

# Early life history of tropical freshwater eels (*Anguilla* spp.) recruiting to Viti Levu, Fiji Islands, in the western South Pacific

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**Abstract.** Abstract. The oceanic early life histories of three species of tropical anguillid glass eels, namely *Anguilla marmorata*, *Anguilla megastoma* and *Anguilla obscura*, recruiting to Viti Levu, Fiji Islands, in the western South Pacific, were studied using otolith microstructures of 94 individuals. *A. megastoma* had the shortest leptocephalus duration, age at recruitment and metamorphosis stage (mean  $\pm$  s.d.  $104.7 \pm 7.2$ ,  $141.3 \pm 8.7$  and  $36.6 \pm 8.5$  days respectively), followed by *A. marmorata* (September–October recruitment peak:  $113.0 \pm 11.9$ ,  $115.1 \pm 8.3$  and  $39.2 \pm 11.1$  days respectively; April recruitment peak:  $152.2 \pm 13.5$ ,  $154.0 \pm 12.5$  and  $38.9 \pm 10.1$  days respectively) and *A. obscura* ( $118.0 \pm 10.2$ ,  $168.4 \pm 16.5$  and  $49.6 \pm 12.5$  days respectively). *A. obscura* also had the shortest oceanic glass eel duration of ( $12.2 \pm 3.4$  days), followed by both peaks of *A. marmorata* ( $23.3 \pm 3.2$  and  $20.2 \pm 3.3$  days) and *A. megastoma* ( $20.7 \pm 6.7$  days). Otolith increment rates during the leptocephalus stage did not differ significantly among the three species, but there were significant differences between the leptocephalus duration and metamorphosis stage of *A. megastoma* compared with *A. marmorata* and *A. obscura*, as well as in the oceanic glass eel duration of *A. obscura* compared with *A. marmorata* and *A. megastoma*. There were also significant differences in the age at recruitment among all three species. Back-calculated hatch dates suggest separate peak seasonal spawning periods for each of the three species.

**Additional keywords:** glass eels, leptocephalus, metamorphosis, otolith, spawning.

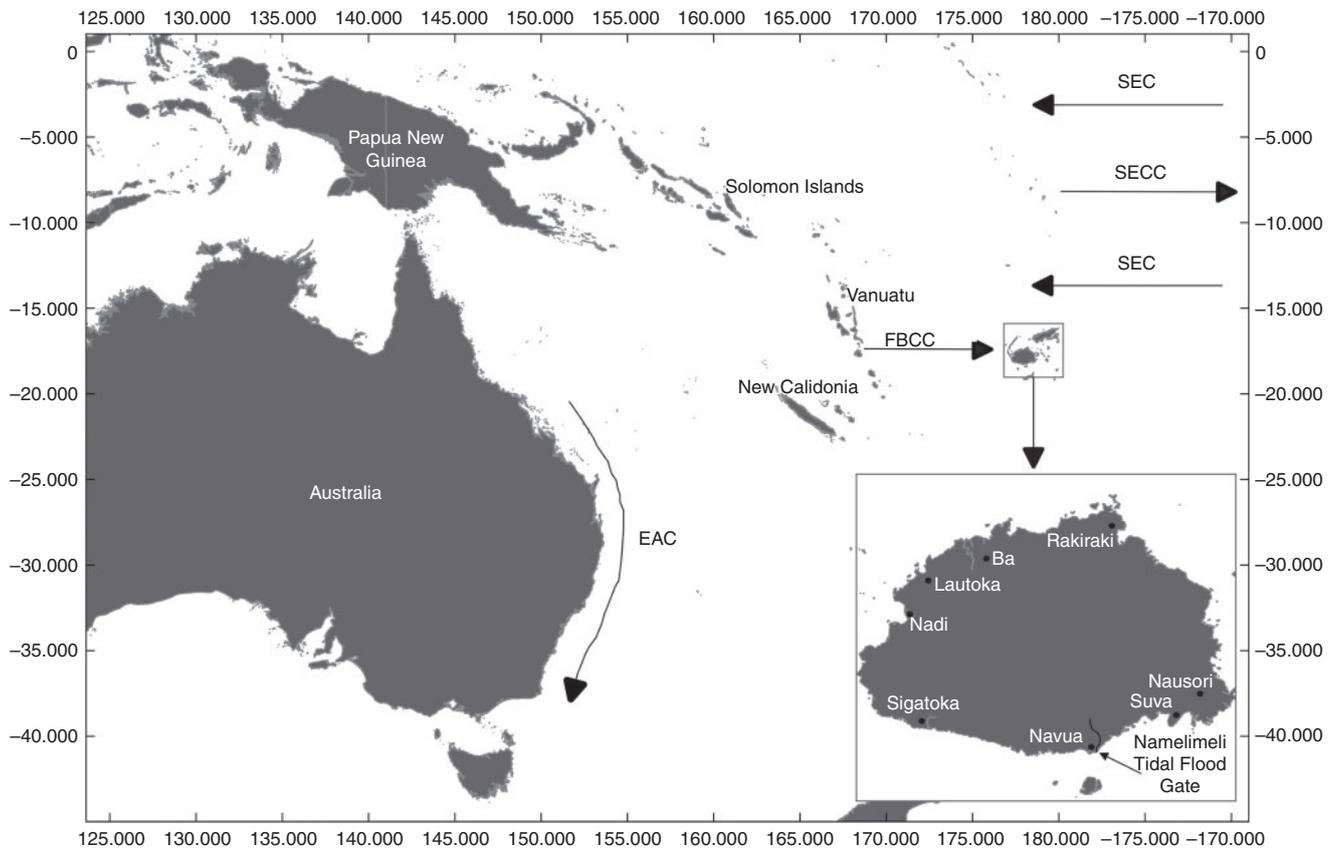
Received 12 February 2019, accepted 30 May 2019, published online 19 August 2019

## Introduction

Progress in otolith analytical techniques has revealed substantial information on the early life histories of several species of anguillid eels. Early life history studies on temperate species, such as *Anguilla japonica*, *Anguilla anguilla*, *Anguilla rostrata*, *Anguilla dieffenbachii* and *Anguilla australis* (Lecomte-Finiger 1992; Otake *et al.* 1994; Marui *et al.* 2001; McCleave 2008), and tropical species, such as *Anguilla bicolor pacifica*, *Anguilla bicolor bicolor*, *Anguilla marmorata*, *Anguilla mossambica* and *Anguilla bengalensis labiata* (Arai *et al.* 1999b; Robinet *et al.* 2003), have provided valuable information on possible spawning locations and migration patterns for these species. Of the 19 species and subspecies of anguillid eels, 13 are found in tropical regions; 6 of these are found in the western South Pacific (WSP) and 3 of the 6 WSP species (*A. marmorata*, *A. megastoma* and

*A. obscura*) in adulthood are distributed along a belt of island groups in the South-west Pacific Ocean (Ege 1939; Beumer *et al.* 1981; Marquet and Galzin 1991). At present only a few studies have focused on the life histories of the three tropical species of anguillid eels recruiting to the WSP (Arai *et al.* 2001; Hewavitharane *et al.* 2017, 2018; Helme *et al.* 2018). With increasing pressure for the procurement of glass eels from the WSP to supply the aquaculture trade in East Asia, it is vital that the biology and life history of anguillid eels in this region is studied to efficiently protect and sustainably utilise this resource.

Therefore the present study examined the otolith microstructure of glass eels from three tropical anguillid species, namely *A. marmorata*, *A. obscura* and *A. megastoma*, recruiting to Fiji Islands in the WSP. Timing and duration of metamorphosis, hatching date, age at recruitment, oceanic glass eel duration and



**Fig. 1.** Map showing the location of Fiji within the western South Pacific Ocean and the sampling location at the Namelimeli Tidal Flood Gate River on Viti Levu Island. The major currents of the region are the South Equatorial Current (SEC), the South Equatorial Counter Current (SECC), the Fiji Basin Counter Current (FBCC) and the East Australian Current (EAC). The map was prepared by Mr Sione Kaituu using online resources from QGIS (ver. 3.6.0, see <https://www.qgis.org/en/site/>).

otolith increment rates during the leptocephalus stage were determined to accumulate baseline data for understanding the migration patterns of anguillid eels in the region.

## Materials and methods

### Collection of glass eels

Glass eels were collected at night every 2 weeks from 18 April 2015 to 6 June 2016 during flood tides on new moon and full moon periods at the tidal flood gates of a small river near Namelimeli, referred to as the Namelimeli Tidal Flood Gate River, ~2 km east of Navua Town, Fiji Islands (Fig. 1). The river is ~30 m wide and 6 km long, and was artificially dug as an 'overflow' channel for the Navua Dam in the Serua District (Hewavitharane *et al.* 2018). The tidal flood gates are a series of 5 gates constructed across the mouth of the river that close and prevent sea water from entering the river during flood tides, but are open during ebb tides to allow river water to flow out to sea. The gates are situated ~60 m from the mouth of the river, which flows directly into the sea at latitude 178.1843°E and longitude 18.2203°S.

Glass eels were collected using two methods. First, using a 'glass eel' fyke net placed at a pre-opened tidal gate on flood tides after sunset, glass eels were collected at the codend of the

net. The second method was hand collection with a fine-mesh scoop net together with a bright light. Glass eels observed on the surface of the water were collected. Glass eels collected were transported live in aerated buckets of brackish water to the laboratory at the School of Marine Studies, The University of the South Pacific, for further analysis.

### Species identification

Hewavitharane *et al.* (2017) collected 1368 glass eels that were morphologically and genetically identified to three species: *A. marmorata*, *A. megastoma* and *A. obscura*. Otoliths of 94 glass eels were used in the present study for otolith microstructure analysis. These 94 specimens were randomly chosen from the collections made during peak recruitment periods, as described by Hewavitharane *et al.* (2018). For morphological identification, glass eels were anaesthetised using a clove oil solution and identified using the anodorsal fin length ratio (ADL%), which is the relative distance between the origins of the dorsal and anal fins as a percentage of total length, together with pigmentation patterns of the caudal cutaneous fin and caudal peduncle, as described by Hewavitharane *et al.* (2017). Thirty-five specimens, having minimum, medium and maximum ADL% values of each of the three species of anguillid glass eels, were initially

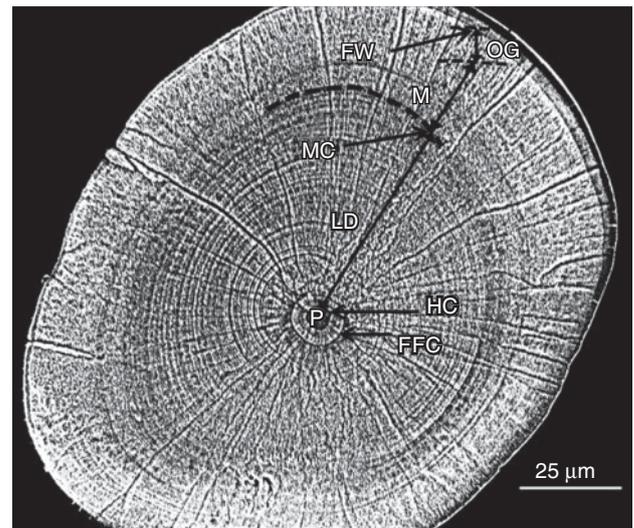
identified morphologically to the species level and subsequently confirmed genetically using a partial sequence of the mitochondrial DNA *16s* rRNA region (Minegishi *et al.* 2005; Tawa *et al.* 2012). DNA sequences within each species were found to be identical, and therefore sequences of 10 randomly selected specimens from each species were submitted to DNA Data Bank Japan (DDBJ) under the accession numbers LC222561–LC222590. Specimens were stored individually in 99.8% ethanol at Kyushu University Museum (voucher numbers KYUM-PI 4753–4782). The pigmentation stage of individual glass eels was assessed according to Strubberg (1913).

#### Otolith preparation and examination

For otoliths of *A. marmorata*, glass eels of two sets were chosen from two separate peak recruitment periods: April 2015 and 2016 ( $n = 21$ ) and September–October 2015 ( $n = 27$ ). For *A. obscura*, a single set was chosen from April 2015 and February–April 2016 ( $n = 22$ ). For *A. megastoma*, samples were chosen from October–December 2015 ( $n = 26$ ). Two sets were chosen for *A. marmorata* because two separate seasonal peaks in recruitment had been observed during the 1-year sampling period, whereas *A. obscura* and *A. megastoma* each exhibited only a single peak in recruitment during the same sampling period (Hewavitharane *et al.* 2018). Otoliths were extracted from individual specimens and mounted on glass slides embedded in epoxy resin. Otoliths were then ground down by hand on abrasive paper 1500 and 2000 cc (Sankyo Waterproof Abrasive Paper, Tokyo, Japan) until the core in the sagittal plane was exposed. The otoliths were further hand polished using 6- and 3- $\mu\text{m}$  diamond paste on a diamond paste polishing cloth (Struers, Tokyo, Japan). Otoliths were then washed in 98% ethanol and distilled water. Following this, otoliths were etched with 0.05 M HCl and vacuum coated in a fine coat ion sputter for observation under a scanning electron microscope (SEM; JCM-6000Plus, Nikon, Tokyo, Japan). SEM photographs of otolith microstructures were taken at a magnification of 2000 $\times$  and were then used to count growth increments along the longest axis of the otolith. Otolith characteristics (Fig. 2), such as primordium and core, first feeding check, leptocephalus zone, metamorphosis zone, oceanic glass eel zone and transition mark to fresh water, were determined as described by Kawakami *et al.* (1998). Ninety of the 94 otoliths in the present study had clearly visible daily increment rings. In the remaining four otoliths examined, daily increment rings could not be distinguished in certain areas within the metamorphosis stage (although they were distinguishable for all other stages). Therefore, areas with indistinguishable increment rings were estimated using areas with clearly visible increment rings within the same otolith zone.

#### Statistical analysis

Datasets with equal variances underwent one-way analysis of variance (ANOVA) followed by a post hoc Tukey test to detect pairwise differences, whereas datasets with unequal variances were analysed using Kruskal–Wallis one-way ANOVA followed by a post hoc test. All statistical analyses were conducted using IBM SPSS Statistics (ver. 22, IBM Corp., Armonk, NY, USA). The significance of correlation coefficients and regression slopes was tested by Fisher's *Z* transformation (Sokal and Rohlf 1969).



**Fig. 2.** Otolith sections of *Anguilla obscura* (51.9-mm total length; specimen KYUM-PI4773). P, primordium; HC, hatch check; FFC, first feeding check; MC, metamorphosis check; LD, leptocephalus duration; M, metamorphosis stage; OG, oceanic glass eel stage; FW, freshwater mark.

## Results

#### Size and stage of glass eels at peak recruitment periods

The total lengths (mean  $\pm$  s.d.) of *A. marmorata* (peak 1 and 2), *A. obscura* and *A. megastoma* were  $51 \pm 1.7$ ,  $51.5 \pm 1.8$ ,  $50.9 \pm 1.6$  and  $51.2 \pm 1.4$  mm respectively (Table 1). No significant differences were observed between the three species ( $P > 0.05$ ) or between peak 1 and peak 2 of *A. marmorata*. Pigmentation stages of glass eels also did not show a significant difference ( $P > 0.05$ ). All glass eels were found to have pigmentation limited to the skull, caudal and rostral regions of the body, and corresponded to classifications of VA and VB stages of Strubberg (1913). The pigmentation stages of glass eels in the present study suggest they had recruited soon after metamorphosis to the river mouth of the Namelimeli tidal flood gate River which agrees with observations reported by Sugeha *et al.* (2001) for tropical eels in the Poigar River on Sulawesi Island.

Unless indicated otherwise, data are given as the mean  $\pm$  s.d.

#### Otolith microstructure analysis

##### Leptocephalus duration

The range of leptocephalus durations for *A. marmorata* (February–April, Peak 1), *A. marmorata* (September–October, Peak 2), *A. obscura* and *A. megastoma* was 101–130, 94–146, 106–142 and 91–124 days respectively (Table 1). The leptocephalus duration of *A. megastoma* ( $104.7 \pm 7.2$  days), was significantly shorter than that of *A. obscura* ( $118.8 \pm 10.2$  days) and both peaks of *A. marmorata* ( $115.1 \pm 8.3$  and  $113.0 \pm 11.9$  days for Peaks 1 and 2 respectively). There were no significant differences between *A. obscura* and *A. marmorata* or between Peaks 1 and 2 of *A. marmorata*. However, there was a significant difference between the leptocephalus duration of *A. megastoma* ( $n = 18$ ) and *A. marmorata* (Peak 2,  $n = 15$ ) that recruited during October 2015 ( $P = 0.035$ ).

**Table 1.** Results of otolith microstructure analysis of three tropical anguillid glass eel species, namely *Anguilla marmorata*, *Anguilla obscura* and *Anguilla megastoma*, recruiting to the western South Pacific, Viti Levu, Fiji

Otolith increment rate was calculated for the leptocephalus stage. The number of samples provided in the 'Oceanic glass eel duration' column are a separate number of samples than those in the species column because not all samples in the former column have oceanic glass eel durations because they simply remain as larvae prior to metamorphosing into glass eels at the river mouth. Peak 1, recruitment period April 2015 and 2016; Peak 2, recruitment period September–October 2015

Species, recruitment period and number of samples	Total length (mm)	Leptocephalus duration (days)	Age at recruitment (days)	Metamorphosis stage (days)	Otolith increment rate ( $\mu\text{m day}^{-1}$ )	Oceanic glass eel duration (days)
<i>A. marmorata</i> Peak 1 ( $n = 21$ )						
Range	48.0–54.5	101–130	134–178	26–72	0.75–0.99	19–29
Mean $\pm$ s.d.	51.0 $\pm$ 1.7	115.1 $\pm$ 8.3	154.0 $\pm$ 12.5	38.9 $\pm$ 10.1	0.86 $\pm$ 0.06	23.3 $\pm$ 3.2
<i>A. marmorata</i> Peak 2 ( $n = 25$ )						
Range	48.5–56.3	94–146	124–183	24–63	0.69–1.06	15–25
Mean $\pm$ s.d.	51.5 $\pm$ 1.8	113.0 $\pm$ 11.9	152.2 $\pm$ 13.5	39.2 $\pm$ 11.1	0.86 $\pm$ 0.09	20.2 $\pm$ 3.3
<i>A. obscura</i> April 2015 and February–April 2016 ( $n = 22$ )						
Range	46.9–53.0	106–142	141–204	30–74	0.63–1.27	8–19
Mean $\pm$ s.d.	50.9 $\pm$ 1.6	118.8 $\pm$ 10.2	168.4 $\pm$ 16.5	49.6 $\pm$ 12.5	0.9 $\pm$ 0.2	12.2 $\pm$ 3.4
<i>A. megastoma</i> October–December 2015 ( $n = 26$ )						
Range	47.8–53.1	91–124	126–163	21–54	0.73–1.03	10–32
Mean $\pm$ s.d.	51.2 $\pm$ 1.4	104.7 $\pm$ 7.2	141.3 $\pm$ 8.7	36.6 $\pm$ 8.5	0.91 $\pm$ 0.09	20.7 $\pm$ 6.7

#### Age at recruitment

The range of age at recruitment for Peaks 1 and 2 of *A. marmorata*, for *A. obscura* and for *A. megastoma* was 134–178, 124–183, 141–204 and 126–163 days respectively (Table 1). There were significant differences across all three species for age at recruitment ( $P < 0.05$ ), with *A. megastoma* having the youngest mean age at recruitment (141.3  $\pm$  8.7 days), followed by Peaks 1 and 2 of *A. marmorata* (154  $\pm$  12.5 and 152.2  $\pm$  13.5 days respectively) and *A. obscura* (168.4  $\pm$  16.5 days). There was no difference between the two peak recruitment periods for *A. marmorata*. There was large variability (>40 days) in the age at recruitment of all three species (Table 1). A significant difference was also observed between *A. megastoma* and *A. marmorata* (Peak 2) that recruited during October 2015 ( $P = 0.005$ ).

Close linear relationships were also apparent between leptocephalus duration and age at recruitment in all three species of anguillid glass eels in the present study (Fisher's Z-transformation  $P < 0.0001$ ; Fig. 3).

#### Metamorphosis stage

The duration of the metamorphosis stage ranged from 21 to 54 days for *A. megastoma*, from 30 to 74 days for *A. obscura* and from 26 to 72 days for *A. marmorata* Peak 1 and from 24 to 63 days for *A. marmorata* Peak 2 (Table 1). The metamorphosis stage took significantly ( $P < 0.05$ ) longer in *A. obscura* (49.6  $\pm$  12.5 days) than in *A. marmorata* Peak 1 and 2 recruitment periods (38.9  $\pm$  10.1 and 39.2  $\pm$  11.1 days respectively) and *A. megastoma* (36.6  $\pm$  8.5 days). In contrast, there was no significant difference in the duration of the metamorphosis stage between the two *A. marmorata* peaks or compared with *A. megastoma* ( $P > 0.05$ ). There was also no significant difference in the metamorphosis stages between *A. megastoma* and *A. marmorata* Peak 2 glass eels that recruited during October 2015 ( $P > 0.05$ ).

#### Otolith increment rate during leptocephalus stage

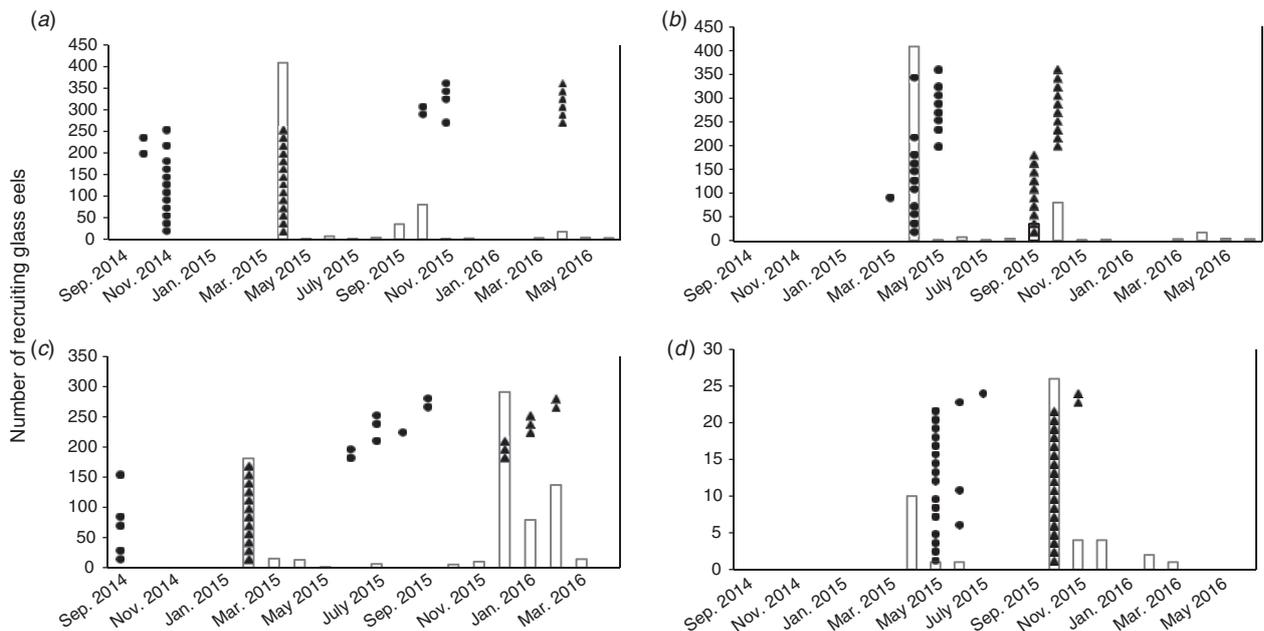
The range of otolith increment rates was 0.73–1.73  $\mu\text{m day}^{-1}$  for *A. megastoma*, 0.63–1.37  $\mu\text{m day}^{-1}$  for *A. obscura* and 0.75–0.99 and 0.69–1.06  $\mu\text{m day}^{-1}$  for *A. marmorata* Peaks 1 and 2 respectively (Table 1). Otolith increment rates did not exhibit any significant differences among all three species ( $P > 0.05$ ), with *A. megastoma* having the largest mean increment rate (0.91  $\pm$  0.01  $\mu\text{m day}^{-1}$ ), followed by *A. obscura* (0.9  $\pm$  0.2  $\mu\text{m day}^{-1}$ ) and *A. marmorata* Peaks 1 and 2 (0.86  $\pm$  0.06 and 0.86  $\pm$  0.09  $\mu\text{m day}^{-1}$  respectively). There were also no significant differences in otolith increment rates between *A. megastoma* and *A. marmorata* (Peak 2) that recruited during October 2015 ( $P > 0.05$ ).

#### Oceanic glass eel duration

Oceanic glass eel durations for *A. obscura* ranged from 8 to 19 days, compared with 10–32 days for *A. megastoma*, 19–29 days for *A. marmorata* Peak 1 and 15–25 days for *A. marmorata* Peak 2 (Table 1). *A. obscura* had a significantly ( $P < 0.05$ ) shorter oceanic glass eel duration than *A. megastoma* and *A. marmorata* Peaks 1 and 2 (12.2  $\pm$  3.4 v. 20.7  $\pm$  6.7, 23.3  $\pm$  3.2 and 20.2  $\pm$  3.3 days respectively). There were no significant differences in the oceanic glass eel duration for both peak recruitment periods of *A. marmorata* and *A. megastoma* ( $P > 0.05$ ). The oceanic glass eel duration of *A. megastoma* and *A. marmorata* Peak 2 that recruited during October 2015 also did not differ significantly ( $P = 0.19$ ).

#### Hatching dates

The estimated hatching dates were back-calculated from sampling dates and ages of each glass eel (Fig. 4). For *A. marmorata* from the Peak 1 recruitment period (April 2015, 2016), hatch dates ranged from late October to late November 2014 and from mid-October to late November 2015 respectively. Hatch dates for *A. marmorata* from Peak 2 (September–October 2015)



**Fig. 3.** Recruitment dates and hatch dates of three species of anguillid glass eels recruiting to Viti Levu, Fiji, in the western South Pacific. (a) *Anguilla marmorata* recruitment season Apr 2015, 2016; (b) *A. marmorata* recruitment season September–October 2015; (c) *Anguilla obscura* recruitment season February–April 2015, 2016; (d) *Anguilla megastoma* recruitment season October–December 2015. Closed circles represent hatch dates of individual glass eels, closed triangles represent corresponding recruitment dates; bar graphs indicate the monthly abundance of each species of anguillid glass eel (Hewavitharane *et al.* 2018).

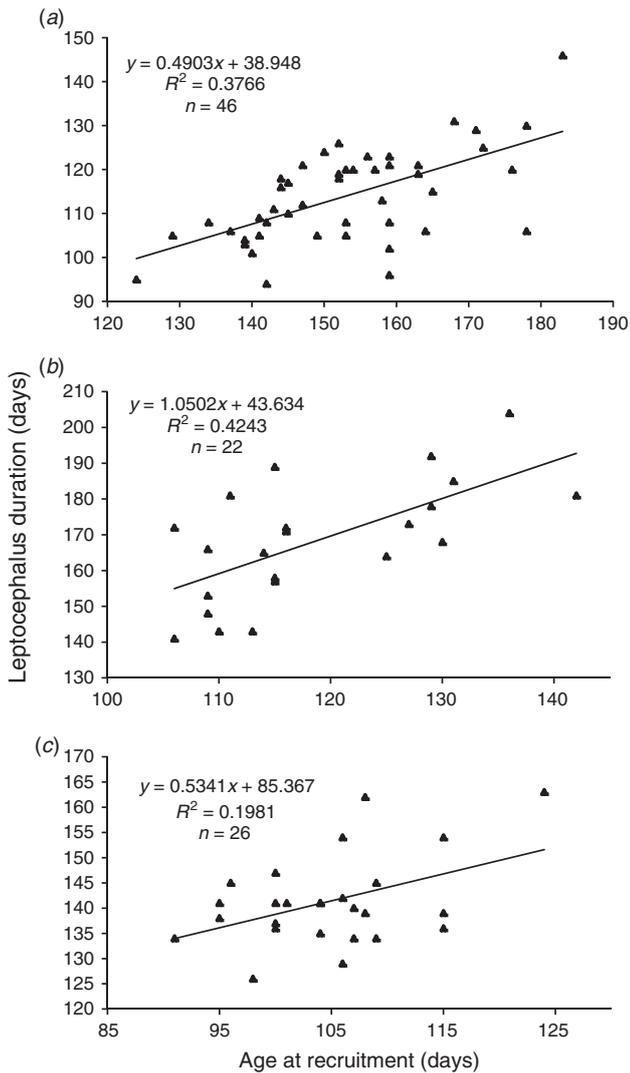
ranged from late March to mid-June 2014. *A. obscura* glass eels that had peak recruitment periods in April 2015 and February–April 2016 had hatch dates ranging from mid-September to late November 2014 and from mid-August to late November 2015 respectively. *A. megastoma* from peak recruitment periods of October–December 2015 had hatch dates ranging from early May to early August 2014. Back-calculated hatch dates for all three of these tropical anguillid eel species show that each species had its own separate peak seasons of spawning (Fig. 4).

## Discussion

There is very little information on the early oceanic life histories of the tropical anguillid eels found in the WSP. Hewavitharane *et al.* (2018) were the first to report the inshore recruitment patterns, species composition and abundance of tropical *Anguilla* glass eels recruiting to Fiji in the WSP. Hewavitharane *et al.* (2018) used recruitment data spanning 14 months (April 2015–June 2016) and reported peak recruitment seasons for three species of anguillid eels, all of which occurred during the hot and rainy seasons in the region. *A. marmorata* was reported to have two peak recruitment periods in April 2015 and September to October 2015, whereas *A. megastoma* had two peak recruitment periods in April 2015 and another in October 2015, and *A. obscura* had a single extended period of recruitment from February to April 2016 (Hewavitharane *et al.* 2018). The present study evaluated the early life histories of the three species of anguillid glass eels recruiting to Fiji by randomly selecting species collected during their peak recruitment periods as reported by Hewavitharane *et al.* (2018) and analysing their otolith microstructures. *A. obscura* is distributed in the WSP

from the eastern coast of Australia to Papua New Guinea, New Caledonia and Tahiti, *A. marmorata* is distributed from Tahiti to New Caledonia and Papua New Guinea, and *A. megastoma* is distributed from Tahiti to New Caledonia and the eastern coast of Papua New Guinea (Watanabe 2003).

Significant differences were found between age at recruitment for all three species of anguillid eels, as well as between the leptocephali duration of *A. megastoma* and *A. obscura*, together with *A. megastoma* and *A. marmorata* of both peaks. There were also significant differences in the duration of the metamorphosis stage between *A. obscura* and both *A. marmorata* and *A. megastoma* (Table 1). Glass eels of *A. megastoma* recruited ~1 month younger and began metamorphosing 2 weeks younger than those of *A. obscura* (Table 1). Leptocephalus duration is the period after hatching and before leptocephalus larvae initiate the onset of metamorphosis into glass eels. This process is indicative of an adaptive shift where the slender leaf-like body form of leptocephalus larvae, adapted to passive pelagic drifting in ocean currents, transforms to a cylindrical body form of glass eels, which are adapted to active swimming and a benthic life (Moran 1994; Miller 2009). Furthermore, the leptocephalus duration of anguillid eels coincides with their oceanic migrations, with species that have shorter leptocephalus durations also having shorter distances to spawning locations (Leander *et al.* 2013). In addition, Kuroki *et al.* (2014) found that tropical anguillid leptocephali tend to metamorphose into glass eels at a much smaller average size than those of temperate species, but indicated that maximum larval sizes for various tropical anguillid eel species in the WSP may be similar. The suggestions of both Leander *et al.* (2013) and Kuroki *et al.* (2014) can be clearly



**Fig. 4.** Linear correlations between age at recruitment and leptocephalus duration for three tropical anguillid glass eel species recruiting to the western South Pacific, Viti Levu, Fiji: (a) *Anguilla marmorata*, (b) *Anguilla obscura* and (c) *Anguilla megastoma*.

observed for *A. japonica* and *A. marmorata* distributed in the western North Pacific (WNP). These two species have similar spawning areas west of the Mariana Islands and use the same oceanic larval transport systems within the North Equatorial Current (NEC) and Kuroshio Current (KC) in the WNP (Kuroki *et al.* 2009; Tsukamoto *et al.* 2011). The NEC bifurcates northward and southward at the western edge of the ocean basin near the Philippines (Lukas *et al.* 1991) and *A. marmorata* are dispersed in both directions, whereas *A. japonica* are carried northward to their areas of distribution (Kuroki *et al.* 2014). However, *A. marmorata* distributed in the northern regions of the WNP do not recruit as far north in Japan or Korea as *A. japonica*. This may be attributed to their different early oceanic life histories and maximum larval size range at metamorphosis (Leander *et al.* 2013; Kuroki *et al.* 2014). It is possible that because of the longer leptocephalus duration and

larger larval size range at the onset of metamorphosis, *A. japonica* is able to metamorphose in more northerly regions of the WNP and is therefore distributed further north in the WNP. *A. marmorata* has a shorter leptocephalus duration and metamorphoses into glass eels at a much smaller larval size, and is therefore not adapted to recruit to more northerly regions (Tesch 1977). Accordingly, the leptocephalus duration and age at recruitment of glass eels may explain distances to spawning locations for each of the three species in the present study. Because all three species in the present study are tropical anguillids, there were no significant differences in the mean size at recruitment, as suggested by Kuroki *et al.* (2014), but there were significant differences for the age at recruitment and leptocephalus duration between *A. megastoma* and *A. obscura*. This may indicate divergent spawning locations in the WSP for these two species recruiting to Fiji. *A. megastoma* had significantly younger age at recruitment and shorter leptocephalus duration than *A. obscura*, indicating that *A. megastoma* may have a closer spawning location to Fiji.

Furthermore, we found a close linear relationship between the leptocephalus duration and age at recruitment for all three species of anguillid eels, which suggests that glass eels with shorter leptocephalus durations also recruit to estuaries earlier in the WSP (Fig. 3). Similar linear relationships have been observed for several other anguillid eel species, such as *A. anguilla*, *A. rostrata* (Arai *et al.* 2000), *A. australis* (Arai *et al.* 1999a) and *A. dieffenbachii* (Marui *et al.* 2001), suggesting that the trend we observed is general across the taxa. Supporting independent evidence further strengthens the hypothesis of divergent spawning locations for *A. megastoma* and *A. obscura* recruiting to Fiji.

In addition, *A. obscura* possessed a significantly longer metamorphosis stage and shorter oceanic glass eel duration than *A. marmorata* and *A. megastoma*. The duration of the metamorphosis stage indicates the length of time taken for leptocephali to metamorphose into glass eels, whereas oceanic glass eel durations indicate the length of time these metamorphosed individuals actively swim at sea before entering estuaries. A significant difference in both durations may indicate that *A. obscura* had a different migration route on distinctive oceanic currents than *A. marmorata* and *A. megastoma* before recruitment in the Namelimele Tidal Flood Gate River. With a mean ( $\pm$ s.d.) oceanic glass eel duration of  $20 \pm 8$  days, *A. japonica* (Kawakami *et al.* 1998) has a similar duration to *A. marmorata* and *A. megastoma* ( $23.3 \pm 3.2$  and  $20.2 \pm 3.3$  days for *A. marmorata* Peaks 1 and 2 respectively and  $20.7 \pm 6.7$  days for *A. megastoma*). In contrast, with a notably shorter oceanic glass eel duration of  $12.2 \pm 3.4$  days, *A. obscura* recruits to areas of adult distribution much earlier after metamorphosis than the other species. It appears there are varying recruitment strategies for glass eels among the tropical anguillid species.

Spawning locations for tropical anguillid eels in the WSP have still not been established. This is due to the limited information on anguillid eels in this region. There have only been a few specimens of *A. megastoma* leptocephali collected in the WSP. Castle (1963) and Jespersen (1942) collected two specimens that were identified through morphological characters to *A. megastoma* from the north-east of Vanuatu, and were relatively small at 23.7 and 26.4 mm respectively. Aoyama *et al.* (1999) also collected two *A. megastoma* leptocephali north-west

of Vanuatu and another south of the Solomon Islands between 10 and 15°S. All leptocephali of *A. megastoma* that have been collected in the WSP were from approximately the same area north-west of Fiji. Kuroki *et al.* (2008) analysed the otolith microstructures of the two *A. megastoma* leptocephali collected by Aoyama *et al.* (1999) in August and September 1995 and reported that back-calculated hatch dates were mid-April and early June 1995 respectively. The interpretation agrees with the hatching dates observed in the present study (early May to early August). Kuroki *et al.* (2008) also suggested that the three temperate and three tropical anguillid eels in the WSP, including *A. megastoma*, may all be using the South Equatorial Current (SEC), region for spawning and larval development. However, because of the small sample number in the limited studies that have collected anguillid leptocephali in the WSP, it is still difficult to accurately infer spawning locations for *A. megastoma* in the region. Kuroki *et al.* (2008) further suggested that *A. megastoma* may have several localised spawning locations in the WSP. This hypothesis was reinforced by Watanabe *et al.* (2011), who reported two populations of *A. megastoma* in the WSP based on differences in vertebral counts. Additional leptocephali collections in the WSP during the spawning seasons proposed here would be the most accurate way to determine spawning locations in the region.

Leptocephali of *A. obscura* have been collected across the WSP from locations near Vanuatu, Fiji and Tahiti (Jespersen 1942; Castle 1963; Kuroki *et al.* 2008). Watanabe *et al.* (2011) suggested that a single panmictic population of *A. obscura* is distributed in the WSP, based on statistically similar vertebral counts of this species collected across the region. Furthermore Schabetsberger *et al.* (2015), who studied the genetic diversity of *A. marmorata*, *A. megastoma* and *A. obscura* from Vanuatu using a combination of nuclear and mitochondrial DNA sequences and microsatellite-generated distances genotype sequencing, found several specimens of *A. megastoma* and *A. marmorata* that were possibly admixed or hybrids, whereas *A. obscura* were distinctly separate. Schabetsberger *et al.* (2015) also used pop-up satellite transmitters on migrating silver eels from Vanuatu to determine possible spawning locations of the three species that resulted in tags from both *A. megastoma* and *A. marmorata* popping up in the same general area. This, in combination with evidence of admixed individuals of *A. megastoma* and *A. marmorata*, led Schabetsberger *et al.* (2015) to suggest these two species may have overlapping spawning locations in the WSP, whereas *A. obscura* may have a separate and distinct spawning location. Although this provides an explanation for separate spawning locations between *A. obscura* and *A. megastoma*, it conflicts with our results that support separate spawning locations for *A. megastoma* and *A. marmorata* as well because of significantly different leptocephalus durations and ages at recruitment between individuals of the two species recruiting to Viti Levu, Fiji. Minegishi *et al.* (2008) investigated the population genetic structure of *A. marmorata* and found that control region *F*-statistics showed significant differences between paired localities in the WSP namely Fiji–Papua New Guinea, Tahiti–New Caledonia and Tahiti–Papua New Guinea. Minegishi *et al.* (2008) also stated that these significant differences between paired localities in the WSP were smaller than among other regions and therefore

*A. marmorata* in the WSP may be of a metapopulation. Ishikawa *et al.* (2004), who also analysed the population structure of *A. marmorata* using mitochondrial DNA, suggested that there were two spawning populations in the WSP, one from Fiji and another from Tahiti. Similarly, Watanabe *et al.* (2008) reported that *A. marmorata* may have two populations within the WSP based on significantly different total vertebral counts in populations from Tahiti and Fiji, New Caledonia, Samoa and Papua New Guinea. Taking these studies into consideration, it would seem that there may be more than two spawning locations for *A. marmorata* in the WSP. Because we analysed otoliths from *A. megastoma* recruiting during October 2015 ( $n = 18$ ) that overlapped with the second peak recruitment period of *A. marmorata* in September–October 2015 (October glass eels,  $n = 15$ ), significant differences were still evident in leptocephalus durations and age at recruitment for *A. marmorata* and *A. megastoma*. Therefore, it is possible that glass eels of *A. marmorata* recruiting to Fiji may be spawning in a different location to *A. marmorata* and *A. megastoma* recruiting to Vanuatu, resulting in the significant differences in age at recruitment and leptocephalus duration observed in this study. It is also possible that *A. marmorata* and *A. megastoma* recruiting to Fiji may be spawning in the same location as suggested by Schabetsberger *et al.* (2015), namely 8.770–10.322°S, 170.462–174.614°E. We suggest that this is a plausible explanation for the significant differences in leptocephalus durations and age at recruitment, and it would thus be possible that *A. marmorata* is using a separate larval migration strategy to that of *A. megastoma*. Miller *et al.* (2002) suggested that differences in the southward distribution of *A. marmorata* and *A. japonica* in the WNP may be due to differences in the depth distribution of the two species while migrating within the NEC. Divergent depths of migration may also be carrying the leptocephali larvae of *A. marmorata* and *A. megastoma* recruiting to Fiji on separate migration routes, which may account for the significant differences in leptocephalus durations and age at recruitment. From the proposed spawning locations by Schabetsberger *et al.* (2015), it is possible that leptocephalus larvae are carried eastwards on the South Equatorial Counter Current (SECC) and at some point join the SEC and are carried south-westwards towards Fiji. The points at which *A. marmorata* and *A. megastoma* join the SEC may be different. This is another possible explanation for significant differences in leptocephalus duration and age at recruitment. With limited information on oceanic early life histories of anguillid leptocephali in the WSP, further leptocephali collections along the SEC and SECC during the proposed hatch dates documented here are required to clarify whether these assumptions are correct.

We also analysed *A. marmorata* glass eels from two separate seasons: April 2015, 2016 and September–October 2015. Hewavitharane *et al.* (2018) suggested that these were two separate peak periods of recruitment of *A. marmorata* to the Namelimeli Tidal Flood Gate River, Viti Levu, Fiji. Combined with current early life history data and back-calculated hatch dates (Fig. 4), it can clearly be observed that leptocephali of the two seasons had distinctly separate hatching seasons and consequently two distinctive peak spawning periods in the WSP, one in late austral spring and one in mid-autumn to early winter. This is consistent with recently observed tropical anguillid eel species

such as *Anguilla celebesensis* and *A. bicolor pacifica* in Tomini Bay, Indonesia (Wouthuyzen *et al.* 2009), where leptocephali of both species demonstrated seasonality of spawning. In addition, comparing the leptocephalus duration and recruitment age of our *A. marmorata* samples from the WSP to those distributed in Taiwan and Japan in the Indo-Pacific (Arai *et al.* 2002), the two groups have similar mean values. The present study found mean ( $\pm$ s.d.) leptocephalus durations of  $115.1 \pm 8.3$  and  $113 \pm 11.9$  days and recruitment ages of  $154 \pm 13$  and  $152.2 \pm 13.5$  days for Peaks 1 and 2 respectively, whereas the leptocephalus duration for those distributed in Taiwan and Japan was  $114 \pm 14$  and  $123 \pm 14$  days respectively, and recruitment age was  $144 \pm 16$  and  $154 \pm 17$  days respectively. These values are similar to those of *A. marmorata* glass eels recruiting to Sulawesi Island in Indonesia (Arai *et al.* 2001) of 129–171 days and 158–201 days for leptocephalus duration and age at recruitment respectively. Although the WSP and Indo-Pacific populations exhibited similarities, these values are notably higher than those for glass eels of *A. marmorata* recruiting to Réunion Island (Robinet *et al.* 2003) of  $96.9 \pm 26.4$  and  $120.2 \pm 24.7$  days for leptocephalus duration and age at recruitment respectively. These disparities in early oceanic life histories between populations within *A. marmorata* are indicative of the many distinct populations across the Indo-Pacific possessing distinctive reproductive strategies.

Oceanic current patterns in the WSP region are highly complex, compounded by varying seasonal current patterns. Further glass eel recruitment studies in other parts of Fiji and the region are required to strengthen the current knowledge of early life histories of anguillids in the region. Because there is very little information on the early life histories of tropical anguillid eels distributed in the WSP, basic information will enable us to better understand their migration patterns and population structures, which will, in turn, help with conservation efforts within Pacific Island nations. The present study was able to add to the limited information on the oceanic early life histories of anguillid eels distributed in the WSP. Two of the three species of anguillid eels recruiting to Fiji, namely *A. marmorata* and *A. megastoma*, may possibly have a similar spawning region north-west of Fiji, whereas *A. obscura* spawns in a separate location. The significant differences in age at recruitment and leptocephalus durations for all three species that we found, together with previous genetic studies, may also indicate divergent spawning locations for all three species. Further leptocephalus collections within the SECC and SEC from March to August north of Fiji and north-east of Vanuatu are recommended if possible spawning locations for *A. marmorata* and *A. megastoma* are to be identified. Back-calculated hatch dates suggest that tropical eels recruiting to Viti Levu, Fiji have separate seasonal spawning peak periods. There are still large knowledge gaps that need to be filled and further life history studies on anguillid eels in the WSP are required. Currently these three species of anguillid eels are on the International Union for Conservation of Nature (IUCN) (see <https://www.iucnredlist.org/species/196302/2443435> for *A. obscura*; <https://www.iucnredlist.org/species/166189/45832585> for *A. marmorata*; and <https://www.iucnredlist.org/species/196301/2443332> for *A. megastoma*, accessed 13 August 2019) list of threatened species. *A. obscura* and *A. megastoma* are both listed as data deficient (DD), whereas *A. marmorata* is listed as of least

concern (LC), highlighting the need to garner greater knowledge on WSP anguillid eels. Accurate ecological and biological data are essential for any conservation and management initiatives.

### Conflicts of interest

The authors declare that they have no conflicts of interest.

### Declaration of funding

This research did not receive any specific funding.

### Acknowledgements

The authors extend their sincere gratitude to their colleagues at The University of the South Pacific for their generous assistance during sampling and laboratory analysis, namely Ramesh Prasad, Marika Rakai, Samisoni Rakai, Joeli Tupou, Jone Lima, Laura Williams and Nanise Bulai.

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Handling Editor: Michael Joy