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# Reproductive Ecology and Biodiversity of Freshwater Eels around Sulawesi Island Indonesia

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Jun Aoyama, Sam Wouthuyzen, Michael J. Miller, Hagi Y. Sugeha, Mari Kuroki, Shun Watanabe, Augy Syahailatua, Fadly Y. Tantu, Seishi Hagihara, Triyanto, Tsuguo Otake, and Katsumi Tsukamoto (2018) Sulawesi Island of north-central Indonesia is located in a region where at least 6 species of tropical anguillid eels are present, but the reproductive ecology and biodiversity of these eels in each area of the Indonesian archipelago remains poorly understood. Some information about these species was obtained from collections of their leptocephalus larvae made during several times of the year and from year-round collections of their recruitment-stage glass eels at a few locations. A sampling survey of anguillid leptocephali was conducted in March 2010 in both the Celebes Sea and Tomini Bay of Sulawesi Island to learn about the biodiversity and reproductive ecology of the eels in the region. Twenty-eight anguillid leptocephali were collected at 13 different stations, with genetic identification indicating that 3 species of eels had spawned in the two areas. Larvae were more abundant in the Celebes Sea (N = 21; 16.0-52.1 mm TL) than in Tomini Bay (N = 7; 9.6-54.8 mm). The abundant 16-21 mm size-class of Anguilla bornensis in the Celebes Sea indicated that species had recently spawned there, and spawning had also occurred in Tomini Bay by A. celebesensis (17.4 mm). These data and previous life history information suggest that A. celebesensis may have two spawning seasons in the Celebes Sea, but only one main spawning season in Tomini Bay. Anguilla borneensis may spawn at several times of the year in the Celebes Sea. Anguilla marmorata and A. biocolor pacifica spawn outside the Indonesian Seas, with A. marmorata recruiting in large numbers in the Sulawesi Island region during much of the year. Other spawning locations of A. celebesensis and A. interioris likely exist in Indonesian waters. Therefore, further research is needed to understand the reproductive ecologies and biodiversity of the tropical anguillid eels in each region of Indonesia in relation to geographic and climatic factors.

Key words: Freshwater eels, Reproductive ecology, Spawning area, Leptocephali, Sulawesi Island.

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# BACKGROUND

Tropical species of freshwater eels are distributed across the Indo-Pacific region from eastern Africa to French Polynesia (Ege 1939; Watanabe 2003), but their reproductive ecology, early life histories and biodiversity in each area are poorly understood (Aoyama 2009; Kuroki et al. 2014; Miller and Tsukamoto 2017). These species have experienced increasing threats from overfishing - particularly in the glass eel stage in many regions after the northern hemisphere temperate anguillid species have been declared as being endangered and an export ban was placed on glass eels from Europe (Jacoby et al. 2015).

The central Indonesian Seas and nearby areas are one region of concern (Crook 2014; Nijman 2015; Shiraishi and Crook 2015) because it has a high biodiversity of tropical anguillid species that overlap in their distributions (Miller et al. 2009; Watanabe and Miller 2012). At least 5 species are present in the region around Sulawesi Island that include Anguilla celebesensis, A. borneensis, A. interioris, A. marmorata, and A. bicolor pacifica. Another species, A. luzonesis, was recently discovered to be present in the northern Philippines (Watanabe et al. 2009; Aoyama et al. 2015). It also appears to recruit to a lesser extent to the southern Philippines (Han et al. 2016; Shirotori et al. 2016) and likely spawns offshore in the North Equatorial Current (Kuroki et al. 2012; Shinoda et al. 2015). Research on the distribution and size of anguillid leptocephali around Sulawesi Island and southern Indonesia has found that A. celebesensis, A. borneensis, and A. interioris appear to spawn locally after short migrations, but A. marmorata and A. bicolor pacifica spawn outside of the region (Aoyama et al. 2003 2007; Wouthyuzen et al. 2009; Kuroki et al. 2014).

Therefore, anguillid eels in the Sulawesi Island region have several different types of reproductive ecologies. For example, *A. borneensis* appears to be endemic to Borneo Island (Kalimantan) and *A. celebesensis* lives on Sulawesi Island and nearby areas, but both spawn in the Celebes Sea (Aoyama et al. 2003). The species range of *A. interioris* is not well known, but it appears to be present across central Indonesia (Aoyama et al. 2000; Kuroki et al. 2006a; Shirotori et al. 2016). *Anguilla bicolor pacifica* is more widely distributed, but its spawning area is not yet known.

Anguilla marmorata is the most widely distributed species of anguillid eel, with a range covering the entire Indo-Pacific region where tropical anguillids are found. The larvae of A. marmorata that recruit to Sulawesi Island, however, appear to be almost exclusively from the northern population of this species (Minegishi et al. 2008), which is distributed from the northern Indonesian Seas region through the Philippines and up to Taiwan (Shiao et al. 2003) and southern Japan (Yamamoto et al. 2001; Mizuno and Nasawa 2010). The northern population spawns in the North Equatorial Current (Miller et al. 2002; Kuroki et al. 2009) in an overlapping area with the Japanese eel, Anguilla japonica (Tsukamoto et al. 2011), but the two species have different larval characteristics and dispersal patterns (Han et al. 2012; Leander et al. 2013). Comparison of the gonadosomatic index (GSI) values of A. celebesesis that spawns locally and A. marmorata that does not have also indicated that the adults of A. marmorata on Sulawesi Island migrate back to the NEC to spawn (Hagihara et al. 2012; Arai 2014). Anguilla marmorata has separate spawning populations in other parts of its range (Ishikawa et al. 2004; Minegishi et al. 2008; Watanabe et al. 2008; Donovan et al. 2012), but exactly where the eels from those populations spawn remains uncertain (Kuroki et al. 2008; Schabetsburger et al. 2015).

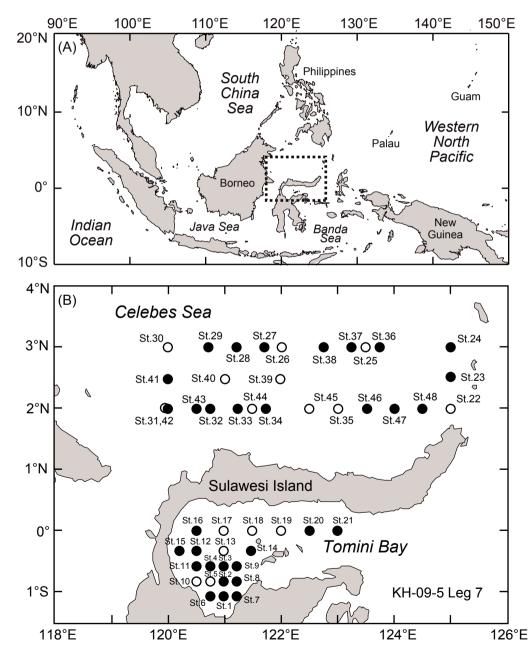
Other information about the reproductive ecology of tropical anguillid eels has been obtained from studies on their recruitment-stage glass eels or their reproductive-stage silver eels. The occurrences of glass eels recruiting to Sulawesi Island suggests spawning might occur throughout much of the year (Arai et al. 2001; Sugeha et al. 2001). Some species living in areas adjacent to the eastern Indian Ocean like A. bicolor bicolor recruit throughout the year (Setiawan et al. 2001) and may spawn during the whole year or during several periods based on their GSI values (Arai et al. 2016; Arai and Kadir 2017). Other species may spawn more seasonally in some areas (Wouthuyzen et al. 2009; Arai and Kadir 2017) depending on factors such as the monsoon cycle of rainfall distribution (Aldrian and Susanto 2003), but these patterns are not vet well understood.

The objective of the present study was to determine the distribution and species composition of anguillid leptocephali around northern Sulawesi Island during March 2010 to contribute to a greater understanding of the biodiversity and reproductive ecology of tropical anguillid eels in this area. Previous surveys around Sulawesi Island were conducted in May 2001 (Aoyama et al. 2003; Wouthuyzen et al. 2005; Miller et al. 2006) and October 2002 (Lee et al. 2008; Wouthuyzen et al. 2009). An additional survey was conducted off west Sumatra in the eastern Indian Ocean in June 2003 (Aoyama et al. 2007; Kuroki et al. 2007; Miller et al. 2011 2013) and a few stations were previously sampled in January 2000 (Aoyama et al. 2003; Miller et al. 2009). The present survey provides new information about the species composition, distribution, and sizes of anguillid larvae and compares it to existing data to examine

the reproductive ecology and biodiversity of anguillid eels in the Sulawesi Island region.

# MATERIALS AND METHODS

The sampling survey for leptocephali in 2010 consisted of sampling in both Tomini Bay (1-13 March) and the Celebes Sea (13-19 March) using the R/V Hahuho Maru of JAMSTEC (Japan) (Fig. 1).



**Fig. 1.** Map of the Indonesian Archipelago region showing the location of the sampling survey (rectangle) (A) for collecting anguillid leptocephali in the Celebes Sea and Tomini Bay in 2 grids of stations (B). Stations sampled at night (black circles) and during daytime (white circles) and the station numbers are shown.

This cruise was the Leg 7 of the extensive KH-09-5 cruise that extended into the Indian Ocean, and the catches of marine eel leptocephali in the Celebes Sea and Tomini Bay have been overviewed recently (Miller et al. 2016).

Sampling was conducted at 48 stations during day and night using an 8.7 m<sup>2</sup> mouth opening Isaacs-Kidd Midwater trawl (IKMT) with 0.5 mm mesh to collect leptocephali in grids of stations in Tomini Bay (21 stations) and the Celebes Sea (26 station locations) (Fig. 1B). 14 of the 21 IKMT tows in Tomini Bay and 17 of the 30 tows in the Celebes Sea were made at night. Oblique tows of the IKMT during the day were made that fished to maximum depths of about 500 m in both Tomini Bay and the Celebes Sea. Two types of IKMT tows were made at night. At the start of the cruise in the south-central region of Tomini Bay where anguillid leptocephali were collected in 2001 (Aoyama et al. 2003; Wouthuyzen et al. 2009), step tows targeted 3 depths of the thermocline layer and one shallow layer after the net went down to about 150 m (Stn. 1-14). Ten minutes of horizontal step-towing was made in the middle of the thermocline, and 10 minutes of step-towing at both 10 m above and below this layer was made to target the layers where anguillid eggs and newlyhatched preleptocephali may accumulate after spawning according to research on the Japanese eel (Tsukamoto et al. 2011; Aoyama et al. 2014). Then a single shallow 10 min step was made at 30 m to collect other leptocephali. Other night stations in Tomini Bay (Stn. 15-21) and all Celebes Sea night stations had step tows that fished to 150 m maximum depths and then had horizontal step-towing at approximately 120, 90, 70, 50, 30 m depths for 10 min (Wouthuyzen et al. 2003) as in previous surveys (Wouthuyzen et al. 2005; Aoyama et al. 2007). Most stations were only sampled once, but two stations in the Celebes Sea were sampled twice at different times (Stn. 31, 42), and 1 extra tow was made at Stn. 32 and 2 extra shallow tows were made at Stn. 36 to collect tuna larvae. The 3 extra tows made for collecting tuna larvae fished down to about 35 m (2 tows) or 96 m (1 tow). A flowmeter was not used with the IKMT in part to reduce net avoidance, so the present study will not make quantitative analyses of anguillid leptocephali catch rates.

After each tow, the plankton samples were visually sorted and all leptocephali were removed and placed in chilled seawater until they were identified onboard. Anguillid larvae were distinguished based on their unique set of morphological features (Fig. 2), which include a total lack of pigmentation except on the tip of the



**Fig. 2.** Photographs of a 16.0 mm *Anguilla borneensis* longfin leptocephalus collected at Stn. 46 in the Celebes Sea (A), and a 54.8 mm *Anguilla marmorata* longfin leptocephalus collected at Stn. 8 in Tomini Bay (B) that shows a deep-body and anteriorly extending dorsal fin (longfin type) that is typical of that species.

tail in preleptocephali, their body shape and the unique position of their last vertical blood vessels and total myomere (muscle segments) (TM) counts (Miller and Tsukamoto 2004). The species of shortfin and longfin eels can be distinguished by the position of the dorsal fin relative to the end of the gut in larger leptocephali (Jespersen 1942; Miller and Tsukamoto 2004; Kuroki et al. 2014).

Each specimen was measured to the nearest 0.1 mm of total length (TL). The morphological features and TM of the larvae and the total number of vertebrae in adults of tropical longfin anguillid species overlap too much to enable species to be distinguished with any certainty based on morphological characteristics for the species in this region. Therefore, genetic identification was conducted to identify the 28 Anguilla larvae using partial nucleotide sequences of their mitochondrial 16S rRNA gene (598-616 bp) as has been done in similar studies in the Indonesian Seas region using that gene (e.g. Aoyama et al. 2007). The sequences were compared with data in the National Center for Biotechnology Information (NCBI) databases by a BLAST search. All specimens had 99.9-100% sequence similarity, except for 2 specimens, which are similar to A. interioris and tentatively labeled as Anguilla spp. in the present study; the sequence data for these two will be used as part of a separate future study. The leptocephali sequences have been submitted to the NCBI GenBank with accession numbers MH289474-MH289501.

### RESULTS

There were 28 anguillid larvae among the 2056 total leptocephali collected at the 48 survey stations around northern Sulawesi Island in Tomini Bay and the Celebes Sea in March 2010. The *Anguilla* leptocephali collected at 13 different stations included 4 different species (Fig. 3), and leptocephali of 2 general size classes were caught (Figs. 4, 5B). The anguillid larvae were widely distributed geographically in the Celebes Sea, but were only caught in the southern portion of Tomini Bay and none were caught in the northern part of the bay (Fig. 3). More leptocephali were collected in the Celebes Sea (N = 21) than in Tomini Bay (N = 7).

The 7 anguillid leptocephali collected in the southern region of Tomini Bay at sizes of 9.6-54.8 mm TL included *A. marmorata* (N = 3; 50.8-54.8 mm), *A. celebesensis* (17.4 mm), and

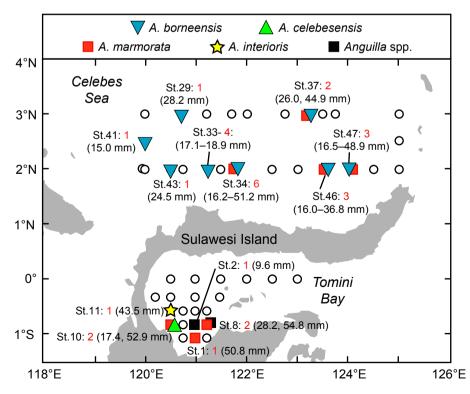


Fig. 3. Map of the stations where anguillid leptocephali were collected showing the number and size range of the anguillid leptocephali at each station in the Celebes Sea and Tomini Bay. Empty circles show stations where no anguillid larvae were collected.

A. interioris or Anguilla spp. (N = 3; 9.6, 28.2, 43.5 mm) (Fig. 3). The larvae were caught at 5 stations, with 2 specimens being collected at both Stn. 8 and Stn. 10. The smallest anguillid leptocephalus (9.6 mm Anguilla spp.) collected during the cruise was found at Stn. 2 near the seamount area (Pasir Laut) in the southern part of the bay. All anguillid leptocephali collected in Tomini Bay had 104 to 106 total myomeres (TM), which is consistent with being the larvae of the longfin eels *A. celebesensis*, *A. interioris*, and *A. marmorata*.

In the Celebes Sea, the 21 anguillid leptocephali were collected at 8 of the 26 stations. There were 2-6 specimens collected at 5 of the stations and a single specimen was caught at the other stations that caught anguillid larvae (Fig. 3). Anguilla borneensis was most abundant (N = 17; 15.0-28.2 mm), and 4 large A. marmorata were also collected and identified (36.8, 44.9, 51.2, 48.9 mm; Stn. 46, 37, 34, 47, respectively). More larvae were caught at the southern stations closer to the base of the northern arm of Sulawesi Island (Fig. 5B). Nine A. borneensis larvae were collected at Stn. 33 and Stn. 34 of the Celebes Sea at sizes of 16.2-21.0 mm along with a 52.1 mm A. marmorata larva. Four A. borneensis were also caught at Stn. 46 and Stn. 47 at size ranges from 16.0-24.5 mm. The leptocephali collected in the Celebes Sea had a TM range of 102 to 109. Anguillid eel larvae (Fig. 5B) were not as abundant or widely distributed as the larvae of the mesopelagic eel family Serrivomeridae - their closest marine eel relatives (Inoue et al. 2010) (Fig. 5C), which also spawn offshore in both areas - or the larvae of several other families of marine eel leptocephali (Miller et al. 2016).

#### DISCUSSION

The survey for anguillid leptocephali in two areas adjacent to northern Sulawesi Island confirmed there is a high anguillid eel biodiversity in this area, because the leptocephalus larvae of 4 species were collected. The small size (9-28 mm) and distribution of collected larvae indicated that spawning occurred in both the Celebes Sea (A. bornensis) and Tomini Bay (A. celebesensis and A. interioris/Anguilla spp.) in the weeks before the cruise in March 2010. This size range of larvae indicated that they had been spawned over about the previous 45 days according to the likely ages of these leptocephali sizes based on age analyses using the otoliths of these species (Kuroki et al. 2006a b 2014). The largest number of small leptocephali was collected in the Celebes Sea at sizes between 15 and 21 mm (A. bornensis), which may have been about 25-35 days old. These specimens were mostly caught in the southern stations that were closest to the northern part of Sulawesi Island.

The grid survey in Tomini Bay provided evidence that most of the spawning activity had occurred in the southern part of the bay in about the previous 10-25 days (Kuroki et al. 2006a b 2014). That finding was somewhat different than the collections in Tomini Bay in May 2001, in which 2 size classes of *A. celebesensis* larvae, 13-23 mm and 30-48 mm, were collected in the areas that included both the southern and northern stations extending to the east as far as Stn. 21 of the present study (Aoyama et al. 2003; Wouthuyzen et al. 2009). A 48.9 mm *A. interioris* leptocephalus was also caught in the southernmost part of the bay in 2001. In contrast, no anguillid leptocephali were collected in Tomini Bay in September 2002

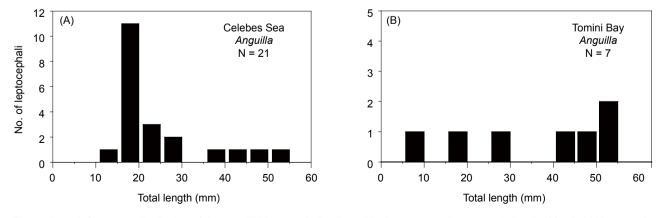
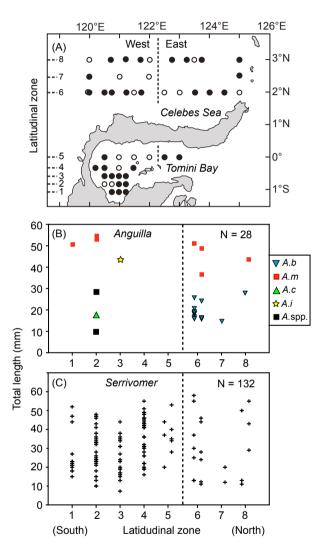


Fig. 4. Length frequency distribution of the anguillid leptocephali collected in the two sampling areas during the March 2010 survey in the northern Sulawesi Island region.

(Wouthuyzen et al. 2009).

Anguillid leptocephali were more abundant in the sampling grid in the Celebes Sea during the 2010 survey than in previous surveys due to the spawning event by *A. borneensis*. Their collection in the southern line of stations in the Celebes Sea suggests they were likely spawned farther out in



**Fig. 5.** Map of the sampling stations for anguillid leptocephali at each of the latitudinal zones of the survey showing stations sampled at night (black circles) and during the day (white) (A), and plots of the total lengths of individual angullid (B) and mesopelagic serrivoverid eel (C) leptocephali collected at each latitudinal zone. The larvae caught in each zone are separated into those in the west (left side) and east (right side) sides of both areas for zones 5-8. The symbols for anguillid species are the same as in figure 3. The dotted lines in (A) separate the east and west sides and in (B) and (C) they separate the larvae caught in Tomini Bay in the south and the Celebes Sea in the north. The data for serrivomerid larvae are adapted from Miller et al. (2016). A.b: *Anguilla borneensis*; A.m: *A. marmorata*; A.c: *A. celebesensis*, A.i: *A. interioris*, *A.*spp.: *Anguilla* spp.

the Celebes Sea and possibly to the northwest based on the likely pattern of ocean currents and large eddies there (Kashino et al. 2001; Masumoto et al. 2001) during and before the survey (see Miller et al. 2016). Small leptocephali of both A. borneensis (8.5 mm) and A. celebesensis (12.3, 17.4, 20.0 mm) were also collected to the northwest and in an area that overlaps with the present study in the Celebes Sea in February 2000 (Aoyama et al. 2003; Miller et al. 2009). In 2001, 31.5 and 37.5 mm A. celebesensis leptocephali were collected in the Celebes Sea and a 36.4 mm larva was collected there in September 2002 (Wouthuyzen et al. 2009). Anguilla borneensis leptocephali (49.0 mm) were also caught in the Celebes Sea area sampled in the present study and also just to the east in the northern Molucca Sea in 2002 (48.6 mm). But the abundance of the larvae of this species was clearly higher in 2010 in the Celebes Sea than the previous surveys in 2002 and earlier.

The survey in 2010 and the previous survey data indicate that tropical eels spawned in two separate areas in the northern Sulawesi Island region. One area is in Tomini Bay, where many of the spawning eels likely come out of the large Poso Lake (323 km<sup>2</sup>) whose outflow enters the southern part of the bay (Sugeha et al. 2006; Hagihara et al. 2012). The presence of small larvae suggest spawning might occur in the bay at least during the February to May rainy season. This spawning activity may be seasonal, however, and dependent on when each species of eels is stimulated to mature and leave the lake during January to July when water levels are higher in the lake (Sugeha et al. 2006; Wouthuyzen et al. 2009), but the process is not well understood. Evidence of spawning during the same seasonal period has been found also in the Celebes Sea for A. borneensis and A. celebesensis, but if the eels spawn in particular areas of the basin is not yet known.

In contrast, all the leptocephali of *Anguilla marmorata* and *A. bicolor pacifica* collected during the surveys around Sulawesi Island have been > 30 mm (Aoyama et al. 2003; Kuroki et al. 2006b; Wouthuyzen et al. 2009) indicating that they likely came from the North Pacific or South Pacific during some seasons and not from local spawning. The Mindanao Current flows directly into the Celebes Sea basin and eddies near its connection with the North Pacific would enable water from various areas to enter the basin (Lukas et al. 1991). Water from the South Pacific also can enter the waters near Tomini Bay (Castruccio et al. 2013). Therefore, as indicated by genetic research (Minegeishi et al. 2008), the *A. marmorata* leptocephali collected in the northern Indonesian seas, as well as the glass eels that recruit there, seem to be from the North Pacific or South Pacific populations of that species, and not from local spawning.

More information is becoming available about the reproductive ecologies of tropical eels through previous and ongoing research in Indonesia and nearby areas on their larvae and especially their recruiting glass eels (Sugeha et al. unpubl. data). The back-calculated hatching dates of A. celebesensis leptocephali from Tomini Bay and the Celebes Sea using otolith microstructure indicate the larvae hatched from January to May, with only one larva hatching in early August (Kuroki et al. 2006b). The smaller number of A. borneensis hatching dates were more widely spread out between February to May and July and September. Back-calculated hatching dates of A. celebesensis glass eels from northern Sulawesi Island adjacent to the Celebes Sea showed that many had hatched during December to April (Arai et al. 2001), which is consistent with estimates from leptocephali. But many glass eels also had hatching dates from July to October, which suggests that A. celebesensis may have 2 spawning seasons around Sulawesi Island. Their glass eels also show seasonal patterns of their abundance during recruitment to northern Sulawesi Island, but the patterns are not always the same among years (Sugeha et al. 2001). The largest number of A. celebesensis glass eels were caught in June of 3 different years (Sugeha et al. 2001) that were likely hatched in January-February if they were about 3-4.5 months old as estimated previously (Arai et al. 2001). Interestingly, there seems to be only one main recruitment season for glass eels in Tomini Bay, from June to November (Sugeha 2010). Considering the 3-4.5-month possible larval duration, that recruitment season is consistent with the data from leptocephali (Kuroki et al. 2006b) and the downstream migration of adults (including A. marmorata; Sugeha et al. 2006; Wouthuyzen et al. 2009) that indicated spawning mostly from January to May.

In nearby areas, catches of *A. celebesenis* glass eels were highest in July and December at the southern edge of Mindanao Island (Shirotori et al. 2016). *Anguilla interioris* glass eels were mostly caught there during November to January, but those species were much less abundant than the

glass eels of *A. marmorata* and *A. bicolor pacifica. Anguilla marmorata* glass eels recruit to southern Mindanao Island, northern Sulawesi Island, and the Poso River in Tomini Bay year-round, whereas the recruitment patterns of *A. biolor pacifica* are much patchier and at lower abundances (Sugeha et al. 2001; Sugeha 2010; Sugeha and Arai 2010; Han et al. 2016; Shirotori et al. 2016). *Anguilla borneensis* appears to have a much smaller recruitment area, restricted to eastern Borneo, but there have been no studies on their glass eels to help evaluate their spawning season.

Although progress has been made to understand the biodiversity, reproductive ecology and early life histories of tropical freshwater eels around Sulawesi Island, there is much more to be learned. The present study collected more A. borneensis leptocephali than ever before and showed that at least two species spawn in Tomini Bay. But how the seasonal patterns of spawning and recruitment might be related to the seasonal cycles of wet and dry seasons in the region (Aldrian and Susanto 2003) and changes in ocean currents during each season is an important subject being examined. It appears that there are at least 3 different spawning and recruitment patterns of the tropical glass eels in Indonesian waters (Sugeha et al. 2008). For example, A. bicolor pacifica and A. marmorata glass eels recruiting in Indonesian waters appear to be coming from 2-3 different spawning sources or spawning seasons, including both north and south Pacific spawning populations (Sugeha and Sasanti 2008; Sugeha and Arai 2010). For A. marmorata this is supported by its population genetic structure in the Indo Pacific (Minegishi et al. 2008). The recruitment patterns of the tropical glass eels recruiting in the western, central and eastern Indonesia waters (Sugeha et al. 2008) also coincides with the 3 regional rainfall patterns across the Indonesian archipelago reported by Aldrian and Susanto (2003).

In addition, from a regional biodiversity perspective, it has been demonstrated that *A*. *celebesensis* spawns in at least 2 different ocean basin areas (Celebes Sea and Tomini Bay), but recent research has shown that *A*. *celebesensis* is an abundant species on the large islands of Buton and Kabaena, which are located at the southeastern end of Sulawesi Island (Tweedley et al. 2013). The long distance to either of the known spawning areas in the Celebes Sea and Tomini Bay suggests that there could be another local spawning area in that part of the Banda Sea. Other regions of the Indonesian archipelago where tropical anguillid eels live have not been sampled for leptocephali; so much remains to be learned about the biodiversity, reproductive ecologies, and early life histories of tropical anguillid eel species living in this interesting region.

# CONCLUSIONS

The sampling survey for anguillid leptocephali conducted in the southern Celebes Sea and Tomini Bay of Sulawesi Island found that the locally endemic species Anguilla borneensis had been spawning in the Celebes Sea and that A. interioris and A. celebesensis had spawned in Tomini Bay. Including information from previous studies, this indicates that 3 of the 5 species known to recruit to these areas spawn locally (A. borneensis, A. celebesensis, and A. interioris), but A. marmorata and A. bicolor pacifica migrate out of the Indonesian Seas to spawn in the North Pacific, and one species, A. celebesensis, spawns in both the Celebes Sea and Tomini Bay. Both A. celebesensis and A. interioris may also have other spawning areas in the Indonesian Seas that have not been found yet. The dynamics of how the reproductive ecology and recruitment pattern of each species interact with the different climate regimes in the Indonesia region and the biodiversity of eel species in each area need further exploration.

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# REFERENCES

- Aldrian E, Susanto RD. 2003. Identification of three dominant rainfall regions within Indonesia and their relationships to sea surface temperature. Int J Climatol **23:**1435-1452.
- Aoyama J. 2009. Life history and evolution of migration in catadromous eels (Genus Anguilla). Aqua-Biosci Monogr 2:1-42.
- Aoyama J, Watanabe S, Ishikawa S, Nishida M, Tsukamoto K. 2000. Are morphological characters distinctive enough to discriminate between two species of freshwater eels, *Anguilla celebesensis* and *A. interioris*? Ichthyol Res 47:157-161.
- Aoyama J, Wouthuyzen S, Miller MJ, Inagaki T, Tsukamoto K. 2003. Short-distance spawning migration of tropical freshwater eels. Biol Bull **204**:104-108.
- Aoyama J, Wouthuyzen S, Miller MJ, Minegishi Y, Minagawa G, Kuroki M, Suharti SR, Kawakami, T, Sumardiharga KO, Tsukamoto K. 2007. Distribution of leptocephali of the freshwater eels, genus *Anguilla*, in the waters off west Sumatra in the Indian Ocean. Environ Biol Fish **80**:445-452.
- Aoyama J, Watanabe S, Miller MJ, Mochioka N, Otake T, Yoshinaga T, Tsukamoto K. 2014. Spawning sites of the Japanese eel in relation to oceanographic structure and the West Mariana Ridge. PLoS ONE 9(2):e88759. doi:10.1371/journal.pone.0088759.
- Aoyama J, Yoshinaga T, Shinoda A, Shirotori F, Yambot AV, Han YS. 2015. Seasonal changes in species composition of glass eels of the genus *Anguilla* (Teleostei: Anguillidae) recruiting to the Cagayan River, Luzon Island, the Philippines. Pac Sci **69**:61-268.
- Arai T. 2014. Evidence of local short-distance spawning migration of tropical freshwater eels, and implications for the evolution of freshwater eel migration. Ecol Evol 4:3812-3819.
- Arai T, Abdul Kadir SR, Chino N. 2016. Year-round spawning by a tropical catadromous eel *Anguilla bicolor bicolor* Mar Biol **163**:1-7.
- Aari T, Kadir SRA. 2017. Opportunistic spawning of tropical anguillid eels *Anguilla bicolor bicolor* and *A. bengalensis bengalensis*. Scientific Rep **7**:41649.
- Arai T, Limbong D, Otake T, Tsukamoto K. 2001. Recruitment mechanisms of tropical eels, *Anguilla* spp. and implications for the evolution of oceanic migration in the genus *Anguilla*. Mar Ecol Progr Ser **216**:253-264.
- Castruccio FS, Curchitser EN, Kleypas JA. 2013. A model for quantifying oceanic transport and mesoscale variability in the Coral Triangle of the Indonesian/Philippines Archipelago. J Geophys Res **118**:6123-6144.
- Crook V. 2014. Slipping away: international *Anguilla* eel trade and the role of the Philippines. TRAFFIC and ZSL, Cambridge.
- Donovan S, Pezold F, Chen Y, Lynch B. 2012. Phylogeography

of *Anguilla marmorata* (Teleostei: Anguilliformes) from the eastern Caroline Islands. Ichthyol Res **59:**70-76.

- Ege V. 1939. A revision of the genus *Anguilla* Shaw, a systematic, phylogenetic and geographical study. Dana Report **16:**1-256.
- Hagihara S, Aoyama J, Limbong D, Tsukamoto K. 2012. Morphological and physiological change of female tropical eels, *Anguilla celebesensis* and *Anguilla marmorata*, in relation to downstream migration. J Fish Biol **81**:408-426.
- Han YS, Yambot AV, Zhang H. 2012. Sympatric spawning but allopatric distribution of *Anguilla japonica* and *Anguilla marmorata*: sieving effects of temperature and oceanic current. PLoS ONE **7**:e37484. doi:10.1371/journal. pone.0037484.
- Han YS, Lin YF, Wu C-R, Iizuka Y, Castillo TR, Yambot IU, Mamalangkap MD, Yambot AV. 2016. Biogeographic distribution of the eel *Anguilla luzonensis*: dependence upon larval duration and oceanic currents. Mar Ecol Prog Ser **551**:227-238.
- Inoue JG, Miya M, Miller MJ, Sado T, Hanel R, López JA, Hatooka K, Aoyama J, Minegishi Y, Nishida M, Tsukamoto K. 2010. Deep-ocean origin of the freshwater eels. Biol Lett 6:363-366.
- Ishikawa S, Tsukamoto K, Nishida M. 2004. Genetic evidence for multiple geographic populations of the giant mottled eel Anguilla marmorata in the Pacific and Indian oceans. Ichthyol Res 51:343-353.
- Jacoby DMP, Casselman JM, Crook V, DeLucia MB, Ahn H, Kaifu K, Durwie T, Sasal P, Silvergrip MC, Smith KG, Uchida K, Walker AM, Gollock MJ. 2015. Synergistic patterns of threat and the challenges facing global anguillid eel conservation. Glob Ecol Conserv **4**:321-333.
- Jespersen P. 1942. Indo-Pacific leptocephalids of the genus Anguilla: systematic and biological studies. Dana Rep 22:1-128.
- Kashino Y, Firing T, Hacker P, Sulaiman A, Lukiyanto. 2001. Currents in the Celebes and Maluku Seas, February 1999. Geophys Res Lett **28**:1263-1266.
- Kuroki M, Aoyama J, Miller MJ, Watanabe S, Shinoda A, Jellyman DJ, Feunteun E, Tsukamoto K. 2008. Distribution and early life-history characteristics of anguillid leptocephali in the western South Pacific. Mar Freshw Res 59:1035-1047.
- Kuroki M, Aoyama J, Miller MJ, Wouthuyzen S, Arai T, Tsukamoto K. 2006b. Contrasting patterns of growth and migration of tropical anguillid leptocephali in the western Pacific and Indonesian Seas. Mar Ecol Progr Ser 309:233-246.
- Kuroki M, Aoyama J, Miller MJ, Yoshinaga T, Shinoda A, Hagihara S, Tsukamoto K. 2009. Sympatric spawning of Anguilla marmorata and Anguilla japonica in the western North Pacific Ocean. J Fish Biol **74**:1853-1865.
- Kuroki M, Aoyama J, Miller MJ, Yoshinaga T, Watanabe S, Tsukamoto K. 2012. Offshore spawning of the newly discovered anguillid species Anguilla luzonensis (Teleostei: Anguillidae) in the western North Pacific. Pac Sci 66:497-507.
- Kuroki M, Aoyama J, Wouthuyzen S, Sumardiharga K, Miller MJ, Tsukamoto K. 2007. Age and growth of Anguilla bicolor bicolor leptocephali in the eastern Indian Ocean. J Fish Biol **70**:538-550.
- Kuroki M, Aoyama J, Wouthuyzen S, Sumardhiharga KO, Miller MJ, Minagawa G, Tsukamoto K. 2006a. Age and growth of *Anguilla interioris* leptocephali collected in Indonesian waters. Coast Mar Sci **30**:464-468.

- Kuroki M, Miller MJ, Tsukamoto K. 2014. Diversity of early life history traits in freshwater eels and the evolution of their oceanic migrations. Can J Zool **92**:749-770.
- Leander NJ, Tzeng WN, Yeh NT, Shen KN, Han YS. 2013. Effects of metamorphosis timing and the larval growth rate on the latitudinal distribution of sympatric freshwater eels *Anguilla japonica* and *A. marmorata*, in the western North Pacific. Zool Stud **52:**30. doi:10.1186/1810-522X-52-30.
- Lee TW, Miller MJ, Hwang HB, Wouthuyzen S, Tsukamoto K. 2008. Distribution and early life history of *Kaupichthys* leptocephali (family Chlopsidae) in the central Indonesian Seas. Mar Biol **153**:285-295.
- Lukas R, Firing ER, Hacker P, Richardson PL, Collins CA, Fine R, Gammon R. 1991. Observations of the Mindanao Current during the western Equatorial Pacific Ocean Circulation Study. J Geophys Res **96**:7089-7104.
- Masumoto Y, Kagimoto T, Yoshida M, Fukuda M, Hirose N, Yamagata T. 2001. Intraseasonal eddies in the Sulawesi Sea simulated in an ocean general circulation model. Geophys Res Lett **28**:1631-1634.
- Miller MJ, Aoyama J, Tsukamoto K. 2009. New perspectives on the early life history of tropical anguillid eels: Implications for management. *In*: Casselman J, Cairns D (eds) Eels at the edge. American Fisheries Society Symposium 58, Bethesda, Maryland, pp. 71-84.
- Miller MJ, Mochioka N, Otake T, Tsukamoto K. 2002. Evidence of a spawning area of *Anguilla marmorata* in the western North Pacific. Mar Biol **140:**809-814.
- Miller MJ, Tsukamoto K. 2004. An introduction to leptocephali: Biology and identification. Tokyo: Ocean Research Institute, University of Tokyo.
- Miller MJ, Tsukamoto K. 2017. The ecology of oceanic dispersal and survival of anguillid leptocephali. Can J Fish Aquat Sci **74:**958-971.
- Miller MJ, Yamaguchi M, Wouthuyzen S, Aoyama J, Suharti S, Ma T, Yoshinaga T, Minegishi Y, Kawakami T, Tsukamoto K. 2013. Ariosoma-type leptocephali (Congridae: Bathymyrinae) in the Mentawai Islands region off western Sumatra, Indonesia. Zool Stud 52:26. doi:10.1186/1810-522X-52-26.
- Miller MJ, Wouthuyzen S, Ma T, Aoyama J, Suharti SR, Minegishi Y, Tsukamoto K. 2011. Distribution, diversity and abundance of garden eel leptocephali off west Sumatra, Indonesia. Zool Stud **50**:177-191.
- Miller MJ, Wouthuyzen S, Minagawa G, Aoyama J, Tsukamoto K. 2006. Distribution and ecology of leptocephali of the congrid eel, *Ariosoma scheelei*, around Sulawesi Island, Indonesia. Mar Biol **148**:1101-1111.
- Miller MJ, Wouthuyzen S, Sugeha HY, Kuroki M, Tawa A, Watanabe S, Syahailatua A, Suharti S, Tantu FY, Otake T, Tsukamoto K, Aoyama J. 2016. High biodiversity of leptocephali in Tomini Bay Indonesia in the center of the Coral Triangle. Reg Stud Mar Sci 8:99-113.
- Minegishi Y, Aoyama J, Tsukamoto K. 2008. Multiple population structure of the giant mottled eel *Anguilla marmorata*. Molec Ecol **17:**3109-3122.
- Mizuno K, Nasawa K. 2010. Occurrence and habitats of the giant mottled eel *Anguilla marmorata* (Anguilliformes, Anguillidae) in rivers of Ehime Prefecture, Japan. Biogeogr **12:**133-139.
- Nijman V. 2015. CITES-listings, EU eel trade bans and the increase of export of tropical eels out of Indonesia. Mar Policy 58:36-41.
- Schabetsberger R, Økland F, Kalfatak D, Sichrowsky U, Tambets M, Aarestrup K, Dall'Olmo G, Sarginson J, Gubili

C, Boufana B, Jehle R, Miller MJ, Scheck A, Kaiser R, Quartly G. 2015. Sympatric spawning of tropical Pacific eels from Vanuatu. Mar Ecol Prog Ser **521**:171-187.

- Setiawan IE, Mochioka N, Amarullah H, Nakazono A. 2001. Inshore migration and spawning season of the tropical eel Anguilla bicolor bicolor recruiting to the Cimandiri River estuary, Java Island, Indian Ocean. *In*: Aida K, Tsukamoto K, Yamauchi K (eds) Proceedings of the International Symposium: Advances in Eel Biology. The University of Tokyo, pp. 125-127.
- Shiao JC, lizuka Y, Chang CW, Tzeng WN. 2003. Disparities in habitat use and migratory behavior between tropical eel *Anguilla marmorata* and temperate eel *A. japonica* in four Taiwanese rivers. Mar Ecol Progr Ser **261**:233-242.
- Shinoda A, Yoshinaga T, Aoyama J, Tsuchida G, Nakazono S, Ishikawa M, Matsugamoto Y, Watanabe S, Azanza RV, Tsukamoto K. 2015. Early life history of the Luzon mottled eel Anguilla luzonensis recruited to the Cagayan River, Luzon Island, the Philippines. Coastal Mar Sci 38:21-26.
- Shiraishi H, Crook V. 2015. Eel market dynamics: an analysis of Anguilla production, trade and consumption in East Asia. TRAFFIC. Tokyo, JAPAN.
- Shirotori F, Ishikawa T, Tanaka C, Aoyama J, Shinoda A, Yambot AV, Yoshinaga T. 2016. Species composition of anguillid glass eels recruited at southern Mindanao Island, the Philippines. Fish Sci **82:**915-922.
- Sugeha HY. 2010. Recruitment mechanism of the tropical anguillid glass eels in the Poso Estuary, Central Sulawesi Island, Indonesia. J Fish Sci UGM **XII(2):**86-100.
- Sugeha HY, Aoyama J, Tsukamoto K. 2006. Downstream migration of tropical anguillid silver eel from Lake Poso, central Sulawesi Island, Indonesia. Limnotec **XIII:**18-25.
- Sugeha HY, Arai T. 2010. Contrasting morphology, genetic, and recruitment pattern of *Anguilla marmorata* glass eels from western, central, and eastern Indonesian waters. J Mar Sci UNDIP, Spec Ed Vol **1:**1-19.
- Sugeha HY, Arai T, Miller MJ, Limbong D, Tsukamoto K. 2001. Inshore migration of the tropical eels, *Anguilla* spp., recruiting to the Poigar River estuary on Sulawesi Island. Mar Ecol Progr Ser **221**:233-243.
- Sugeha HY, Suharti SR. 2008. Discrimination and distribution of two tropical short finned eel *A. bicolor bicolor* and *A. bicolor pacifica* in the Indonesian waters. The NAGISA WESTPAC CONGRESS, pp. 1-14.
- Sugeha HY, Suharti SR, Wouthuyzen S, Sumadhiharga K. 2008. Biodiversity, distribution, and abundance of the

tropical anguillid eels in the Indonesian waters. Mar Res Indonesia **33**:129-137

- Tsukamoto K, Chow S, Otake T, Kurogi H, Mochioka N, Miller MJ, Aoyama J, Kimura S, Watanabe S, Yoshinaga T, Shinoda A, Kuroki M, Oya M, Watanabe T, Hata K, Ijiri S, Kazeto Y, Nomura K, Tanaka H. 2011. Oceanic spawning ecology of freshwater eels in the western North Pacific. Nat Commun 2:179. doi:10.1038/ncomms1174.
- Tweedley JR, Bird DJ, Potter IC, Gill HS, Miller PJ, O'Donovan G, Tjakrawidjaja AJ. 2013. Species compositions and ecology of the riverine ichthyofaunas in two Sulawesian islands in the biodiversity hotspot of Wallacea. J Fish Biol 82:1916-1950.
- Watanabe S. 2003. Taxonomy of the freshwater eels, genus Anguilla Schrank, 1798. In: Aida K, Tsukamoto K, Yamauchi K (eds) Eel Biology. Springer, Tokyo, pp. 3-18.
- Watanabe S, Aoyama J, Miller MJ, Ishikawa S, Feunteun E, Tsukamoto K. 2008. Evidence of population structure in the giant mottled eel, *Anguilla marmorata*, using total number of vertebrae. Copeia **2008**:680-688.
- Watanabe S, Aoyama J, Tsukamoto K. 2009. A new species of freshwater eel, Anguilla luzonensis (Teleostei: Anguillidae) from Luzon Island of the Philippines. Fish Sci 75:387-392.
- Watanabe S, Miller MJ. 2012. Species, geographic distribution, habitat and conservation of freshwater eels. *In*: Sachiko N, Fujimoto M (eds) Eels: Physiology, habitat and conservation. Nova Science Publishers, New York, pp. 1-43.
- Wouthuyzen S, Aoyama J, Sugeha YH, Miller MJ, Kuroki M, Minegishi Y, Suhartati SR, Tsukamoto K. 2009. Seasonality of spawning by tropical anguillid eels around Sulawesi Island, Indonesia. Naturwissenshaften 96:153-158.
- Wouthuyzen S, Miller MJ, Aoyama J, Minagawa G, Sugeha YH, Suhartati S, Inagaki T, Tsukamoto K. 2005. Biodiversity of anguilliform leptocephali in the central Indonesian Seas. Bull Mar Sci 77:209-224.
- Wouthuyzen S, Miller MJ, Minagawa G, Aoyama J, Fukamachi T, Kuroki M, Suhartati SR, Azanza R, Tsukamoto K. 2003. An evaluation of the effectiveness of oblique and step tows for collecting leptocephali. Oseanologi dan Limnol di Indon 35:1-10.
- Yamamoto T, Mochioka N, Nakazono A. 2001. Seasonal occurrence of anguillid glass eels at Yakushima Island, Japan. Fish Sci **67**:530-532.