

The short-lived neon damselfish *Pomacentrus coelestis*: implications for population dynamics

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Daily increments of *Pomacentrus coelestis*, an abundant and well-studied fish, were validated for the life of the fish and depending on the location, age-maxima were estimated to be 127–160 days on reefs separated by tens to hundreds of kilometres on the Great Barrier Reef. This contrasts with congeners and other damselfishes that live for 5 years or more. Otoliths of *P. coelestis* were thinner and had different patterns of banding when compared with relatively long-lived congeners. It is suggested that banding patterns in *P. coelestis* may be related to patterns of maturation and spawning. The consequences of a short life would have a great influence on the population dynamics of this widespread species. Further, the demographics and habitat preferences of this species suggest rapid colonization and establishment of breeding populations that would quickly change the relative abundance of sympatric fishes.

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Key words: age; age structure; coral-reef-fish; otolith; Pomacentridae; population-dynamics.

INTRODUCTION

Determination of the age structure of fish populations is critical for understanding population dynamics (Doherty & Fowler, 1994a). The accumulation of year classes within a population provides a level of resilience ('storage effects'; Doherty & Williams, 1988) that would not be found in short-lived fishes. Long-lived fishes, combined with strong recruitment, should result in more stable populations than fishes with very short lives. Although many tropical reef fishes live for decades (Acanthuridae, Choat & Axe, 1996; Serranidae, Pears *et al.*, 2006; Chaetodontidae, Berumen *et al.*, 2012), recent studies have shown that a number of reef fishes may live for a year or less (Gobiidae, Depczynski & Bellwood, 2005; Apogonidae, Kingsford *et al.*, 2014). There is, therefore, a high risk of recruitment failure, major fluctuations in population size and even potential for rapid changes in the genotypic makeup of populations for fish species with short lives (Lefevre *et al.*, 2016).

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Members of the Pomacentridae (damselfishes) represent a family of highly speciose tropical reef fishes. Pomacentrids belong to trophic groups that include herbivores and planktivores (Allen, 1991) and often rank first or second in regional and local species diversity (Bellwood & Wainright, 2002). The abundance of damselfishes and the diverse range of habitats in which they are found has resulted in great focus on the family for studies on the dynamics of reef fish populations and communities (Sale, 1991; Doherty, 2002; Jones *et al.*, 2009).

Despite their small size (Munday & Jones, 1998) many damselfishes from tropical and subtropical regions are surprisingly old. For example (maximum standard length, L_S , in cm is indicated; Allen, 1991): *Pomacentrus moluccensis* Bleeker 1853 (70 mm, 14–18 years; Fowler & Doherty, 1992); *Pomacentrus amboinensis* Bleeker 1868 (80 mm, 6 years; McCormick, 2016); *Pomacentrus wardi* Whitley 1927 (8 cm, 8 years; Fowler & Doherty, 1992); *Stegastes fuscus* Cuvier 1830 (c. 8 cm, 16 years; Schwamborn & Ferreira, 2002); *Stegastes planifrons* Cuvier 1830 (85 mm, 18 years; Caldow & Wellington, 2003); *Hypsypops rubicundus* Girard 1854 (310 mm, 13 years; Clarke, 1970); *Parma microlepis* Günther 1862 (160 mm, 37 years; Tzioumis & Kingsford, 1999).

One of the most common and species diverse pomacentrid genera is *Pomacentrus* (Allen, 1991) and, as a result there have been numerous studies on these fishes. Many species have broad distributions in tropical waters. *Pomacentrus coelestis* Jordan & Starks 1901 is widespread and has a largely tropical distribution that includes, Japan, Taiwan, Philippines, Indonesia, Papua New Guinea, Melanesia, Oceania, the Great Barrier Reef (GBR) and western and eastern Australia to southern New South Wales (Allen, 1991; Liggins *et al.*, 2015). There have been studies on this species that have focussed on: life history (Suzuki *et al.*, 1985), spawning (Jan, 1997), early life history (Patterson *et al.*, 2005), Planktonic Larval Duration (PLD) and the age of juveniles (Kingsford *et al.*, 2011); settlement (Ohman *et al.*, 1998); biogeography (Williams, 1982), recruitment (Doherty *et al.*, 1996), connectivity (Patterson *et al.*, 2004), genetic structure among reefs (Gerlach *et al.*, 2007) and at broader spatial scales (Lacson, 1994; Liu *et al.*, 2010; Mirams *et al.*, 2011; Liggins *et al.*, 2015, 2016). With the exception of estimates of age maxima from high latitude reefs in Japan (c. 2 years, based on scales; Suzuki *et al.*, 1985), however, there are no similar data from tropical waters. Information regarding the age-structure and age maxima of tropical *P. coelestis* populations is important for understanding the meta-population dynamics of this species and in reconciling fine scale population genetic patterns. Preliminary genetic data (G. Gerlach *et al.* unpubl. data) suggest that spatio-temporal genetic patterns in this species resemble those recently described for the short-lived apogonid *Ostorhinchus doederleini* Jordan & Snyder 1901 (Gerlach *et al.*, 2016). Accordingly, investigators need reliable data on the age of fishes to help interpret the dynamics of reef-based populations.

The objective of this study was to determine the age and growth of *P. coelestis* and estimate age maxima of local populations at different locations. The aims were as follows. (1) The ageing technique for *P. coelestis* was validated, especially for the largest size classes as the validation of daily rings had only been done previously for pre-settlement fish and early settlers (Kingsford *et al.*, 2011). Furthermore, because Suzuki *et al.* (1985) concluded that annual rings were laid down in the scales of *P. coelestis* collected at high latitude sites in Japan the scales of a sub-set of *P. coelestis* were examined from tropical sites on the GBR, Australia. (2) To provide growth curves and von Bertalanffy statistics for *P. coelestis* and make comparisons of age and growth

at reefs separated by up to 1200 km and in different years. Unexpectedly, based on (1) and (2) only daily increments were found and no annual rings in *P. coelestis*, yet there was a pattern of intermittent dark banding (discontinuous areas) along the radius extending from the primordium to the outer edge of the otolith. (3) Consequently, the aim was to further validate the observation that only daily increments were present in *P. coelestis* otoliths by comparing their banding with congeners where annual rings have been validated or found (e.g. *P. moluccensis*). This involved a comparison of band spacing from the primordium to the edge (annuli are widely spaced early in life and narrower as fish get older), comparisons of otolith height v. width; where long lived fish would be expected to have wider otoliths. (4) Once it was demonstrated that the dark bands in the otoliths of *P. coelestis* were not annuli a detailed analysis of band structure in *P. coelestis* was carried out to investigate sub-daily possibilities. Because sub-month banding has been found in other groups [e.g. lunar patterns in molluscs (Cerrato, 2000)], the frequency of deposition of bands in the fish was described. The broader implications of the results are discussed with respect to the dynamics of *P. coelestis* and potential changes to species representation in assemblages of reef fishes.

MATERIALS AND METHODS

SAMPLING

A total of 227 *P. coelestis* of a broad size range were collected from One Tree Island and Fitzroy Reef (southern GBR), and Lizard Island (northern GBR; Table I) in order to compare patterns of age and growth among reefs. The most distant reefs (Lizard and Fitzroy) were separated by 1200 km, while One Tree Island and Fitzroy are 15 km apart. Additional fish were collected from Keeper and Arlington reef (central GBR) for the validation experiment ($n=40$) and, for an estimate of the egg hatching dates of fish collected in the austral winter–spring in 2015 ($n=11$) and 2016 ($n=24$).

All fish were collected by scuba divers using diluted clove oil (5:1, ethanol:clove oil) and hand nets or hand spears. All samples were preserved in ethanol, then dissected and L_S measured later in the laboratory.

PREPARATION OF OTOLITHS

All fish were weighed (± 0.01 g) and measured ($L_S \pm 1$ mm). Sagittal otolith pairs were dissected, and dried overnight. An otolith from each pair of sagittae was randomly selected, embedded to the edge of a glass microscope slide using Crystal Bond thermoplastic glue (<https://www.emsdiasum.com/microscopy/default.aspx>). The sulcal groove of the otolith was orientated perpendicular to the edge of the slide and the outer edge of the primordium was flush with the inside edge of the slide. Slides were then held vertically and the posterior section was removed by grinding to the transverse mid-plane using a Gemmasta GF4 Faceting Machine (<http://www.shell-lap.com.au/>) with a 1200 μm diamond encrusted steel disc. The glue was then reheated and otoliths were remounted with the newly ground surface placed face down in the centre of the glass slide. Otoliths were ground with a disk with 3000 μm grit until a thin section (c. 20 μm thick) was translucent enough to see structure including the primordium.

VALIDATION OF INCREMENT PERIODICITY

Daily growth increments have been found and validated in the sectioned sagittae of pre-settlement and early juvenile *P. coelestis* (to a maximum age of 27 days and L_S of

TABLE I. Details for the collection of *Pomacentrus coelestis* (PC), *Pomacentrus amboinensis* (PA) and *Pomacentrus moluccensis* (PM)

Dates sampled	Reefs	Latitude	Longitude	Species	Stage
16 December 2005	North Direction Island	14° 44' 37'' S	145° 30' 17'' E	PC	New settlers
17 December 2005	Day Reef	14° 29' 82'' S	145° 30' 79'' E	PC	New settlers
17 December 2005	MacGillvary Reef	14° 39' 18'' S	145° 29' 24'' E	PC	New settlers
17–25 January 2006	One Tree Island	23° 29' 42'' S	152° 05' 53'' E	PC	All stages
February 2011 and 2012	Lizard Island	14° 42' 12'' S	145° 33' 57'' E	PC	Adults
19 January 2013	Fitzroy Reef	23° 36' 11'' S	152° 09' 58'' E	PC	All stages
30 January to 1 February 2013	One Tree Island	23° 29' 42'' S	152° 05' 53'' E	PC	All stages
7–9 January 2014	Arlington Reef	16° 40' 40'' S	145° 58' 7'' E	PC	All stages
20 August 2013	Keeper Reef	18° 44' 33'' S	147° 16' 59'' E	PC, PM, PA	All stages
8–9 June 2014					
16 October 2016					
2 February 2015	One Tree Island	23° 29' 42'' S	152° 05' 53'' E	PC, PM, PA	Adults
18 April 2016	Fitzroy Reef	23° 36' 59'' S	152° 09' 41'' E	PA	Adults

11–21 mm; Kingsford *et al.*, 2011). An initial examination of the sagittae of fish that were approaching maximum size suggested that only daily increments were visible. Accordingly, it was decided that it was prudent to repeat the validation experiment for fish of the largest sizes.

It was determined that fish had a translocation mark from when they were placed in tanks (Fig. 1). It was possible therefore, to measure the number of increments to a tetracycline mark (administered 25 days later) and from the tetracycline mark to the edge of the otoliths. The translocation mark was validated by knowing the date of translocation and the date when the tetracycline was administered.

The validation procedure required the exposure of *P. coelestis* otoliths to a broad spectrum antibiotic tetracycline hydrochloride (TCL). Fish were allowed to acclimate for 14 days or more in a flow through seawater aquarium at the Marine and Aquaculture Research Facilities Unit (MARFU) at James Cook University. Following the acclimation period, fish were then anaesthetized with 10 ml 10:1 (ethanol:clove oil) per 5 l of sea water for 10 s and then, L_S was measured for an estimate of body mass, and fish were then injected with one of the following doses: (1) low dose injected with 173–216 mg kg⁻¹ TCL or, (2) high dose injected with 346–461 mg kg⁻¹ TCL. Fish were then kept in a flow through seawater facility at MARFU for a known period of time.

All experimental fish were fed on a diet of live *Artemia* sp. and INVE Aquaculture Nutrition NRD pellets (<http://www.inveaquaculture.com/>) daily. TCL marks could be viewed under fluorescent light with a compound microscope (Leica DMLB fitted with an Osram HBO

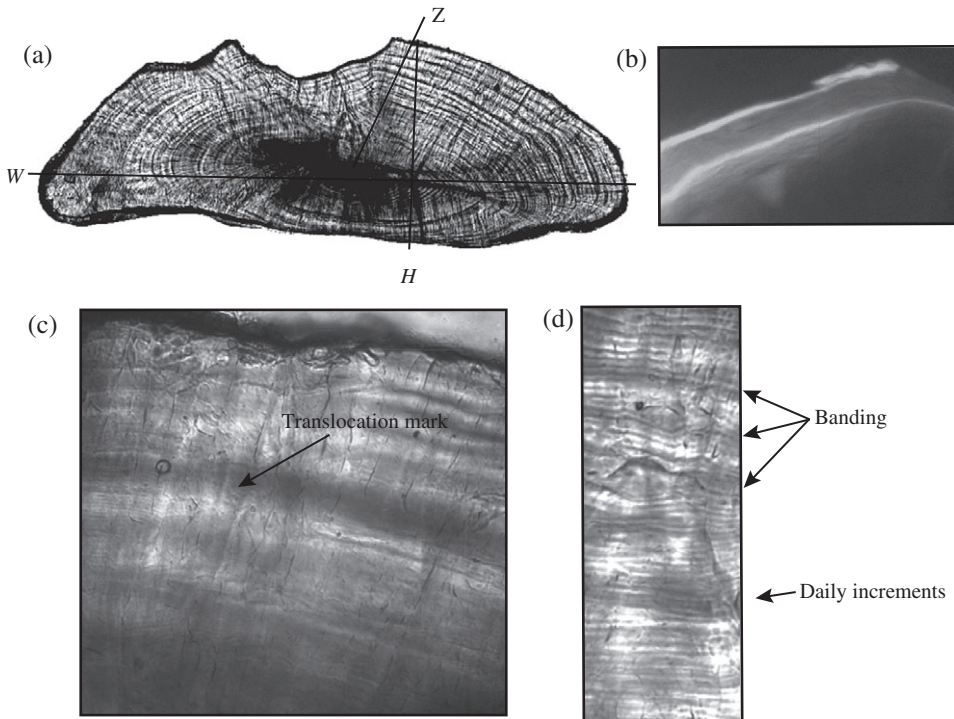


FIG. 1. Features of *Pomacentrus coelestis* otolith sections. (a) A transverse section of an otolith from a 53 day-old Heron Reef fish (27.5 mm, standard length, L_S) showing axis (W) used for measuring width (1200 μm) and the height ($H = 380 \mu\text{m}$) of otoliths used for morphometrics. Z, the axis along which age bands were counted. (b) Segment of otolith treated with tetracycline 28 days before being sacrificed (25 μm post-treatment growth). (c) The translocation mark indicates the time fish were transferred from the wild to the aquarium system. (d) An enlarged segment of otolith showing detail of daily increments and banding pattern.

fluorescent light and a Leica DC300 digital camera) and measurements were made with the Leica IM50 image measurement module (www.leica.com).

Suzuki *et al.* (1985) used scales to gain estimates of the age of *P. coelestis* and concluded that they deposit annual rings. Annual rings were not observed in the sagittae of the fish collected here. It was cross checked whether annuli were present in the ctenoid scales of large ($n = 20$ fish and 43–64 mm L_S). Scales were sampled in four areas of the fish as follows: under the pectoral fin, dorsal to the pectoral fin, mid-way down the body and close to the caudal peduncle and five scales were sampled at each position on each fish.

There is strong spawning and recruitment of *P. coelestis* during the Austral summer (Doherty *et al.*, 1996), and this is the period that most of the fish were collected. Recruitment is considered largely over by April to May (Sale *et al.*, 1984). Given how short-lived fish were estimated to be in this study (≤ 160 days) it was predicted that spawning must take place more often for populations to persist. Accordingly, the age of some fish collected in the Austral winter at Keeper Reef in 2015 and 2016 were back-dated to determine the time of spawning (2015, $n = 11$; 2016, $n = 24$).

ANALYSIS OF GROWTH INCREMENTS

Daily increments of *P. coelestis* of a wide size range were counted along a radius from the primordium to the outer edge of the otolith using a compound microscope with a light field

(Leica DMLB; Fig. 1 using axis Z). Alternating translucent and opaque daily increments were counted; no annuli were observed, even in the largest fish. The counting of increments began from the first clear increment closest to the primordium. Sections were coded and examined in random order and the increments counted on two occasions by the same observer separated by at least 4 days. Counts of daily rings from the two occasions were compared in order to assess the confidence in the accuracy of age estimates. If increment counts differed by >10% between counting occasions, then the otoliths were re-polished and then re-examined; 122 fish of 207 fish required a recount. If, following a third reading, agreement between the third and one of the two other counts was not reached, then the fish was rejected ($n=2$ of 122 fish); following a recount accuracy was 4%, or better. For estimates of age, an average of the two closest age estimates was used.

GROWTH

Patterns of growth were described using the von Bertalanffy growth function (VBGF), according to:

$$L_t = L_\infty [1 - e^{-K(t-t_0)}] \quad (1)$$

where L_t is the length at age t (days), L_∞ the mean asymptotic L_S (average maximum length), K the rate at which the growth curve approaches L_∞ , and t_0 the age at which the fish have a theoretical length of zero as described by K ; the approximate size at hatching (3.0 mm) was used. Growth curves were constrained to the size of smallest fish collected at each reef. Initial estimates of the growth function were then altered with 100 permutations using the Solver function of Microsoft Excel to minimize variation between the model and the data by altering L_∞ , K and t_0 .

INTERSPECIFIC COMPARISON OF OTOLITHS

Annuli have been found in other species of *Pomacentrus* (Doherty & Fowler, 1992, 1994a), but were not observed in *P. coelestis*. It was predicted that fish living for a short period of time would have thinner otoliths, than those taxa that accumulate aragonite in the otoliths over many years. Accordingly, the otolith morphometrics of *Pomacentrus* species with annuli were compared with those of *P. coelestis*. The maximum width (W) and height (H) were measured for otolith sections of *P. coelestis*, *P. amboinensis* and *P. moluccensis* (Fig. 1). Interspecific comparisons of otolith height to width, as a proportion were made (Fig. 1 using axes H and W). As this relationship may vary with the size of fish, comparisons were done using ANCOVA where fish of a broader size range were available (*i.e.* 20–70 mm L_S). A prerequisite of a test of adjusted means was to determine that the slope of the lines were not significantly different (using Systat ver. 13; <https://systatsoftware.com/>).

Although it was concluded that *P. coelestis* only had daily increments in the otoliths, dark bands were found in some fishes. To confirm these bands were not annuli, the dark band spacing of *P. coelestis* was compared with the yearly bands found in other *Pomacentrus* species. Yearly bands are generally widely spaced in the first few years and rapidly narrow in following years (Fowler, 1990). It was predicted therefore, that the relationship of band spacing (*i.e.* distance between discontinuous zones) verses increment number would be different for fish with annuli than without. The distances between dark bands were measured from the primordium of otoliths for the three *Pomacentrus* species as above. Fish were collected from Keeper Reef, One Tree Island and Fitzroy Reef (Table I).

ANALYSIS OF BANDS IN *P. COELESTIS* OTOLITHS

It was possible that these dark bands found in *P. coelestis* related to sub-monthly influences such as spawning cycles (Kanazawa & Sato, 2008), and phase of the moon (Cerrato, 2000; Schone *et al.*, 2002), as has been found with molluscs. Accordingly, samples of fish from One Tree Island, Keeper Reef and Fitzroy Island were selected where the fish from each reef had been collected on the same day. This allowed a direct comparison of the placement, by day, of

darkened bands in relation to lunar phase and tidal amplitude (maximum difference between high and low tide in daylight hours). Because the width of increments could also have been affected within months (Schone *et al.*, 2002), the spacing of increments over 40 days from the time of collection was measured. To further test for periodicity in banding the data from all three locations were concatenated, whereby they were joined at the same lunar date, and analysed for autocorrelation (Systat ver. 13).

RESULTS

AGE VALIDATION

There was a close agreement between the number of increments represented between the translocation mark and injection with TCL and the number of days between treatments. From the outside edge of the mark (TM) to the TCL the mean percentage agreement was 85% (Table II), while from the inside edge of the TM mean percentage agreement was 104% (*i.e.* a slight overestimate of the known value); mean increment count 25.90 ± 1.32 . Increment spacing before and during the experiment indicated that otolith growth, and presumably fish growth, was similar. It was concluded therefore, that increments were daily in large fish as demonstrated for juveniles (Kingsford *et al.*, 2011). The otoliths of most *P. coelestis* treated with TCL injection showed a bright fluorescent band close to the otolith edge [Fig. 1(c)]. Tetracycline, however, appeared to slow growth, as average growth (*i.e.* increment spacing) both before translocation, between translocation and the TCL mark were greater than that measured after (TCL to edge measurements, Table II). No annuli were found in the scales of large *P. coelestis* and this was regardless of position sampled on the body of the fish.

AGE AND GROWTH

The maximum age of *P. coelestis* from all reefs ranged from 127 to 160 days (Table III). Average maximum ages (upper 5%) ranged from 127 to 147, so long-lived fish did not even attain an age of 6 months. Age maxima of the top 5–10% of fish collected was similar at One Tree Island and Fitzroy, while fish from Lizard Island only reached 86–88% of the age of their southern conspecifics. Youngest fish were new settlers from 14 to 25 days. Growth was more rapid over the first 60 days at Lizard Island (Fig. 2) as indicated by a higher *K* (Table IV). Categorical analysis of fish in the size range of 35–45 mm L_S showed that fish from the two southern most reefs (One Tree Island and Fitzroy Reef) attained this size at a similar age of 78–93 days (Fig. 3). In contrast, fish from Lizard Island were young for their size, which matches the estimate of *K* from the V-B analysis (Table IV). L_∞ was estimated to be 60 mm at Lizard Island and 67–69 mm at One Tree Island and Fitzroy Island (Table IV). Of the top 5% of fish at each location fish were longer on southern reefs [maximum L_S mm (range)]; One Tree Island [64 (62–65)], Fitzroy Reef [62 (61–63)], when compared to Lizard Island [55 (54–56)].

Pomacentrus coelestis caught in August 2015 were 27–112 days old at capture ($n=11$, L_S 16–49 mm) and collected in September 2016 were 22–116 days old ($n=24$, L_S 15–55 mm). Back-dating age for 2015 fish indicated that these individuals would have hatched between mid-May to early August and the 2016 fish from late

TABLE II. Summary of validation experiments to determine the frequency of increment deposition in *Pomacentrus coelestis*. Fish were held for 25 days before administration of tetracycline (TCL); *i.e.* the predicted difference between the outer edge of the transition mark (TM) and TCL. Range of ages of fish in the experiment with TCL, 58–104 days

Increment count TM–TCL				Mean ± S.E.	Mean ± S.E. (range)	Calculated mean ± S.E.
<i>n</i>	TCL treatment	Increments represented (%)	10–TM (μm)	TM–TCL (μm)	(range) increment width	
Translocation mark present						
6	A	82 ± 5.14 (64–100)	2.23 ± 0.11 (1.5–2.3)	2.23 ± 0.18 (1.75–3.0)	0.29 ± 0.04 (0.16–0.47)	
10	B	87.2 ± 4.25 (60–100)	2.33 ± 0.18 (1.4–3.1)	2.33 ± 0.17 (1.4–2.92)	0.26 ± 0.04 (0.14–0.32)	
	Mean	84.6 ± 4.69	2.26 ± 0.17	2.28 ± 0.18	0.28 ± 0.04	
All fish with TCL						
		Increment count TCL–edge	Average increment width TCL–edge (μm)			
9	A	8.3 ± 1.8 (3–11)	0.27 ± 0.04 (0.16–0.47)			
15	B	11 ± 1.09 (6–20)	0.41 ± 0.07 (0.16–1.07)			
	Mean	9.69 ± 1.46	0.34 ± 0.05			

A, low-dose tetracycline = 173–216 mg kg⁻¹; B, high-dose tetracycline = 346–461 mg kg⁻¹.

TABLE III. Estimates of age maxima and average maximum age of the oldest 5 and 10% for *Pomacentrus coelestis* from each location

Reef	<i>n</i>	Maximum age (days)	Maximum mean \pm S.E. 5% (<i>n</i>)	Maximum mean \pm S.E. 10% (<i>n</i>)
Lizard Island	77	127	116 \pm 6 (4)	107 \pm 4 (8)
One Tree Island	85	160	147 \pm 6 (4)	133 \pm 5 (9)
Fitzroy	65	152	143 \pm 5 (3)	136 \pm 3 (7)

n, sample size.

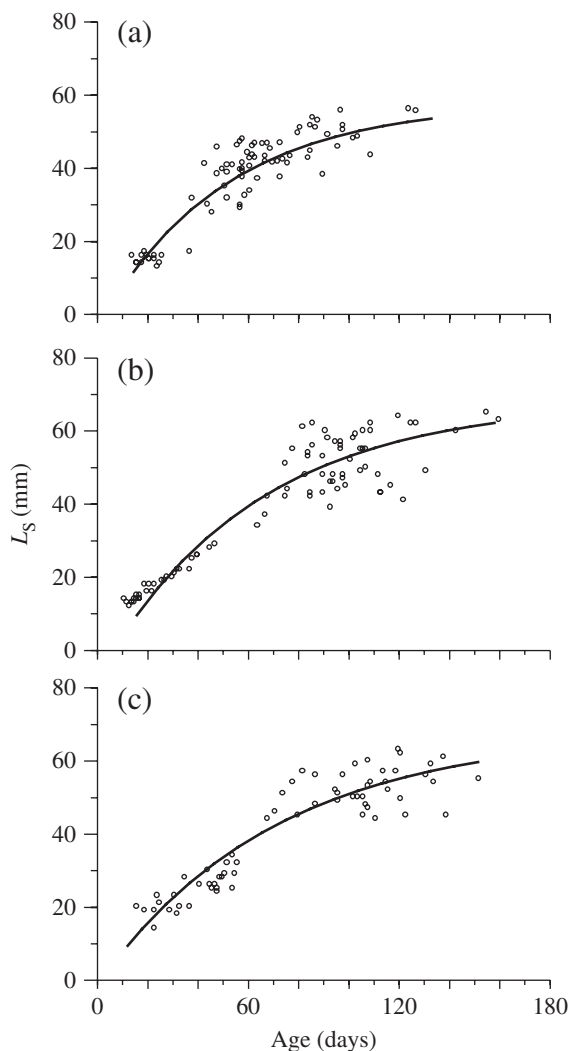


FIG. 2. Standard length (L_S) von Bertalanffy growth curves for *Pomacentrus coelestis* for (a) Lizard Island ($n=77$), (b) One Tree Island ($n=85$) and (c) Fitzroy Reef ($n=65$) from the Great Barrier Reef, Australia. Growth parameters are given in Table IV.

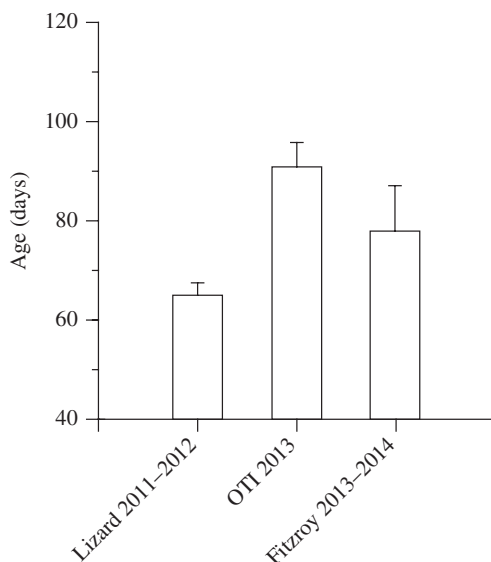


FIG. 3. Comparison of mean \pm S.E. age v. 35–45 mm standard length (L_S) for *Pomacentrus coelestis* from three Great Barrier Reef sites (Lizard, Lizard Island, $n = 31$; OTI, One Tree Island, $n = 14$; Fitzroy, Fitzroy Reef, $n = 13$) and years.

May to late August indicating that at least some spawning is taking place during late autumn and the winter months.

INTERSPECIFIC COMPARISON OF OTOLITH SIZE AND THICKNESS

The bands (=annuli) in *P. amboinensis* and *P. moluccensis* were initially wide (*P. amboinensis*, 60 μm and *P. moluccensis*, 98 μm) and rapidly narrowed with increment number (Fig. 4). In contrast, the width of bands in *P. coelestis* ranged between 19 and 23 μm and there was no steep decline in spacing with age, especially between the first and second band, as for the other species.

The *Pomacentrus* species that lived for >1 year generally had wider and thicker otoliths for a given size (Figs 5 and 6). ANCOVA demonstrated that the slopes of the relationships between L_S and otolith width were not significantly different for *P. coelestis* and *P. moluccensis* (test for slopes: d.f. = 1,54; $F = 0.110$, >0.05). *Pomacentrus moluccensis*, however, had significantly wider otoliths for a given size [adjusted means: d.f. = 1,55; $F = 106.46$, $P < 0.001$; Fig. 6(a)].

Visually, the transverse sections of *P. amboinensis* and *P. moluccensis* otoliths were greater in width and the sulcal groove was deeper than for *P. coelestis* [Fig. 5(a)–(c)]. *Pomacentrus amboinensis* and *P. moluccensis* that lived for multiple years had otolith length/width ratios that were greater than the maximum measured for *P. coelestis* [0.4, Fig. 6(b)].

BANDING IN *P. COELESTIS*

Dark bands (that were not daily or annual) were present in 58% of sectioned otoliths for fish with $L_S > 25$ mm ($n = 164$). The mean \pm S.E. number of broadbands for the

TABLE IV. Summary of von Bertalanffy growth parameters for *Pomacentrus coelestis* from Lizard Island, One Tree Island and Fitzroy Reef

Reef	<i>n</i>	L_{∞}	<i>K</i>
Lizard Island	77	60.2	0.019
One Tree Island	85	67.2	0.015
Fitzroy	65	68.8	0.013
All fish	227	68.1	0.015

n, sample size; L_{∞} , the mean maximum length; *K*, the growth rate.

largest (L_S) 10% of *P. coelestis*, from Fitzroy was 5.17 ± 2.11 ($n = 6$), One Tree Island was 10.00 ± 1.28 ($n = 6$) and Lizard Island was 4.67 ± 0.67 ($n = 6$).

There was variation in the frequency of band occurrence by day, during a lunar month ('bubbles' in Fig. 7). Although there was temporal overlap of bands for many fish (a maximum of eight of 10 on a day), this overlap was less than five of 10 on many days. These findings were consistent within and among reefs, by lunar phase and tidal amplitude. When the data were concatenated for all reefs, no significant periodicity in frequency of band occurrence was detected using autocorrelation analysis.

Average daily increment widths were similar among days prior to capture. On average increment widths were $1.94 \mu\text{m}$ wide at One Tree Island, $2.31 \mu\text{m}$ at Keeper Reef and $2.60 \mu\text{m}$ at Fitzroy Reef. Bands within individual fish on average had a clear continuous zone of about seven increments wide and the discontinuous dark zone was three increments wide ($n = 20$ fish). There were no consistent differences in the width of daily increments between discontinuous and continuous zones [in order: $2.29, 2.36 \mu\text{m}$ at Keeper Reef, $1.99, 1.93 \mu\text{m}$ at One Tree Island and $2.41, 2.60 \mu\text{m}$ at Fitzroy Reef (100 or more increments were counted per reef and zone)].

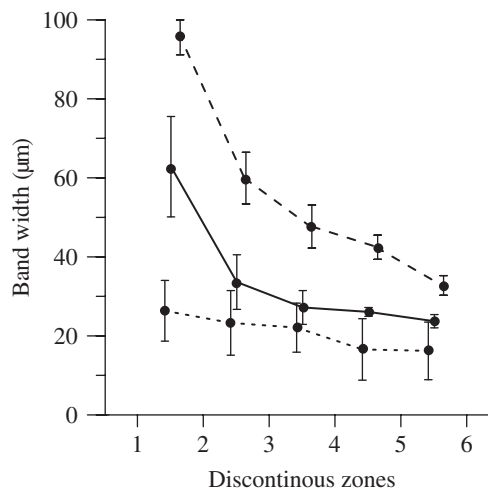


FIG. 4. Comparison of mean \pm S.E. band width between discontinuous bands (or annuli) for *Pomacentrus coelestis* (.....), *Pomacentrus amboinensis* (—) and *Pomacentrus moluccensis* (---). $n = 5$ fish per sample, but $n = 3$ for *P. coelestis* when discontinuous bands > 4 .

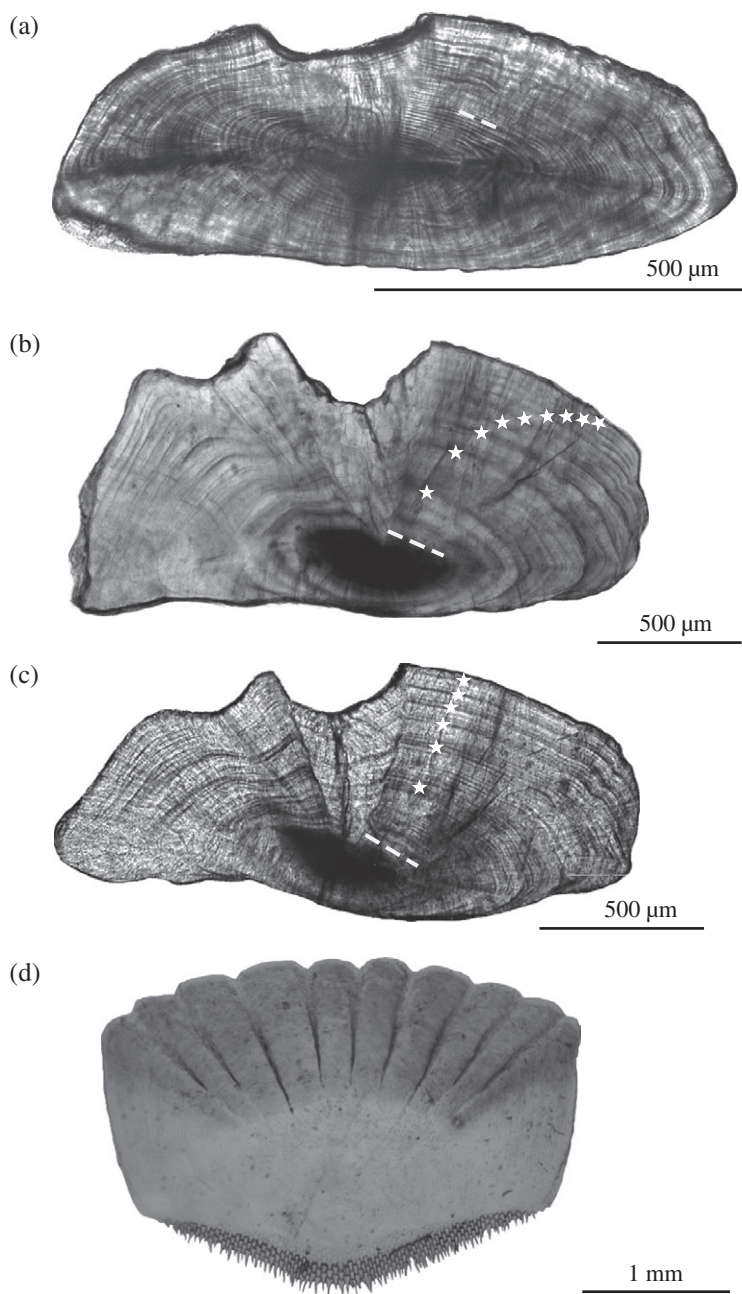




FIG. 5. Transverse sections of sagittal otoliths from (a) *Pomacentrus coelestis*, 52 days old (24 day old core), (b) *Pomacentrus moluccensis*, 9 years old (24 day old core); (c) *Pomacentrus amboinensis*, 6 years old (24 day old core); (d) lateral body scale of a 96 day old *P. coelestis* with no observable annuli.  the Presettlement core; , annual age bands.

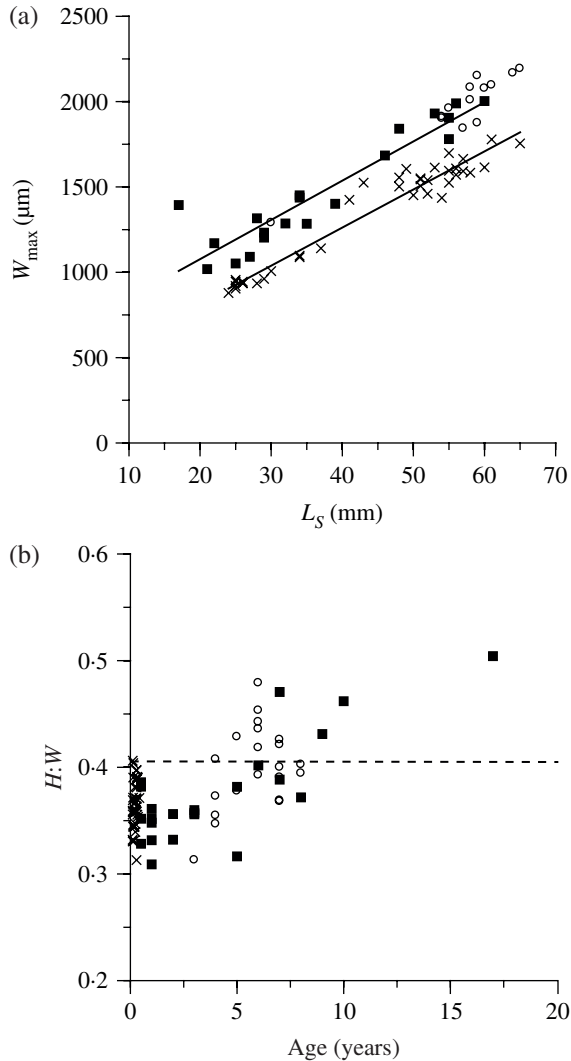


FIG. 6. (a) Comparison of maximum otolith width (W_{\max} ; Fig. 1) and standard length (L_S) for three *Pomacentrus* species and (b) the ratio of otolith height:width (H:W; Fig. 1) for the three species: *Pomacentrus coelestis* [X, $y = 22.328x + 368.915$; $r^2 = 0.945$, $F_{1,36} = 623$, $P < 0.001$, $n = 38$], *P. moluccensis* [■, $y = 22.992x + 617.129$, $r^2 = 0.859$, $F_{1,18} = 109.58$, $P < 0.001$, $n = 20$] and *P. amboinensis* (O, $n = 21$). - - -, Maximum value for *P. coelestis* H:W.

DISCUSSION

Pomacentrus coelestis, associated with tropical reefs of the GBR, live for <6 months. This is in stark contrast to congeners that live for years and in the case of *P. amboinensis* to 6 years (McCormick, 2016) and *P. moluccensis* for up to 17 years in the same area (Doherty & Fowler, 1994a). The daily ageing technique had previously been validated for young *P. coelestis* (up to 27 days; Kingsford *et al.*, 2011); the present study validates this technique for all size classes present on reefs of the GBR. It is unclear

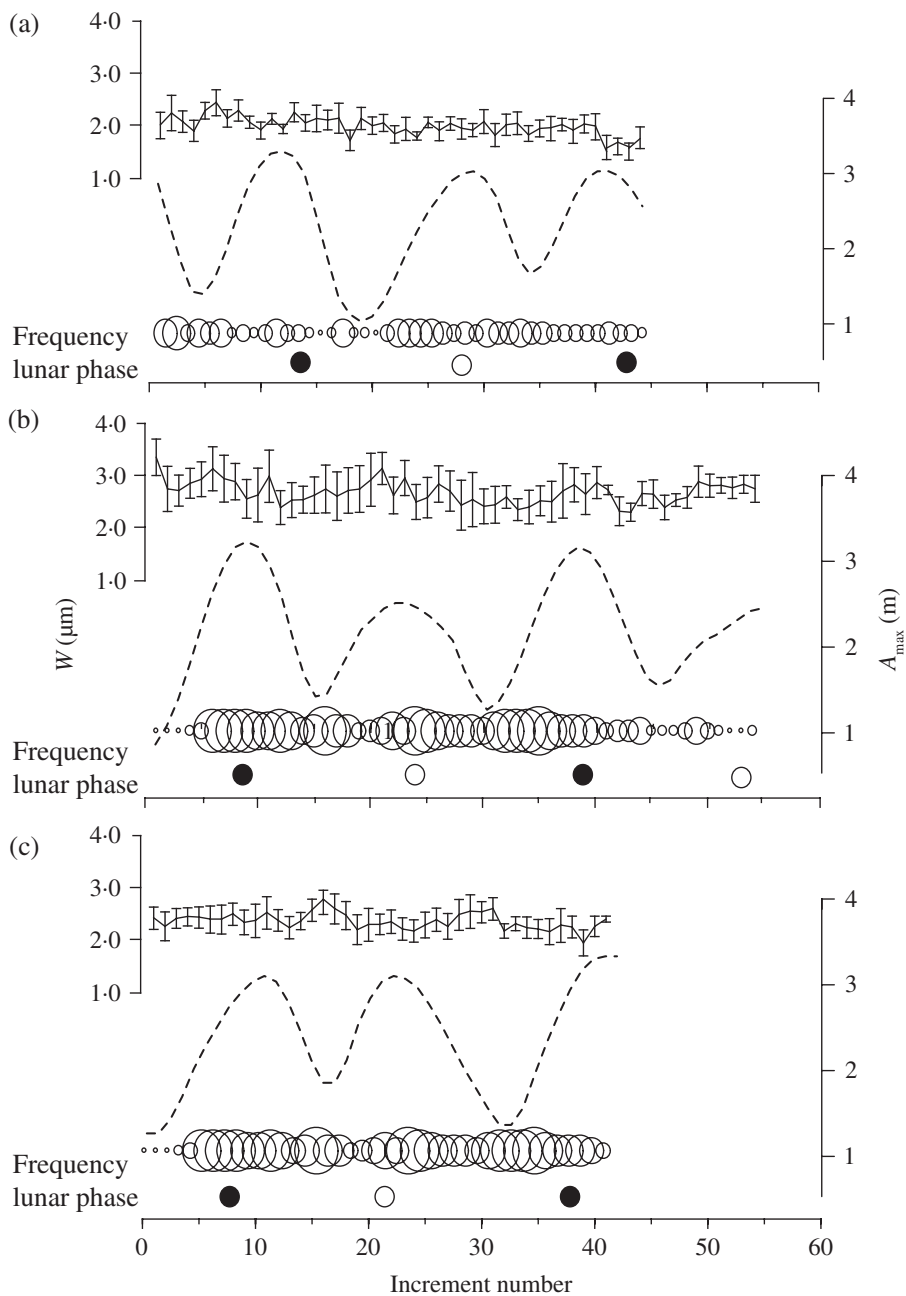


FIG. 7. Mean \pm S.E. increment width (W , μm) from the otolith edge towards the primordium (see Fig. 1) showing relationship with the presence of dark bands on a given day, expressed as a bubble plot for frequency (the biggest bubble shown is eight of 10) of all fish at (a) One Tree Island, (b) Fitzroy Reef and (c) Keeper Reef. Lunar phase: \bullet , new moon; \circ , full moon. ---, Maximum tidal amplitude (A_{max}). The fish from each location were collected on a single day; $n = 10$ fish for all reefs.

why tetracycline affected increment deposition, this may be dose-dependent or relate to the conditions in which fish were kept. The strongest evidence here of daily rings later in life, however, came from the translocation mark. Further evidence of a short life and, fundamental differences in otolith deposition patterns among congeners were found in the shape of otoliths. The otoliths of *P. coelestis* were thinner and not as wide for a given L_S than those of congeners that lived for years. Furthermore, the relative spacing of dark banding in *P. coelestis*, from primordium to otolith edge, was different from that of annuli in other species. Suzuki *et al.* (1985) concluded for *P. coelestis* from a high latitude location in Japan, that fish are presumed to reach 2 years of age or less. It is possible that fish live longer in cooler waters and a pattern of greater age maxima with increased latitude has been found in other fishes [e.g. *Sebastes diploproa* Gilbert 1890 (Boehlert & Kappenman, 1980)]. Suzuki *et al.* (1985) also used scales for ageing and they commented that a comparison with the more robust otolith technique would be judicious. Multiple scales from the largest size classes of *P. coelestis* were examined and, regardless of position on the body, there were no annuli. Accordingly, this concurred with present interpretation of the otoliths, that *P. coelestis* of the GBR live <6 months.

Pomacentrus coelestis is very common on reefs of the Indo-Pacific (Allen, 1991). Present findings indicate that turnover rates within reef-based populations must be very high as for other short-lived fishes that include gobies (Lefevre *et al.*, 2016) and apogonids (Kingsford *et al.*, 2014). Densities of recruits can be very high on the GBR and some of the highest rates of recruitment have been found on the southern GBR. Furthermore, there is great variation in the number of recruits by reef among years (Sale *et al.*, 1984; Doherty *et al.*, 1996). As argued for other short-lived fishes (Lefevre *et al.*, 2016), for populations to persist there must be almost continuous spawning and recruitment. It is known that spawning and subsequent settlement, c. 14–27 days later (Kingsford *et al.*, 2011), on the GBR is greatest over the austral spring and summer months. Given a short age maximum, however, some spawning would be expected at other times of the year if populations are to persist. Strong evidence was found for this in that small fish were collected in late-winter to early spring (September) on the GBR in two separate years and had hatching days of May to July.

There is great spatial variation in numbers of *P. coelestis* on reefs (Williams, 1982; Sale *et al.*, 1984). This is probably due to a combination of temporal variation in recruitment (Doherty *et al.*, 1996), availability of suitable habitat and vulnerability to predation (Doherty & Fowler, 1994b). *Pomacentrus coelestis* prefer coral rubble habitat (pers. obs.) and although they can be observed in other habitats where live coral is abundant they are generally rare or absent in these areas (Bell & Galzin, 1984). The abundance of *P. coelestis* can vary greatly in time due to disturbance (e.g. cyclones) and the related abundance of coral rubble habitat. Halford (2004) demonstrated that the number of *P. coelestis* increased greatly after major disturbance events and decreased as the reef recovered and disturbance related habitats (*i.e.* rubble) decreased.

The PLD of *P. coelestis* is similar to many other damselfishes, but short compared to other families of reef fishes (Brothers *et al.*, 1983). Their short PLD and very short lives, combined with their rapid response to the availability of suitable habitat suggests that founder effects in this species would be profound (Halford, 2004). The probability of recruitment failure and the potential collapse of local populations, however, must be also be high. Consequently, the local genotypic composition of reef populations will have the potential to change quickly, as has been found for short-lived

O. doederleini (Gerlach *et al.*, 2016). Although earlier population genetic research indicated that *P. coelestis* populations were panmictic (separated by kilometres to tens of kilometres; Gerlach *et al.*, 2007), it has recently been found that the distribution of genotypes can vary within and among reef populations of *P. coelestis* across years (Gerlach *et al.*, unpubl. data). A model for these patterns would be that local populations can become quickly established in a mosaic of reefs as suitable disturbed habitat becomes available. The persistence of the local population would depend on reproductive output, successful recruitment from natal sources and elsewhere, longevity and mortality. *Pomacentrus coelestis* clearly has the potential to disperse widely, as recruiting fish are found hundreds of kilometres south of the reefs where adults are known to spawn (Booth *et al.*, 2007). Further, late stage pre-settlement *P. coelestis*, probably from multiple source reefs, have been shown to accumulate around reefs (Doherty *et al.*, 1996; Patterson *et al.*, 2005). Given the short lives of *P. coelestis*, the proportional representation of genotypes in a larval pool would be expected to determine the subsequent genetic makeup of populations.

Within the otoliths of *P. coelestis* banding was found that could only be structures laid down over short periods of time (less than a year). Similar patterns have been found in molluscs that relate to phase of the moon and related tides (Cerrato, 2000; Schone *et al.*, 2002), and spawning (Kanazawa & Sato, 2008); this could be due to differences in food intake and reproduction related stress (Campana & Neilson, 1985). It is hypothesized that variation in increment spacing, and banding could result from altering the feeding regime of planktivorous *P. coelestis*. Accordingly, increment spacing and otolith bands could relate to tidal amplitude and related changes in tidal currents that could vary the supply of food (Bray, 1981). A hypothesis that tide influenced the width of daily increments was rejected as was a model that tide influences the position of bands, as there was often overlap in the occurrence of bands by individual fish; this was true according to lunar phase and amplitude of the tide at three reefs. An alternative hypothesis is that the banding pattern could relate to individual maturation and spawning cycles. Given the short life of *P. coelestis*, once they reach maturity, almost constant spawning would be expected, as for short-lived gobies (Lefevre *et al.*, 2016). The lack of synchrony in banding among fish from the same reef is suggestive that individual-based cycles of maturation and spawning and related changes in blood chemistry (Kalish, 1991) could influence banding in otoliths. Lunar or semilunar periodicity of spawning is common in many pomacentrid species (Doherty, 1983) and temporal variation in spawning was noted by Jan (1997) for *P. coelestis* in Taiwan. If banding does reflect changes in blood chemistry in *P. coelestis*, with bouts of spawning, then there may be some smearing of individual-based maturation and spawning that is not detected in population based assessment of spawning frequency; this suggestion requires closer investigation. Further, it is acknowledged that an assessment of short-term variation in increment spacing, and how it relates to tides and feeding, should be repeated when spawning does not interfere with feeding activity.

In conclusion, *P. coelestis* is an abundant and well-studied fish that lives for <6 months. This is in contrast to congeners that live for years and in some cases for almost two decades. The otoliths of these short-lived fish were generally thinner and narrower when compared with congeners that live for more than a year. Although bands were observed in *P. coelestis* their patterns of deposition varied from the annuli of congeners. It is suggested that banding patterns in fish may be related to patterns of maturation and spawning. The consequences of a low age-maximum

would have a great influence on the population dynamics of this widespread species.

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References

- Allen, G. R. (1991). *Damselfishes of the World*. Melle: Mergus.
- Bell, J. D. & Galzin, R. (1984). Influence of live coral cover on coral-reef fish communities. *Marine Ecology Progress Series* **15**, 265–274.
- Bellwood, D. R. & Wainright, P. C. (2002). The history and biogeography of fishes on coral reefs. In *Coral Reef Fishes: Dynamics and Diversity in a Complex Ecosystem* (Sale, P. F., ed), pp. 5–32. Amsterdam: Academic Press.
- Berumen, M. L., Trip, E. D. L., Pratchett, M. S. & Choat, J. H. (2012). Differences in demographic traits of four butterflyfish species between two reefs of the Great Barrier Reef separated by 1,200 km. *Coral Reefs* **31**, 169–177.
- Boehlert, G. W. & Kappenman, R. F. (1980). Variation of growth with latitude of two species of rock fishes (*Sebastes pinniger* and *S. diploproa*) from the North Eastern Pacific Ocean. *Marine Ecology Progress Series* **3**, 1–10.
- Booth, D. J., Figueira, W. F., Gregson, M. A., Brown, L. & Beretta, G. (2007). Occurrence of tropical fishes in temperate southeastern Australia: role of the East Australian Current. *Estuarine, Coastal and Shelf Science* **72**, 102–114.
- Bray, R. N. (1981). Influence of water currents and zooplankton densities on daily foraging movements of blacksmith, *Chromis punctipinnis*, a planktivorous reef fish. *Fishery Bulletin U.S.* **78**, 829–841.
- Brothers, E. B., Williams, D. M. & Sale, P. F. (1983). Length of larval life in twelve families of fishes at "One Tree Lagoon", Great Barrier Reef, Australia. *Marine Biology* **76**, 319–324.
- Caldow, C. & Wellington, G. M. (2003). Patterns of annual increment formation in otoliths of pomacentrids in the tropical western Atlantic: implications for population age-structure examination. *Marine Ecology Progress Series* **265**, 185–195.
- Campana, S. E. & Neilson, J. D. (1985). Microstructure of fish otoliths. *Canadian Journal of Fisheries and Aquatic Science* **42**, 1014–1032.
- Cerrato, R. M. (2000). What fish biologists should know about bivalve shells. *Fisheries Research* **46**, 39–49.
- Choat, J. H. & Axe, L. M. (1996). Growth and longevity in acanthurid fishes: an analysis of otolith increments. *Marine Ecology Progress Series* **134**, 15–26.
- Clarke, T. A. (1970). Territorial behavior and population dynamics of a pomacentrid fish, Garibaldi, *Hypsypops rubicunda*. *Ecological Monographs* **40**, 189–212.
- Depczynski, M. & Bellwood, D. R. (2005). Shortest recorded vertebrate lifespan found in a coral reef fish. *Current Biology* **15**, R288–R289.
- Doherty, P. J. (1983). Diel, lunar and seasonal rhythms in the reproduction of two tropical damselfishes: *Pomacentrus flavicauda* and *P. wardi*. *Marine Biology* **75**, 215–224.
- Doherty, P. J. (2002). Variable replenishment and the dynamics of reef fish populations. In *Coral Reef Fishes: Dynamics and Diversity in a Complex Ecosystem* (Sale, P. F., ed), pp. 327–358. London: Academic Press.

- Doherty, P. & Fowler, T. (1994a). An empirical test of recruitment limitation in a coral reef fish. *Science* **263**, 935–939.
- Doherty, P. & Fowler, A. (1994b). Demographic consequences of variable recruitment to coral reef fish populations: a congeneric comparison of two damselfishes. *Bulletin of Marine Science* **54**, 297–313.
- Doherty, P. J. & Williams, D. M. (1988). The replenishment of coral reef fish populations. *Oceanography and Marine Biology: An Annual Review* **26**, 487–551.
- Doherty, P. J., Kingsford, M. J., Booth, D. & Carleton, J. (1996). Habitat selection before settlement by *Pomacentrus coelestis*. *Marine and Freshwater Research* **47**, 391–399.
- Fowler, A. J. (1990). Validation of annual growth increments in the otoliths of a small, tropical coral reef fish. *Marine Ecology Progress Series* **64**, 25–38.
- Fowler, A. J. & Doherty, P. J. (1992). Validation of annual growth increments in the otoliths of two species of damselfish from the southern Great-Barrier-Reef. *Australian Journal of Marine and Freshwater Research* **43**, 1057–1068.
- Gerlach, G., Atema, J., Kingsford, M. J., Black, K. P. & Miller-Sims, V. (2007). Smelling home can prevent dispersal of reef fish larvae. *PNAS* **104**, 858–863.
- Gerlach, G., Atema, J., Raupach, M. J., Deister, F., Muller, A. & Kingsford, M. J. (2016). Cryptic species of cardinalfish with evidence for old and new divergence. *Coral Reefs* **35**, 437–450.
- Halford, A. (2004). Resilience to large-scale disturbance in coral and fish assemblages on the GBR. *Ecology* **85**, 1892–1905.
- Jan, R.-Q. (1997). Sympatric spawning of the damselfishes *Chromis fumea* and *Pomacentrus coelestis* on the northern coast of Taiwan. *Zoological Studies* **36**, 26–32.
- Jones, G. P., Almany, G. R., Russ, G. R., Sale, P. F., Steneck, R. S., van Oppen, M. J. H. & Willis, B. L. (2009). Larval retention and connectivity among populations of corals and reef fishes: history, advances and challenges. *Coral Reefs* **28**, 307–325.
- Kalish, J. M. (1991). Determinants of otolith chemistry: seasonal variation in the composition of blood plasma, endolymph and otoliths of bearded rock cod *Pseudophycis barbatus*. *Marine Ecology Progress Series* **74**, 137–159.
- Kanazawa, T. & Sato, S. (2008). Environmental and physiological controls on shell microgrowth pattern of *Ruditapes philippinarum* (Bivalvia: Veneridae) from Japan. *Journal of Molluscan Studies* **74**, 89–95.
- Kingsford, M. J., Smith, F. J. A. & Flood, M. J. (2011). Growth and pelagic larval duration of presettlement and newly settled neon damselfish, *Pomacentrus coelestis*, at multiple spatial scales. *Coral Reefs* **30**, 203–214.
- Kingsford, M. J., Finn, M. D., O'Callaghan, M. D., Atema, J. & Gerlach, G. (2014). Planktonic larval duration, age and growth of *Ostorhinchus doederleini* (Pisces: Apogonidae) on the southern Great Barrier Reef, Australia. *Marine Biology* **161**, 245–259.
- Lacson, J. M. (1994). Fixed allele frequency differences among Palauan and Okinawan populations of the damselfishes *Chrysiptera cyanea* and *Pomacentrus coelestis*. *Marine Biology* **118**, 359–366.
- Lefevre, C. D., Nash, K. L., Gonzalez-Cabello, A. & Bellwood, D. R. (2016). Consequences of extreme life history traits on population persistence: do short-lived gobies face demographic bottlenecks? *Coral Reefs* **35**, 399–409.
- Liggins, L., Booth, D. J., Figueira, W. F., Trembl, E. A., Tonk, L., Ridgway, T., Harris, D. A. & Riginos, C. (2015). Latitude-wide genetic patterns reveal historical effects and contrasting patterns of turnover and nestedness at the range peripheries of a tropical marine fish. *Ecography* **38**, 1212–1224.
- Liggins, L., Trembl, E. A., Possingham, H. P. & Riginos, C. (2016). Seascape features, rather than dispersal traits, predict spatial genetic patterns in co-distributed reef fishes. *Journal of Biogeography* **43**, 256–267.
- Liu, S. Y. V., Wang, C. H., Shiao, J. C. & Dai, C. F. (2010). Population connectivity of neon damsel, *Pomacentrus coelestis*, inferred from otolith microchemistry and mtDNA. *Marine and Freshwater Research* **61**, 1416–1424.
- McCormick, M. I. (2016). Protogyny in a tropical damselfish: females queue for future benefit. *PeerJ* **4**. doi: 10.7717/peerj.2198

- Mirams, A. G. K., Treml, E. A., Shields, J. L., Liggins, L. & Riginos, C. (2011). Vicariance and dispersal across an intermittent barrier: population genetic structure of marine animals across the Torres Strait land bridge. *Coral Reefs* **30**, 937–949.
- Munday, P. L. & Jones, G. P. (1998). The ecological implications of small body size among coral-reef fishes. *Oceanography and Marine Biology: An Annual Review* **36**, 373–412.
- Ohman, M. C., Munday, P. L., Jones, G. P. & Caley, M. J. (1998). Settlement strategies and distribution patterns of coral-reef fishes. *Journal of Experimental Marine Biology and Ecology* **225**, 219–238.
- Patterson, H. M., Kingsford, M. J. & McCulloch, M. T. (2004). Elemental signatures of *Pomacentrus coelestis* otoliths at multiple spatial scales on the Great barrier Reef, Australia. *Marine Ecology Progress Series* **270**, 229–239.
- Patterson, H. M., Kingsford, M. J. & McCulloch, M. T. (2005). Resolution of the early life history of a reef fish using otolith chemistry. *Coral Reefs* **24**, 222–229.
- Pears, R. J., Choat, J. H., Mapstone, B. D. & Begg, G. A. (2006). Demography of a large grouper, *Epinephelus fuscoguttatus*, from Australia's Great Barrier Reef: implications for fishery management. *Marine Ecology Progress Series* **307**, 259–272.
- Sale, P. F. (1991). Reef fish communities: open nonequilibrium systems. In *The Ecology of Fishes on Coral Reefs* (Sale, P. F., ed), pp. 564–600. San Diego, CA: Academic press.
- Sale, P. F., Doherty, P. J., Eckert, G. J., Douglas, W. A. & Ferrell, D. J. (1984). Large-scale spatial and temporal variation in recruitment to fish populations on coral reefs. *Oecologia* **64**, 191–198.
- Schone, B. R., Lega, J., Flessa, K. W., Goodwin, D. H. & Dettman, D. L. (2002). Reconstructing daily temperatures from growth rates of the intertidal bivalve mollusk *Chione cortezi* (northern Gulf of California, Mexico). *Palaeogeography Palaeoclimatology Palaeoecology* **184**, 131–146.
- Schwamborn, S. H. L. & Ferreira, B. P. (2002). Age structure and growth of the dusky damselfish, *Stegastes fuscus*, from Tamandare reefs, Pernambuco, Brazil. *Environmental Biology of Fishes* **63**, 79–88.
- Suzuki, K., Hioki, S. & Kashihara, M. (1985). Life history of the damselfish *Pomacentrus coelestis* in Suruga Bay, Japan. *Journal of the Faculty of Marine Science and Technology, Tokai University* **21**, 99–114.
- Tzioumis, V. & Kingsford, M. J. (1999). Reproductive biology and growth of the temperate damselfish *Parma microlepis*. *Copeia* **1999**, 348–361.
- Williams, D. M. (1982). Patterns of distribution of fish communities across the central Great Barrier Reef lagoon. *Coral Reefs* **1**, 35–43.