel spawning time for samples collected during its main recruitment periods (June and October in Luzon and Mindanao, Table 1) was between February and May (Table 2), which is in good agreement with the estimate from field leptocephalus samples (Kuroki et al. 2012). For *A. marmorata* glass eels, which were more abundant between February and May on Luzon Island, the estimated main spawning dates were likely to be between September and December (Table 2).

Table 2. Otolith daily growth ring counts (mean ± SD) and estimation of presumed season of birth of *Anguilla marmorata* and *A. luzonensis* glass eels. Different letters beside the age of each eel species indicate that the differences are statistically significant ($p < 0.05$). The 9 d of the preleptocephalus stage were added to the total age.

Location	Sampling date	n	Age (d)	Presumed birth season
A. marmorata				
Baler, Philippines	Sep 2013	10	110.1 ± 6.0^{a}	May/Jun
Cagayan, Philippines	May 2008	13	143.0 ± 11.1^{b}	Dec
Gen. San., Philippines	Sep 2013	15	140.7 ± 6.6^{b}	Apr/May
Siouguluan River, Taiwan	May 2008	12	146.1 ± 14.1^{b}	Dec
A. luzonensis				
Baler, Philippines	Sep 2013	20	116.2 ± 6.4^{a}	May
Cagayan, Philippines	Sep 2009	15	127.2 ± 7.5^{b}	Apr/May
Gen. San., Philippines	Jul 2013	13	137.9 ± 10.7^{b}	Feb/Mar
Siouguluan River, Taiwan	Oct 2010	16	137.0 ± 8.3^{b}	May/Jun

Numerical model and tracer experiments

Monthly averages of the 20 yr time series from the NPO model of the NEC bifurcation latitude (blue curve), Kuroshio transport off Taiwan along 23° N latitude (red curve), and MC transport off Mindanao along 8° N latitude (green curve) are shown in Fig. 2. The NEC bifurcation latitude shifted to the north in the winter (the northernmost position appeared in December), and reached its southernmost latitude in June. The seasonal shifts of the bifurcation latitude agree with those inferred from hydrographic data (Qu & Lukas 2003) and satellite measurements (Wang & Hu 2006). Furthermore, Kuroshio transport was related to the NEC bifurcation latitude. The Kuroshio intensified during the summertime, which was associated with the southward shifting of the NEC bifurcation latitude. However, the Kuroshio transport was weaker in winter, when the bifurcation latitude shifted north. On a seasonal timescale, the MC transport was stronger in summer but was weaker in spring.

Two tracer experiments were then conducted to simulate the possible passive behavior of the larvae. The tracers were released at a 100 m depth around 139.5, 140, and 140.5° E along the lines of 12 and 13° N latitude in March and October to simulate the main spawning times of *A. luzonensis* and *A. marmorata*, respectively (Fig. 3). The tracers (larvae) drifted in the westward-flowing NEC, reaching the eastern coast of Luzon Island, where some of the tracers entered the northward-flowing Kuroshio and some were transported into the southward-flowing MC (the dashed box in Fig. 3). In March, when tracers were released around 12° N, they had an equal probability of entering either the Kuroshio or MC (24.6% for both; Fig. 3a). However, if tracers were released around 13° N in March, they had a much higher probability of entering the Kuroshio than

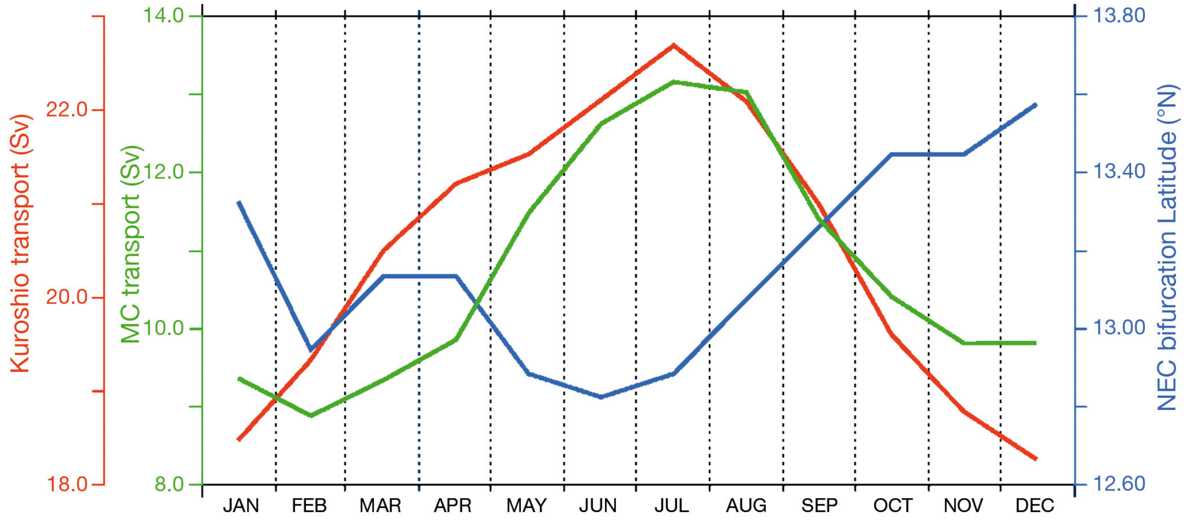

Fig. 2. Monthly fluctuation of the North Equatorial Current (NEC) bifurcation latitude (blue curve), Kuroshio transport along 23° N latitude (red curve), and Mindanao Current (MC) transport along 8° N latitude off Mindanao (green curve). The values are based on the NPO model and averaged from 1990 to 2009 in the upper ocean. One Sverdrup (Sv) = 10^6 m^3 s^{-1}

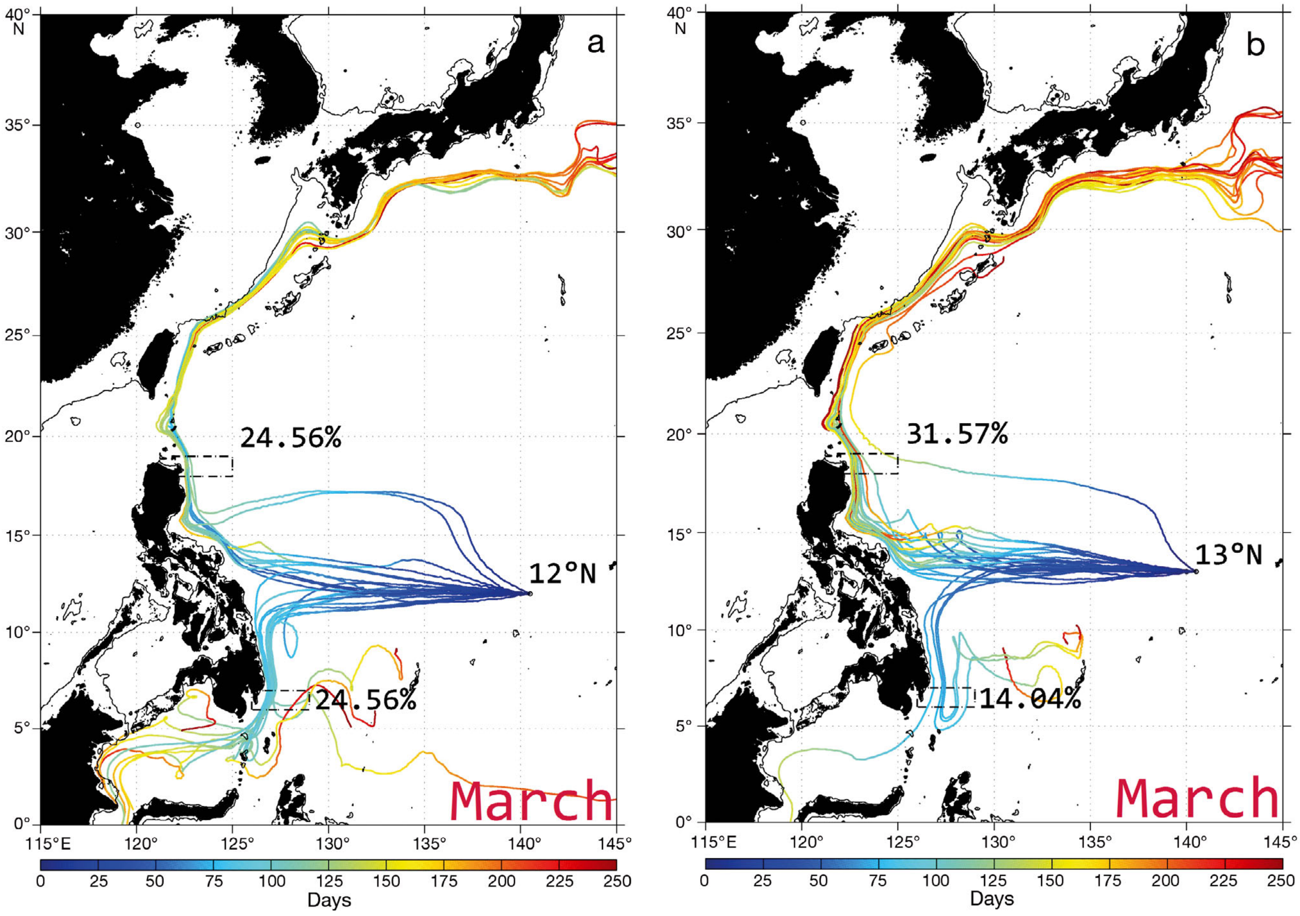

Fig. 3. Trajectories over time of particles released at the presumed spawning sites of (a,b) *Anguilla luzonensis* in March and (c,d) *A. marmorata* in October. Particles were released over a fixed area at (a,c) 12° N or (b,d) 13° N, and 139.5, 140, or 140.5° E. Color scale shows cumulative drift days. The percentage of the particles that successfully passed the dashed boxes of the Kuroshio (18–19° N and 122–125° E) and MC (6–7° N and 126–129° E) was regarded as the successful recruitment rate

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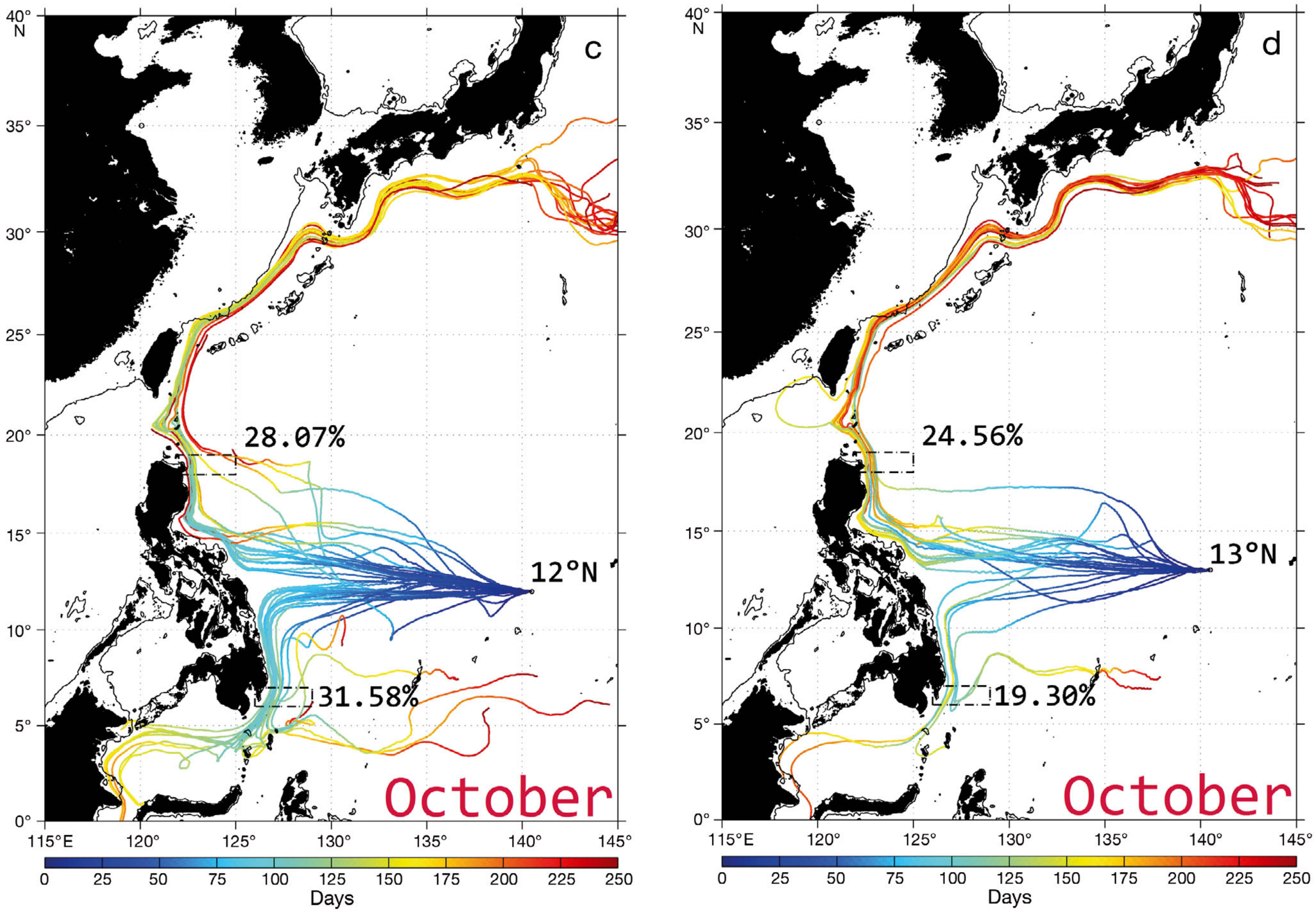

Fig. 3 (continued)

the MC (31.6 and 14.0%, respectively; Fig. 3b). In October, when tracers were released around 12° N, they had a slightly higher probability of entering the MC than the Kuroshio (31.6 and 28.1%, respectively; Fig. 3c). In contrast, if tracers were released around 13° N, they had a slightly higher probability of entering the Kuroshio than the MC (24.6 and 19.3%, respectively; Fig. 3d).

DISCUSSION

A 1970–1974 study by Tabeta et al. (1976) of anguillid elvers in the Cagayan River of northern Luzon identified 4 eel species based on conventional morphological taxonomy, namely *Anguilla marmorata*, *A. celebesensis*, *A. bicolor pacifica*, and *A. japonica*. Identification based solely on morphology is not always reliable because of the overlapping interspecies variation (Jellyman et al. 1996, Han et al. 2002, Watanabe et al. 2009). Using both morphometric characteristics and DNA sequencing identification, 5 eel species (*A. marmorata*, *A. luzonensis*, *A. bicolor pacifica*, *A. japonica*, and *A. celebesensis*) were identified on Luzon Island in the present study. Tabeta et al. (1976) identified the individuals with fin differences between 10 and 12.5% as *A. celebesensis*, and observed that it was the second most dominant eel species in the Cagayan River. *A. luzonensis* has a similar fin ratio (Watanabe et al. 2009). Furthermore, *A. luzonensis* and *A. celebesensis* have the same caudal cutaneous pigmentation in glass eels and a mottled back in juveniles/adults. In the present study, *A. luzonensis* was far more common than *A. celebesensis* on Luzon Island. Thus, most eel samples that were previously identified as *A. celebesensis* in northern Luzon (Tabeta et al. 1976) may actually be *A. luzonensis*. *A. luzonensis* is presumed to spawn in the waters of the NEC (Kuroki et al. 2012). *A. celebesensis*, however, mainly spawns in Indonesian waters such as the Celebes Sea and Tomini Bay (Aoyama et al. 2003, Kuroki et al. 2006b, Miller & Tsukamoto 2006). Thus, *A. celebesensis* should be transported to the Celebes Sea areas instead of being transported to places passed by the Kuroshio. However, the large-scale sampling investigation in the present study found that *A. celebesensis*, although rare, was indeed found in Luzon and Taiwan, so a possible spawning site of *A. celebesensis* in the NEC cannot be excluded.

According to the results of otolith increment counts of *A. marmorata* and *A. luzonensis*, both species had generally comparable ages in the same collection sites (Table 2), suggesting that they may have at least partially overlapping spawning areas in the NEC (Fig. 4). Previous studies indicate that the estimated spawning area of *A. marmorata* (12–17° N, 131–143° E) (Kuroki et al. 2009) overlaps that of *A. japonica* (12–16° N, 137–143° E) (Tsukamoto 1992, 2006, Kuroki et al. 2006a, 2009). The spawning area of *A. luzonensis* may also be in these ranges. In addition, the mean ages of *A. marmorata* and *A. luzonensis* in Baler were lower than those in other locations (Table 2), probably because it is closer to their presumed spawning site. Furthermore, the main recruitment months of the *A. luzonensis* population in Luzon were between June and October. In Mindanao and Taiwan, *A. luzonensis* occurred mainly between June and November (Table 1), suggesting a comparable travel time from the bifurcation of the NEC, with some eels going southward to southern Mindanao and the rest going northward off the coast of Luzon and Taiwan (Fig. 1). However, *A. marmorata* was widely distributed and far more abundant than *A. luzonensis* in Taiwan and the Celebes Sea areas, whereas *A. luzonensis* was concentrated only on Luzon Island (Table 1, Fig. 1). Some biotic and/or environmental factors must be involved in shaping their biogeographic range size in Southeast Asia.

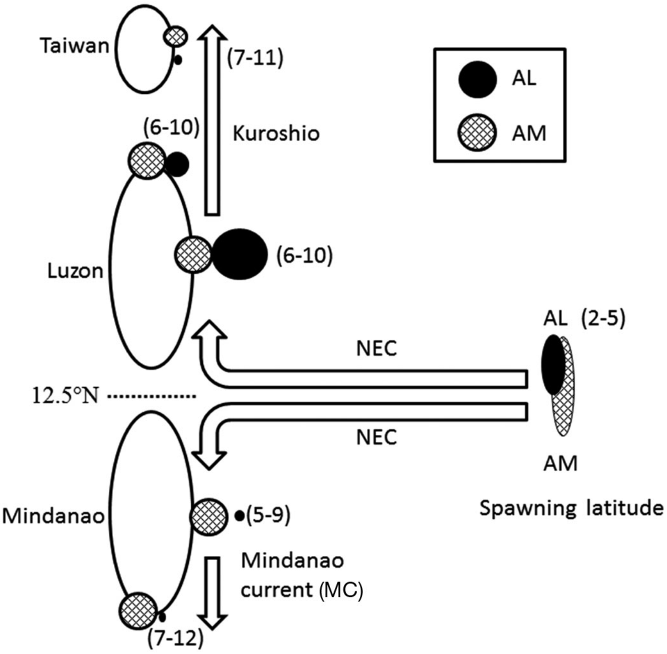

Fig. 4. Hypothesis of the biogeographic distribution of *Anguilla luzonensis* (AL) and *A. marmorata* (AM) in Southeast Asia. *A. luzonensis* mainly spawns north of the North Equatorial Current (NEC) between February and May, and larvae reach the NEC bifurcation site from May through August; most *A. luzonensis* larvae enter the Kuroshio and are restricted to Luzon owing to the shorter/narrower mean larval duration. *A. marmorata*, however, is dispersed in both the MC and the Kuroshio, with a wider distribution range owing to its wider spawning area, protracted spawning period, and a longer/wider mean larval duration. Numbers in parentheses indicate the main months of occurrence of *A. luzonensis*

Kuroki et al. (2009) indicated that *A. japonica* might tend to spawn farther north in the NEC than *A. marmorata*, thus enhancing their northward transport into the Kuroshio. A tracer simulation of *A. japonica* also supported the importance of spawning latitude and season on larval dispersal destination (Kimura et al. 1994, 2001). In the present study, the tracers that were released around 13° N had a much higher probability of entering the Kuroshio than the MC (Fig. 3). In contrast, when tracers were released around 12° N, they had a higher probability of entering the MC than the Kuroshio (Fig. 3). The unusually low percentage of occurrence of *A. luzonensis* in Mindanao (with low CPUE) suggests that *A. luzonensis*, like *A. japonica*, may also spawn farther north in the NEC (Fig. 4).

Back-calculations from the numbers of otolith increments of *A. luzonensis* glass eels revealed that its main spawning time is from February to May (Table 2). A simulated tracer experiment indicated that *A. luzonensis* larvae should reach the NEC bifurcation site from May to August (Fig. 3), when the NEC bifurcation latitude reaches the southernmost position (Fig. 2). Furthermore, Kuroshio transport intensified during the summertime, which is associated with the southward shifting of the NEC bifurcation latitude (Fig. 2). Thus, *A. luzonensis* larvae may have been more likely to be transported into the Kuroshio (Fig. 4). For *A. marmorata*, its main recruitment season was between February and May on Luzon Island, and between June and October on Mindanao Island (Table 1), suggesting the different spawning seasons of *A. marmorata* on Luzon and Mindanao Islands. Thus, a wider spawning latitude and protracted spawning period for *A. marmorata* would guarantee its abundance in both the Kuroshio and MC (Fig. 4).

The differences in larval duration are an important factor in determining the continental distributions of anguillid eels. For example, *A. rostrata* and *A. anguilla*, which share overlapped spawning areas in the

Sargasso Sea and current systems for larval transport, have significantly different lengths of larval duration (Wang & Tzeng 1998). The delay in metamorphosis of about 12 to 15 mo in *A. anguilla* over that in *A. rostrata* is responsible for its long-distance dispersal across the Atlantic Ocean (McCleave 1993). Similarly, for *A. japonica* and *A. marmorata* in the northwestern Pacific, the longer larval duration of *A. japonica* causes it to disperse to higher latitude areas (Leander et al. 2013). The glass eels of *A. marmorata*, which prefer warm water temperature for recruitment and would die in low temperature waters, are mostly screened out in East Asia in winter (Han et al. 2012b). In this study, the main recruitment season of *A. luzonensis* in Taiwan was in the hot summer/autumn, so water temperature seemed unlikely to limit its recruitment. *A. luzonensis*, however, seemed to have a shorter/narrower range of larval duration than *A. marmorata* (Table 2). This restricted *A. luzonensis* to disperse only in tropical areas. Only rare individuals with longer larval durations can be transported to more distant sites such as Taiwan. This also explains why *A. luzonensis* was not found in the Sulawesi.

Phylogenetic analysis of the genus *Anguilla* using 13 mitochondrial genes indicates that *A. luzonensis* and *A. interioris* are closest to each other (Teng et al. 2009). *A. interioris* is a tropical eel species distributed mainly in the Indo-West Pacific Ocean, including Sumatra/Java, New Guinea, and areas around the Celebes Sea (Kuroki et al. 2006b, this study). The tropical Indo-West Pacific is a biodiversity hotspot with numerous archipelagos and many ocean currents passing by, resulting in its high niche complexity and species diversity (Briggs & Bowen 2013). The ancestor of *A. luzonensis* may have originated in the Indo-West Pacific Ocean, and then somehow migrated and established a new spawning population in the NEC. The potentially overlapped spawning sites among *A. luzonensis*, *A. marmorata*, *A. bicolor pacifica*, and *A. japonica* in the NEC suggest potential niche competition among them. Interestingly, the main spawning seasons of *A. marmorata* and *A. luzonensis* populations along the Kuroshio pathway were estimated to be between September and December, and between February and May, respectively (Table 2). A previous study found that the main spawning period of *A. japonica* was between May and August (Han 2011). These 3 eel species appear to have isolated main spawning seasons in the NEC. This may be the result of long-term evolution resulting in the reduction of the inter-species hybridization of spawning adults, food competition among the leptocephali when drifting, or habitat competition for the glass eels when recruiting. In addition, *A. bicolor pacifica* and *A. japonica* prefer to stay in the lower reach of rivers, while *A. luzonensis* and *A. marmorata* prefer to inhabit mountain rivers (Shiao et al. 2002, Watanabe et al. 2009, Arai et al. 2013). The different habitat preferences could also efficiently reduce the inter-species competition for food and space. *A. luzonensis* apparently had a narrower distribution range than *A. marmorata*, suggesting that *A. luzonensis* might possess specific habitat preferences and/or larval/adult traits that are well-adapted to Luzon Island. However, more studies are needed to resolve its unique life history characteristics.

CONCLUSIONS

The northward spawning latitudes in the NEC, the selective transport of eel larvae into the Kuroshio due to the south shift of the NEC bifurcation in summer, and the shorter/narrower larval duration of *A. luzonensis*, together with other unknown larval/adult traits, appear to shape its main biogeographic distribution on Luzon Island (Fig. 4). Its glass eels were commonly fished, along with those of *A. marmorata* and *A. bicolor pacifica*, in the Cagayan River estuary of the Philippines (Crook 2014, Aoyama et al. 2015). The Philippines has supplied East Asian farms with increasing quantities of glass eels in recent years owing to declining eel stocks worldwide, which highlights the potential threat of overexploitation. Based on the narrow distribution of *A. luzonensis*, it is suggested that it be categorized as a 'Vulnerable' fish species in the IUCN Red List, and suitable exploitation regulations should be introduced.

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