

Spatial and temporal validation of settlement-marks in the otoliths of tropical reef fishes

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ABSTRACT: The link between the timing of settlement (transition from the pelagic to the benthic environment) and the formation of the 'settlement-mark' in fish otoliths was examined in order to validate the use of this microstructural feature for the back-calculation of settlement patterns and planktonic larval durations. The formation of the settlement-mark was validated to occur at settlement in 2 pomacentrids, *Pomacentrus amboinensis*, *P. nagasakiensis*, and less conclusively in 13 other species from 7 families. Within these species, 3 distinct settlement-mark durations were identified: a single-increment mark (*P. amboinensis*, *P. nagasakiensis*); a 2-increment mark (*P. coelestis*); and a zonal, 14-increment mark (*Acanthurus* sp. 2, probably *A. blochi*). The structure of settlement-marks for *P. amboinensis* and *P. nagasakiensis* were compared among 4 to 5 locations on the northern Great Barrier Reef, Australia (maximum of 20 km apart). Otolith increment profiles differed minimally over spatial scales of 20 km in these species, with Lizard Island fish possessing increment profiles different to those from other locations. This pattern of minimal variation in profiles occurred despite the fact that the planktonic larval duration of one species (*P. nagasakiensis*) differed among locations (range of mean values: 22.4 to 26.3 d). Further, at Lizard Island, increment profiles of *P. amboinensis* showed statistically significant, although minor, differences among local populations around the island and between successive lunar recruit cohorts. Our evidence suggests that the structure of the increment transitions associated with settlement are taxon specific, although the optical contrast of settlement-marks (determined by increment width) may show subtle spatial and temporal variation.

KEY WORDS: Settlement-mark validation · Tropical reef fish · Metamorphosis · Otolith · Recruit

INTRODUCTION

Two decades of research on the Great Barrier Reef, Australia, and in the Caribbean have indicated that the dynamics of many populations of coral reef fishes are strongly influenced by spatial and temporal variability in recruitment (Doherty & Williams 1988, Doherty 1991, Caley et al. 1996). Information on recruitment patterns has enabled researchers to speculate on processes that deliver propagules to the reef and the scale upon which they operate (Doherty 1991). Moreover, we can use information on recruitment dynamics to speculate on abundance patterns and adult population dynamics (Doherty & Fowler 1994).

Replenishment of reef fish populations has been quantified by 2 main methods. Most commonly, visual censuses have been used to enumerate young-of-the-year juveniles. Censuses for new recruits have been carried out daily (e.g. Williams 1980, Brothers et al. 1983, Schroeder 1987, Robertson et al. 1988), weekly (e.g. Milicich et al. 1992), monthly (e.g. Meekan et al. 1993, Robertson et al. 1993) or by a single census at the end of the main recruitment season (e.g. Fowler et al. 1992, Williams et al. 1994). Unfortunately, visual censuses are time consuming since daily censuses are required for high temporal resolution.

An alternative method that has gained increasing popularity since its first use by Victor (1982) uses life-history information stored in the otoliths of the juvenile population. This technique involves back-calculating settlement patterns from collections of juveniles using transition zones in the regular daily increment struc-

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ture that are believed to be coincident with metamorphosis and/or settlement (the transition from the pelagic environment to the benthic environment) (e.g. Brothers & McFarland 1981, Campana 1984, Radtke et al. 1988, Cowen 1991, Wellington & Victor 1992, Kawase et al. 1993, Tyler et al. 1993, Francis 1994, Jenkins & May 1994, Sponaugle & Cowen 1994). Use of these increment transitions or 'settlement-marks' (sensu Victor 1982) has allowed settlement patterns to be reconstructed with near-daily resolution (Pitcher 1988a). Once properly validated, this method has the advantage of being able to obtain recruitment histories of populations over a whole spectrum of spatial and temporal scales (Victor 1983, Pitcher 1988b, Meekan 1993, Meekan et al. 1993).

Despite researchers' enthusiasm for this technique, few studies have validated that increment transitions are temporally or functionally linked to the settlement event (see Pitcher 1988a for an exception). Most have simply assumed that a close link exists and, in doing so, are potentially incorporating large errors into reconstructed settlement histories or calculated larval durations (e.g. Brothers et al. 1983, Victor 1986). For example, errors may occur if increment deposition ceases or reduces to a level not optically discernible at settlement. Problems arise when attempting to validate the settlement-marks' temporal link with settlement when back-calculated settlement histories are compared to visual census data of non-daily resolution. For example, Fowler (1989) collected chaetodontids after 4 to 7 d of settlement and examined the otoliths for a settlement-mark coinciding with a weekly visual recruitment history. This comparison only enabled a resolution of 4 to 7 d and a loose relationship between the settlement-mark and settlement was suggested. Similarly, Pitcher (1988a) was able to achieve a 2 to 4 d resolution using non-daily visual recruitment surveys and transitions present in pomacentrid otoliths. These and other studies lack the daily resolution of visual censuses required when attempting to establish a direct link between the settlement event and the settlement-mark.

Two sources of information have been used as evidence for the coincidence of a repeatable otolith mark with the settlement event. Firstly, fish have been collected immediately prior to or on the day of settlement from isolated patch reefs, followed by otolith examination to determine the presence or absence of a settlement-mark (e.g. Victor 1983, 1986). Secondly, the assumed settlement-marks in the otoliths of individuals of known recruitment history have been used and a recruitment history back-calculated to compare with the field-measured history (e.g. Pitcher 1988a). We used both of these methods to validate the coincidence of increment marks and settlement in a number of tropical reef fish species.

Studies that have used a settlement-mark in the otolith profile to back-calculate recruitment patterns and larval durations have also assumed that the structure of the settlement-mark is consistent within a species (e.g. Pitcher 1988a, Wellington & Victor 1989, 1992, Meekan et al. 1993). Rigorous validation of the use of the settlement-mark requires an examination of the consistency of structural formation across a range of temporal and spatial scales. This study aims to investigate the levels of error in aging associated with using the settlement-mark as a temporal marker of the settlement event in a number of reef fishes. The research builds on a study that validated the use of settlement-marks to back-calculate recruitment histories in 2 damselfishes by Pitcher (1988a).

Specifically, the present study aimed to: (1) determine the temporal link between the settlement-mark in fish otoliths and the settlement event for a number of tropical fish species; (2) determine how long the characteristic settlement-mark takes to form in the otolith; (3) validate consistencies in the structure of the settlement-mark over a range of spatial scales for 2 common damselfish species, *Pomacentrus amboinensis* and *P. nagasakiensis*; (4) examine the relationship between variability in structure of settlement-marks and planktonic larval duration over scales encompassing tens of kilometers; and (5) interpret increment changes in relation to otolith and somatic growth histories.

MATERIALS AND METHODS

Temporal link between settlement and an otolith mark. To determine whether there was a temporal link between a conspicuous transition in the otolith microstructure and the settlement event, we used 3 field treatments. These involved the manipulation and collection of newly settled fishes on small patch reefs ($0.5 \times 0.5 \times 0.5$ m) on the backreef of Lizard Island, northern Great Barrier Reef (GBR) ($14^{\circ}40'E$, $145^{\circ}28'S$, Fig. 1). Previous studies at Lizard Island have found that many reef fish species recruit soon after each new moon between October and March (Milicich & Doherty 1994). Patch reefs provide natural settlement habitats that can be efficiently cleared of all newly settled fish on a daily basis. Fifteen patch reefs were constructed 5 d prior to new moon in November 1994. Each reef was composed of a variety of coral species, including branching corals and plate corals, to encourage recruitment of a variety of fish species. The reefs were positioned on sand in 3 m depth and 10 to 20 m from contiguous reef. All reefs were cleared daily until there was a large recruitment pulse. Three treatments were then randomly assigned to the 15 patch reefs: (1) 5 reefs were covered with a fiberglass mesh cage ($0.8 \times 0.8 \times$

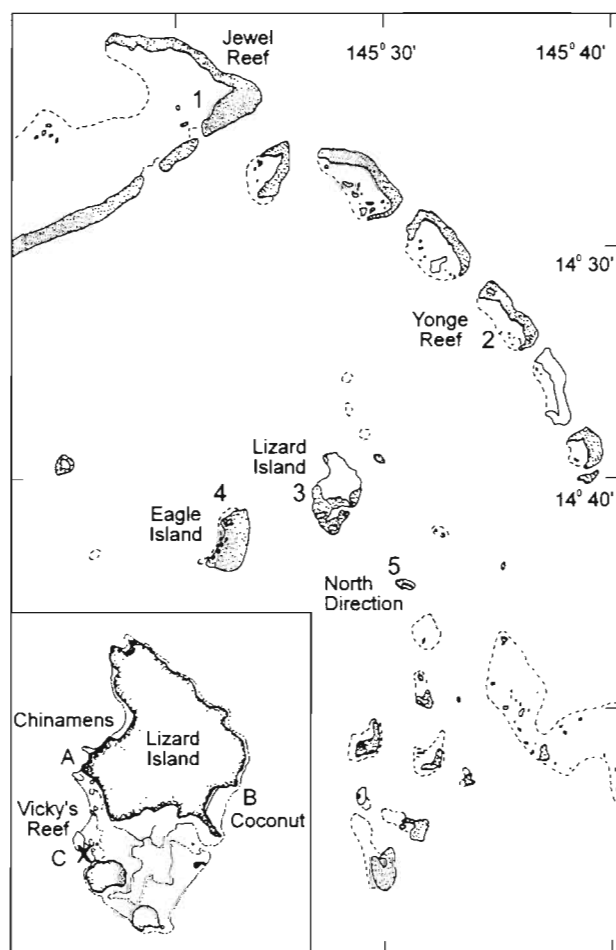


Fig. 1 Study sites used in the analysis of spatial variation in increment growth profiles around settlement. Numbers refer to locations used in the broad spatial comparison (20 km); letters identify sample sites for the Lizard Island comparison and the cross indicates the location of the patch reef manipulation

0.8 m, see below) to prevent further recruitment, thereby confining recruits of known settlement date to enable the back-calculation of the settlement-mark from the fish otoliths; (2) 5 reefs were not caged to act as controls for the possible influence of caging on otolith growth; and (3) 5 reefs were immediately cleared of newly settled fish to determine whether a settlement-mark was laid down prior to the settlement event.

The caged and non-caged patch reefs were left for 10 to 13 d [*Acanthurus* sp. 2 (probably *A. blochi*) was left on a patch reef for 20 d to enhance species identification]. To obtain a history of the recruitment of fish to the non-caged reefs daily visual censuses were conducted for the duration of the cage manipulation. Newly settled fish were recorded separately from recruits from previous days. New recruits were identified by size, colouration and behaviour (i.e. new recruits tended to be smaller, less pigmented and remain well

within the safety of the patch reefs). At the completion of the manipulation all reefs were cleared using fence nets, small hand nets and the fish anaesthetic quinaldine. All fish collected were killed by cold shock soon after capture.

For those individuals removed from the non-caged patch reefs, the recruitment history derived from the daily visual censuses was compared to the recruitment history back-calculated from fish otoliths using frequency histograms. The small number of fish recruiting to each patch reef each day after the initial recruitment pulse on 10 November (mean $<2 \text{ d}^{-1}$ per patch reef *Pomacentrus amboinensis*, mean 2 d^{-1} per patch reef *P. nagasakiensis*) enabled reliable identification of individual fish in the field. This provided a solid basis for identifying those individuals that possessed otolith settlement-marks not coincident with the day of settlement.

Mesh enclosures used in these manipulations were constructed from high grade, 50% shade, fibreglass mesh (hole diameter 1.5 mm^2). The mesh cages were $0.8 \times 0.8 \times 0.8 \text{ m}$, with an access door located in the top for fish removal. Corners of each cage were secured to 2 m metal pickets, and the mesh skirt around the bottom edge was weighted with metal rods and buried in sand at the base.

Analysis of spatial and temporal variation of settlement-mark structure. Settlement-mark structure was examined in samples of juvenile *Pomacentrus amboinensis* and *P. nagasakiensis* collected from 5 locations (separated by up to 20 km) in November 1994, in order to determine the spatial consistency of mark structure. Three mid-shelf reefs (Lizard Island, North Direction and Eagle Island), and 2 outer-shelf reefs (Jewel and Yonge reefs) were sampled (Fig. 1). At each location recently settled fish were taken from the backreef lagoons. At Lizard Island spatial differences in settlement-mark structure at different sites on the windward, leeward and oblique sides of the island (Fig. 1) were examined in detail for *P. amboinensis* collected during November 1994. Consecutive lunar pulses of *P. amboinensis* were collected at Lizard Island from November through to December 1994 and analysed for temporal consistencies in settlement-mark structure.

Otolith preparation. Sagittae were extracted from each fish, cleaned of endolymph tissue using alcohol and stored dry. One sagitta was selected from each fish and mounted on a glass slide using the thermoplastic cement, Crystal bond™, such that the distal end protruded over the edge of the slide. The otolith was then ground using lapping films (12 to $0.3 \mu\text{m}$ grit-size). The polished face of the otolith was then mounted face down on the slide and the rostral end of the otolith ground down in a similar way to produce a thin transverse section incorporating the nucleus. Otoliths were

then viewed under immersion oil with transmitted light using a polarising compound microscope at 1000 \times magnification. The number of increments was determined from 3 replicate increment counts. If the counts deviated by less than 10%, the mean count was accepted; otherwise the otolith was rejected. Increment widths were measured along a transect following the longest axis of the otolith using an image analysis system. To examine the coincidence of the otolith settlement-mark with the settlement event, the number of days between the known date of settlement and the date of field collection was compared to the number of increments between the otolith settlement-mark and the otolith periphery.

Analysis. A comparison of increment profiles from caged and non-caged fish was only possible for *Pomacentrus amboinensis* and *P. nagasakiensis*, the only species that recruited onto the experimental patch reefs in sufficient numbers. The increment profiles of these fish were compared using a repeated measures analysis of variance. Each increment on the otolith profile was measured as a replicate of consecutive increment growth over time for a number of individual fish. The assumption of compound symmetry (Winer 1971) was tested by a sphericity test. If this assumption was violated, then a Greenhouse-Geisser *F*-correction was used (Winer 1971). A significance level of 0.05 was used.

All other species only recruited onto some of the 15 patch reefs and were compared using the number of increments between the known settlement date and date of removal, and the number of increments between the optical density/increment transition and the otolith periphery.

A repeated measures ANOVA was used to test for differences in the shape of the mean increment width profiles of *Pomacentrus amboinensis* and *P. nagasaki-*

ensis among 'Locations' (5 locations for the inter-reef study, 3 locations for the within-Lizard Island study) over 'Time' (repeated measure of increment width). The widths of 10 consecutive increments prior to and from the increment transition mark were used in the analysis. A subset of the other fish were used in a comparison of spatial variation in increment profile shapes, as some fish did not possess sufficient numbers of increments post-settlement.

Planktonic larval durations of each of *Pomacentrus amboinensis* and *P. nagasakiensis* were compared among locations, using a 1-way ANOVA. Tukey's (HSD) means comparisons were used to examine the nature of any significant differences found among locations. Assumptions of homogeneity of variance and normality were examined by residual analysis.

RESULTS

Analysis of otolith increment profiles and the structure of settlement-marks

The traditional method of presenting an average otolith increment profile (Fig. 2a) masks how increment widths change at a particular life history transition period, such as settlement. This occurs because larval duration can vary considerably among individuals within a species, and the features of settlement-marks are masked by averaging increment width profiles that are plotted chronologically from the nucleus. When represented as the mean otolith increment profile, the result is a smearing effect, leading to a false impression that the transition occurs over a number of days.

An alternative method of displaying increment trends at a life-history transition is to centre the indi-

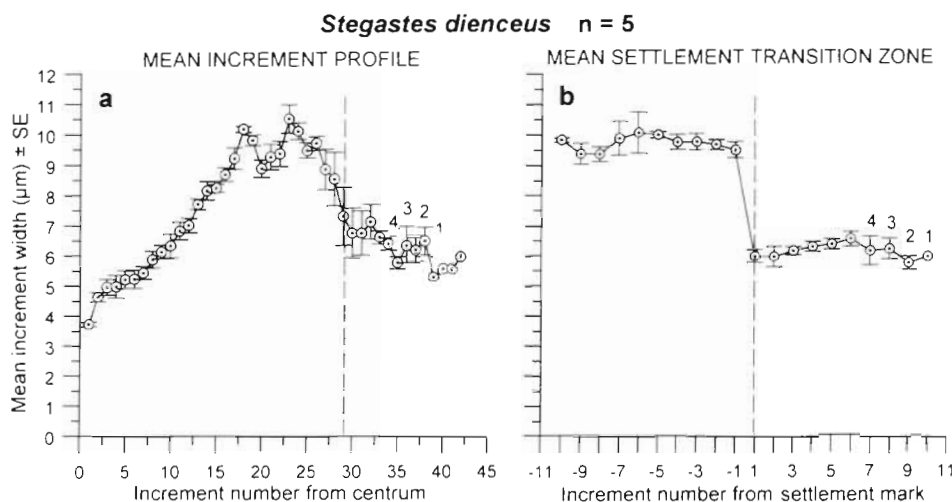


Fig. 2. Comparison between (a) the traditional otolith increment profile versus (b) the 'transition-centred' profile for describing life history events recorded in fish otoliths, in this example *Stegastes dienceaus*. Vertical dashed line indicates the location of the first increment of the settlement-mark (= larval duration + 1 d). Numbers shown on the profile represent decreasing increment replication

vidual increment profiles on the increment that represents the start of the transition. This involved identifying the first increment of the transition zone in a given fish otolith, and taking 10 increment readings before and 10 from (including the transition increment) the transition. We term this the 'transition-centred' method. The increment mark that represented the start of the transition was identified by a distinct change in optical contrast (authors' unpubl. data). This was done for all individuals collected for a species, the mean value obtained for each pre- and post-transition increment, and then plotted as a mean settlement transition profile (Fig. 2b). This method gave an accurate representation of the settlement-mark, and when compared with the overall increment profile (Fig. 2a), provided a clear indication of the magnitude of change in increment width associated with the transition.

Temporal link between settlement and an otolith mark

Pomacentrus amboinensis

The settlement-mark of *Pomacentrus amboinensis* was represented by 1 increment on the otolith profile. There was a slight, though statistically significant, difference in increment width profiles between fish from the caged and non-caged treatments (Fig. 3a, Table 1). The difference stemmed from the increment widths of the otoliths from caged fish being larger than those of the non-caged fish over several days prior to settlement, and again 9 d after settlement.

None of the 10 individuals collected within 12 h of settlement possessed a settlement-mark. Eighty-five percent of fish in the caged treatments (17 of 20 individuals) and 90% for the non-caged treatments (18 of 20 fish, Fig. 4) had settlement-marks on otoliths that were coincident with the settlement event. Five individuals failed to possess a settlement-mark coinciding with the expected date of settlement. The otoliths of these fish were 1 to 5 d (or increments) short of the expected number of increments. We suspect that these fish may in fact have been immigrants that entered the cages through holes eroded in the sand at the base of the cages, or by our failure to detect individuals recruiting on the non-caged patch reefs. Two caged patch reefs developed holes that, although covered as soon as noticed, may have allowed recruitment into the cages.

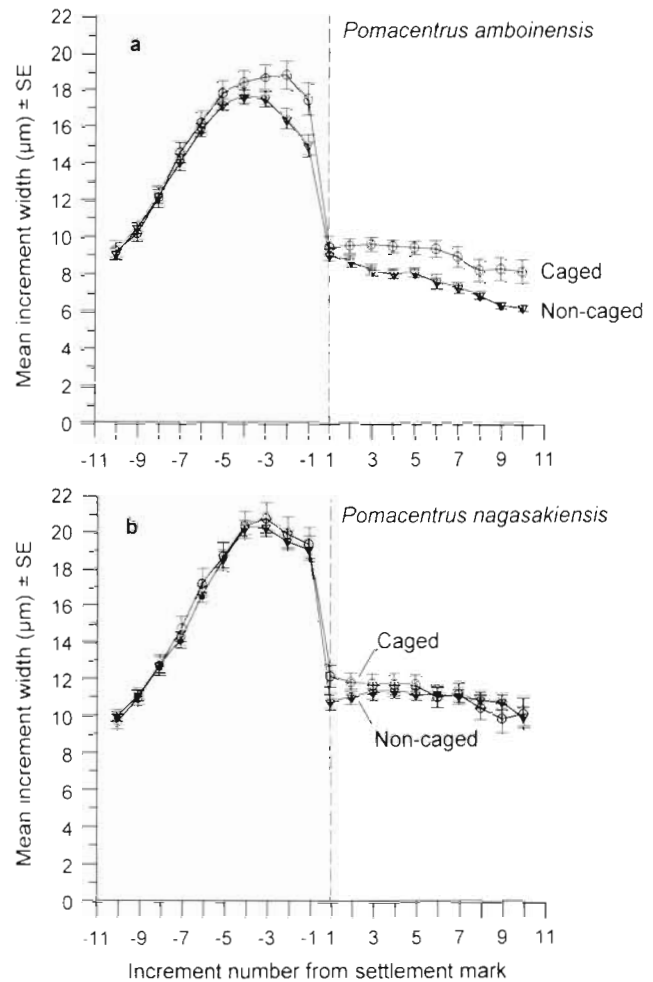


Fig. 3. *Pomacentrus amboinensis* and *P. nagasakiensis*. Comparison of transition-zone increment profiles from caged and non-caged fish for (a) *P. amboinensis* (average age at settlement $23 \text{ d} \pm 0.22 \text{ SE}$), (b) *P. nagasakiensis* (average age at settlement $23 \text{ d} \pm 0.48 \text{ SE}$) ($n = 20$ for each treatment)

Table 1 *Pomacentrus amboinensis* and *P. nagasakiensis*. Results of a repeated measures ANOVA comparing mean increment profiles through settlement-marks of caged and non-caged fish

Species	Effect	Source of variation	df	Mean square	F-ratio	p
<i>Pomacentrus amboinensis</i>	Between	Cage	1	137.5744	2.54	0.1256
		Residual	22	54.2574		
	Within	Time	13	439.7414	194.82	0.0001
		Time \times Cage	13	7.0800	3.14	0.0002
<i>Pomacentrus nagasakiensis</i>	Between	Cage	1	13.8090	0.18	0.6759
		Residual	42	77.9172		
	Within	Time	13	705.4017	139.26	0.0001
		Time \times Cage	13	1.7812	0.35	0.9830
		Residual	546	5.0655		

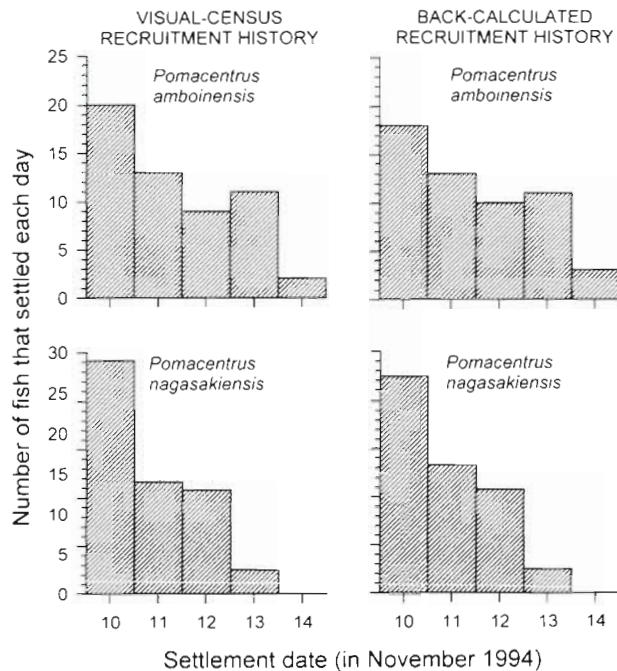


Fig. 4. *Pomacentrus amboinensis* and *P. nagasakiensis*. Frequency histograms of recruitment histories derived from daily visual censuses and otolith back-calculation for non-caged fishes collected up to 2 wk after settlement. Settlement dates in November 1994

Pomacentrus nagasakiensis

The settlement-mark of *Pomacentrus nagasakiensis* was represented by 1 increment on the otolith profile. There was no significant difference in increment profiles between caged and non-caged treatments for *P. nagasakiensis* ($F_{1,42} = 0.18$, $p = 0.6759$) (Table 1, Fig. 3b). Predictably, there was a significant difference in increment width over time, driven by the higher pre-settlement increment width and rapid drop at settlement. There was no significant interaction (Table 1). Ninety percent of the 30 fish in the cages (27 of 30 fish) had the number of increments after the settlement-mark equal to the actual number of days after settlement. Similarly, 93% of the 29 fish from the non-caged treatments (27 of 29 fish) had increment numbers matching expected values post-settlement (Fig. 4). Those fish that possessed less than the expected number of increments may be explained by the reasons given for *P. amboinensis* (i.e. recruitment through cage holes). None of the 9 individuals taken within 12 h of settlement had a settlement-mark on their otoliths.

Other species

A total of 16 species settled onto the caged and non-caged patch reefs. Fifteen were validated as having a

settlement-mark coincident with the settlement event (*Acanthurid* sp. 1 exception). Table 2 presents a comparison of the number of days between settlement and date of removal, and the number of increments between the settlement-mark and the otolith periphery. Of the 142 individuals from 7 families that settled on caged (75 fish) and non-caged (67 fish) patch reefs, 15 individuals from 6 species possessed a settlement-mark that was not coincident with the apparent settlement event (Table 2). Six of these individuals were 1 increment short of the expected total (*Acanthurid* sp. 1, *Pomacentrus amboinensis*, *P. nagasakiensis*). Differences of a day have been previously explained as an 'edge effect' (Fowler 1989, Tyler et al. 1993), where the outermost increment is not distinguishable due to light refraction. Two *P. amboinensis* had settlement-marks that were not coincident with settlement by 1 increment. Similarly, 3 *P. nagasakiensis* had settlement-marks not coincident with settlement by 1 increment.

Dascyllus melanurus, *D. reticulatus*, *Chaetodon plebius* and those species for which only 1 individual was caught (see Table 2) possessed increment transitions coincident with the day of settlement (*Acanthurid* sp. 1, exception). *Pomacentrus coelestis*, *Neopomacentrus cyanomos* and *Heniochus acuminatus* exhibited high coincidence between settlement and the otolith mark, with 1 to 2 individual fish short of the expected increment total (Table 2). *P. coelestis* had a settlement-mark composed of 2 increments in a slightly lighter zone, with these increments still distinguishable. Fig. 5 illustrates the 3 types of settlement-mark durations found on the otolith profiles examined. With the exception of *P. coelestis* and *Acanthurus* sp. 2 all species possessed the single day transition (Fig. 5a). Of the 35 individuals from 5 species taken from the cleared treatment (i.e. removed within 12 h of settlement) none possessed a settlement-mark on the otolith profile.

Spatial and temporal consistency of increment growth profiles over settlement

Pomacentrus amboinensis

Our comparison of settlement transition profiles among fish from the 5 study locations for *P. amboinensis* indicated that fish collected at Lizard Island had a different pattern of otolith growth and increment deposition than fish from the other 4 locations (Fig. 6a). The increment width was found to differ significantly among the 5 locations in a repeated measures analysis ($F_{4,53} = 6.29$, $p = 0.0003$). The interaction between location and time (repeated measures of consecutive increment widths) was also significant ($F_{36,477} = 3.87$, $p = 0.0001$). This interaction was driven by the incre-

Table 2. Extent of coincidence of the settlement-mark with settlement for 16 coral reef fish species. Number of days between settlement and removal of fish [Settlement–Removal (d)] is compared to number of increments between the start of the settlement-mark and otolith periphery [S.Mark–Edge (increments)]. Determination of the presence of a mark formed prior to settlement was achieved using fish collected immediately after their arrival on the patch reefs. Values in table represent the absolute number of fish present in each category

Species	Caged		Non-caged		Presence of S.Mark at settlement
	Settlement– Removal (d)	S.Mark– Edge (increments)	Settlement– Removal (d)	S.Mark– Edge (increments)	
<i>Pomacentrus amboinensis</i>	17/20	coincident with settlement (85%) ^a	18/20	coincident with settlement (90%) ^a	0 out of 10
<i>P. nagasakiensis</i>	27/30	coincident with settlement (90%) ^a	27/29	coincident with settlement (93%) ^a	0 out of 9
<i>P. coelestis</i>	8	coincident with settlement	–	–	0 out of 9
	12	10 ^a	–	–	–
	12	6 ^a	–	–	–
<i>Dascyllus melanurus</i>	15	15	14	14	0 out of 4
	6	6	2	2	–
	6	6	2	2	–
	5	5	–	–	–
<i>D. reticulatus</i>	–	–	9	9	0 out of 3
	–	–	7	7	–
	–	–	7	7	–
	–	–	5	5	–
<i>Neopomacentrus cyanomos</i>	6	6	–	–	–
	6	4 ^a	–	–	–
	6	6	–	–	–
<i>Heniochus acuminatus</i>	13	13	4	4	–
	12	12	4	4	–
	9	5 ^a	3	3	–
	6	6	–	–	–
<i>Chaetodon plebius</i>	18	18	3	3	–
	8	8	–	–	–
	5	5	–	–	–
<i>Epinephelus corallicola</i>	10	10	–	–	–
<i>Pseudochromis quinquedentatus</i>	–	–	13	13	–
<i>Zebrafish scopas</i>	–	–	14	14	–
<i>Acanthurus</i> sp. 1	–	–	10	9 ^a	–
<i>Acanthurus</i> sp. 2	–	–	20	20	–
<i>Acanthurus</i> sp. 3	–	–	4	4	–
<i>Lutjanus gibbus</i>	–	–	9	9	–
<i>Parupeneus barberinus</i>	–	–	10	10	–
No. fish sampled	75		67		35
^a See 'Results' for discussion of anomalous cases					

ment curve for the Lizard Island location overlapping with the other curves at 8 d prior to settlement and being distinctly separated from the other curves 3 d later (Fig. 6a). *A posteriori* re-analysis excluding the Lizard Island location found no difference among the remaining 4 locations. This suggests that the Lizard Island locality produced the significant location by time interaction.

An examination of the increment profiles from fish collected from 3 locations around Lizard Island (Vicky's, Coconut and Chinamens reefs) during the November recruitment pulse found significant variability in otolith growth among locations ($F_{2,39} = 31.99$, $p = 0.0001$, Fig. 7). The interaction between time and location was significant ($F_{26,507} = 2.29$, $p = 0.0144$). This

interaction was driven by the profiles for the 3 locations overlapping 8 to 10 d prior to settlement, after which the Vicky's location became separated. All profiles then fell to a common increment width of 8 μm at settlement before diverging post-settlement, with Vicky's reef maintaining its higher pre-settlement increment widths (Fig. 7).

Increment profiles of fish that arrived in the November and December recruitment pulses were significantly different ($F_{1,37} = 55.34$, $p = 0.0001$, Fig. 8). The interaction term Time \times Pulse was found to be non-significant ($F_{13,481} = 1.60$, $p = 0.1646$). The morphology of the settlement-mark for both pulses remained the same, with a sharp drop in increment width occurring over a single day.

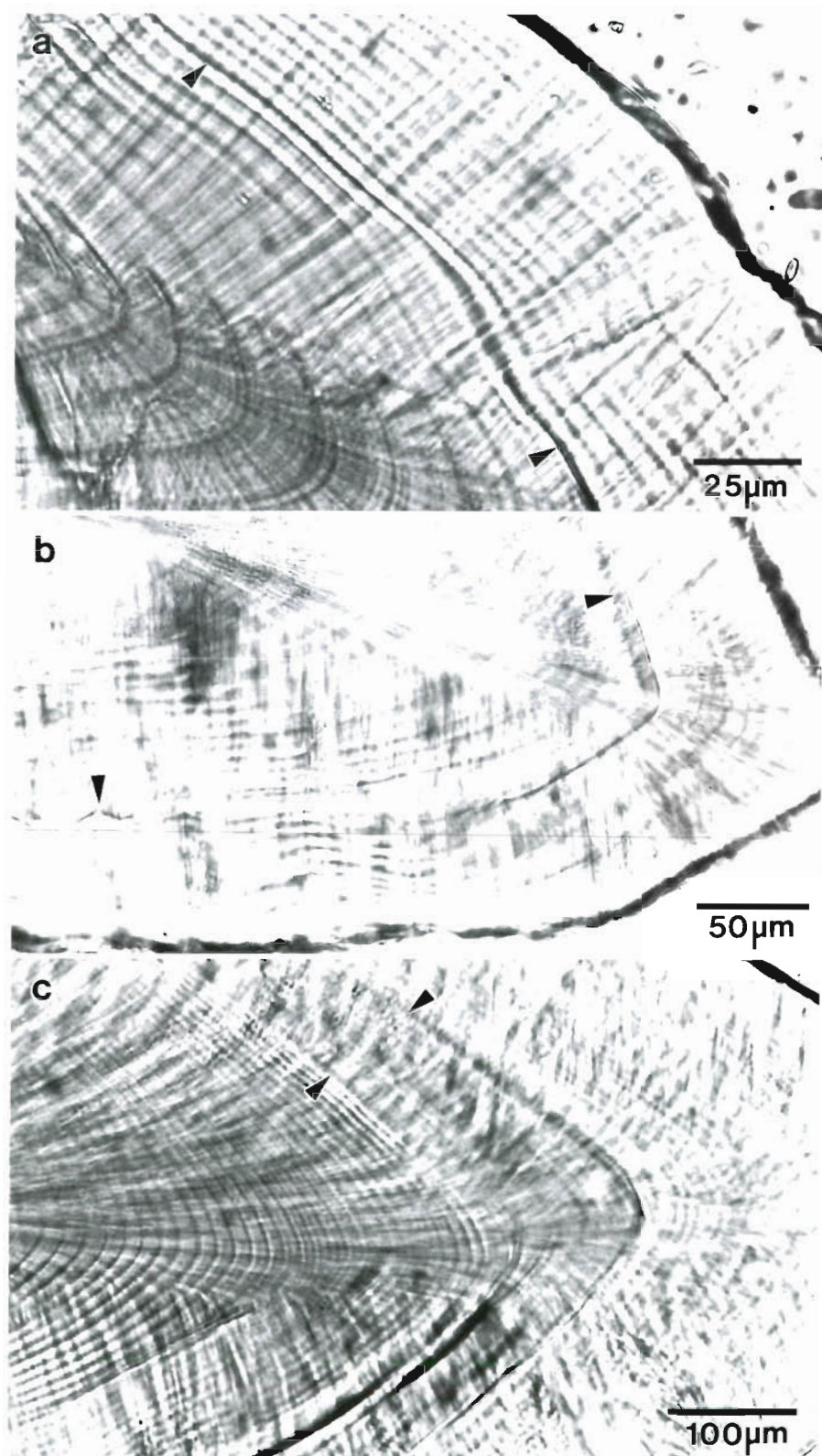


Fig. 5. *Pomacentrus amboinensis*, *P. coelestis* and *Acanthurus* sp. 2. Variations found in the form of the settlement-mark among species. (a) A single increment or day settlement-mark from *P. amboinensis*, (b) 2 increment or day settlement-mark from *P. coelestis*, and (c) a multiple increment or day zonal settlement-mark from *Acanthurus* sp. 2. (probably *A. blochi*). Arrows indicate location of settlement-mark

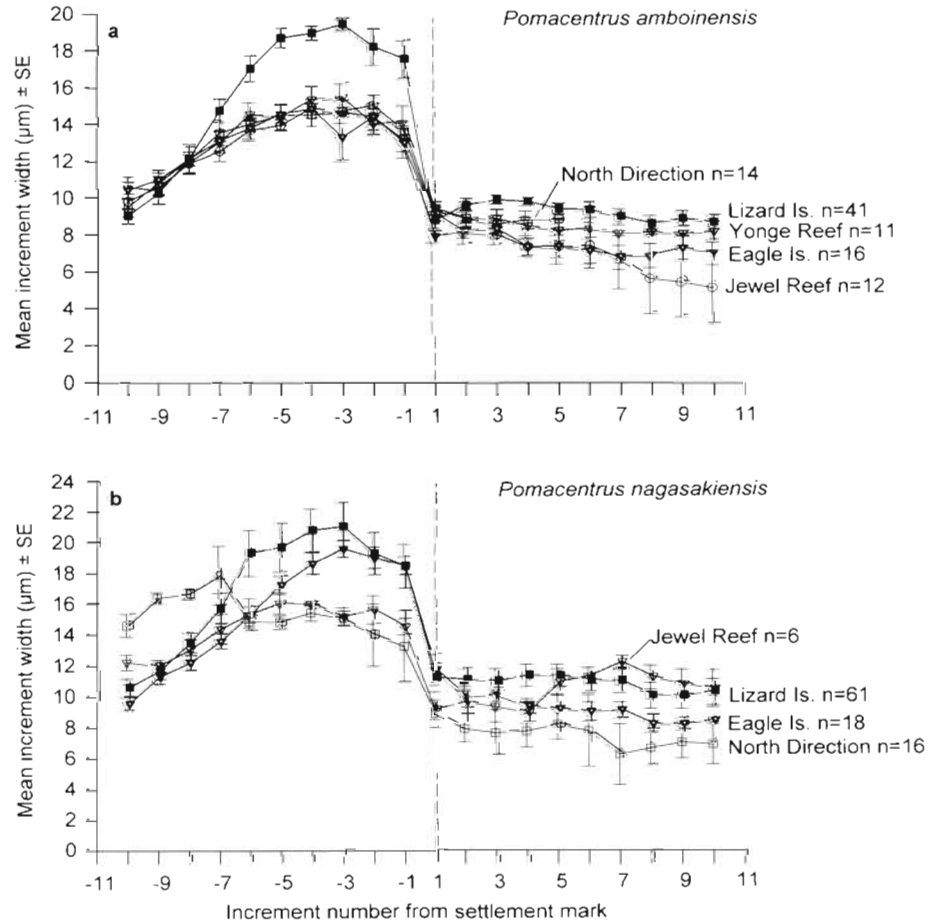


Fig. 6. *Pomacentrus amboinensis* and *P. nagasakiensis*. Large scale spatial variation in increment transition profile. Variation over 20 km, encompassing mid-shelf (Lizard Island, Eagle Island, North Direction) and Outer Barrier (Jewel, Yonge) reefs. (a) *P. amboinensis* (North Direction otolith increment profile ends 5 d post-settlement), and (b) *P. nagasakiensis*

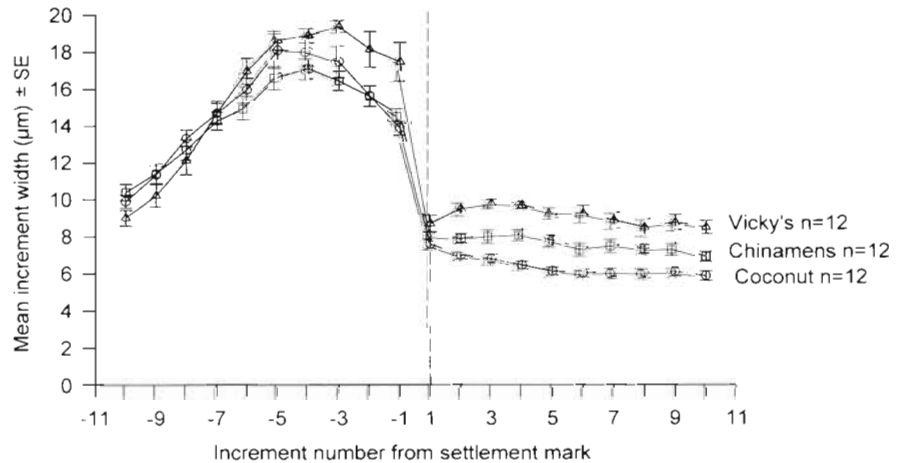


Fig. 7. *Pomacentrus amboinensis*. Small scale spatial variation in increment transition profiles. Variation among 3 locations around Lizard Island

Pomacentrus nagasakiensis

In contrast to *Pomacentrus amboinensis* the shape of the increment profiles for *P. nagasakiensis* showed high levels of variability among the 4 locations sampled

($F_{3,21} = 4.47$, $p = 0.014$; Fig. 6b). Yonge Reef was excluded from the analysis due to insufficient fish with 10 post-settlement increments ($n = 1$). Fig. 6b indicates that fish from Eagle Island and Lizard Island locations had larger increment widths pre-settlement than the other 2 loca-

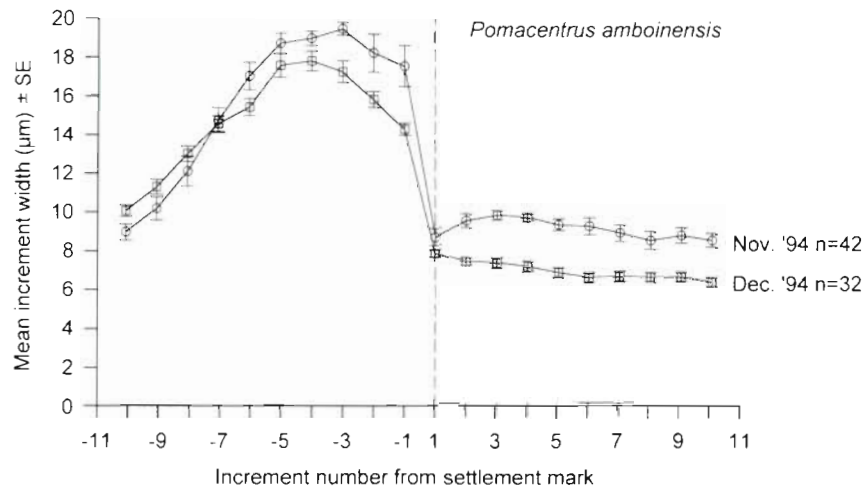


Fig. 8. *Pomacentrus amboinensis*. Temporal variation in increment transition profiles at the same locality. Comparison of variation between 2 consecutive recruitment cohorts at Lizard Island (October–November 1994)

tions. The repeated measures 'Time' factor was predictably significant ($F_{7,147} = 56.98$, $p = 0.0001$) due to the dramatic drop in increment width associated with the settlement-mark. There was no significant interaction (Time \times Location) ($F_{21,147} = 1.04$, $p = 0.4195$). In a similar way to *P. amboinensis*, the structure of the settlement-mark (i.e. width in relation to pre- and post-settlement increments) was consistent among the 4 locations.

Spatial variations in larval durations

Planktonic larval durations (PLDs) varied little for *Pomacentrus amboinensis*, ranging from means of $23.15 (\pm 0.28 \text{ SE})$ d for Lizard Island to $24.25 (\pm 0.48 \text{ SE})$ d for Jewel reef (Table 3). There was no significant variation in PLD of this species among the 5 locations ($F_{4,89} = 1.84$, $p = 0.1283$). The PLD of *P. nagasakiensis* ranged from 19 to 28 d with the 2 Outer Barrier locations having higher mean larval durations than the 3 lagoonal locations (Table 3). There was a significant variation among locations ($F_{4,97} = 5.71$, $p = 0.0004$).

DISCUSSION

Our results show that caging of recruits of known settlement history is a useful way of determining the temporal link between settlement and the formation of a settlement-mark on fish otoliths. Of the 16 species obtained, we were able to examine sufficient numbers of *Pomacentrus amboinensis*, *P. nagasakiensis* and *P. coelestis* to conclusively confirm that link. For *P. coelestis* the presence of a settlement-mark composed of 2 increments requires the counts for larval duration and post-settlement age to be ended and begun, respectively, at the first increment of the 2 d settlement-mark. This was also the case for *Acanthurus* sp. 2 (*A. blochi*) with the wide settlement-mark encompassing 14 d. Victor (1982) found a similarly wide settlement-mark of 5 d duration on the otoliths of the Caribbean wrasses *Thalassoma bifasciatum* and *Halichoeres bivittatus*. For the other 12 species examined in this study, evidence suggests that distinctive changes in increment structure occurred during the settlement event, although the small sample sizes

Table 3. *Pomacentrus amboinensis* and *P. nagasakiensis*. Variation in the Planktonic Larval Durations (PLD) of *P. amboinensis* and *P. nagasakiensis* among 5 study locations. PLDs were determined from back-calculation of otolith increments prior to the settlement-mark. Tukey's (HSD) means comparisons were used to examine the nature of any significant differences found among locations

Species	Location	n	Larval duration (range, d)	Mean larval duration \pm SE	Tukey grouping
<i>Pomacentrus amboinensis</i>	Lizard Island	41	21–32	23.15 ± 0.28	A
	North Direction	14	22–27	24.21 ± 0.47	A
	Eagle Reef	16	20–28	24.13 ± 0.53	A
	Jewel Reef	12	22–27	24.25 ± 0.48	A
	Yonge Reef	11	22–26	24 ± 0.4	A
<i>Pomacentrus nagasakiensis</i>	Lizard Island	61	19–27	23.28 ± 0.23	BA
	North Direction	16	20–28	23.56 ± 0.56	BA
	Eagle Reef	18	19–26	22.39 ± 0.5	B
	Jewel Reef	6	25–28	26.33 ± 0.56	A
	Yonge Reef	1	27	27 ± 0	A

examined for some species meant that further examination is required before strong conclusions can be drawn. Our results indicate that other studies, such as those of Brothers et al. (1983), Fowler (1989), Wellington & Victor (1989) and Tyler et al. (1993), are correct in assuming that the settlement-mark is directly associated temporally with the settlement event.

Otolith increment profiles were found to differ among locations for both pomacentrid species examined. These differences in otolith profile shape, while statistically significant, were relatively minor and the overall increment profile showed a very similar morphological pattern among locations for both species. Interestingly, all fish within a species exhibited similar settlement-mark structures, although the contrast of these marks differed among locations. This suggests that settlement-marks can be reliably used in these species as temporal indicators of settlement.

The repeatability of the settlement-mark structure suggests that, although the otolith increment profile is affected by the processes that influence larval durations, the morphology of the settlement-mark remains constant. The increment width profiles around settlement displayed similar patterns of change despite the larval duration of *Pomacentrus nagasakiensis* differing among reefs by up to a mean difference of 5 d. The morphology of the settlement-mark was also robust at smaller spatial scales and between consecutively sampled lunar recruitment pulses for *P. amboinensis*. Although the increment profiles from samples of fishes significantly differed both among 3 locations around Lizard Island and at 1 location between pulses, the morphology of the settlement-mark remained similar. The finding of species-specific signatures in otolith increment profiles is not unique to the species in the present study. Sponaugle & Cowen (1994) found similar repeatable increment profiles for 2 Caribbean gobies from samples collected over a 15 km stretch of Barbados coastline.

The increment width profiles of both species showed that location differences were attributable to the pre-settlement part of the otolith growth profiles, while post-settlement curves were generally similar among locations. For both *Pomacentrus amboinensis* and *P. nagasakiensis*, fishes that settled in the Lizard Island location had markedly higher otolith growth during the pelagic stage than for the other locations (with the exception of the Eagle Island location for *P. nagasakiensis*). Recent laboratory studies that demonstrate a transient link between otolith and somatic growth urge cautious interpretation of the relationship between increment growth and fish growth (Molony & Choat 1990, Milicich & Choat 1992). However, if we assume, for the purpose of discussion, that there is some less than perfect positive relationship between otolith and

somatic growth, these increment profiles advance some interesting hypotheses.

Firstly, *Pomacentrus amboinensis* reaching Lizard Island appear to be growing potentially faster during their late larval phase than those that settled in the other localities. This may be the result of characteristics of the water masses that larvae travelled through to reach Lizard Island (Lobel & Robinson 1983, Lewis & Boers 1991). Recent evidence suggests that late-stage reef fish larvae may aggregate close to the reef for a number of days prior to settlement (P. Doherty pers. comm.). Water temperature and food availability are known to influence growth in many marine species (Buckley 1982, Bailey & Stehr 1988, Francis 1991, McCormick & Molony 1992, 1995, Kerrigan 1996). Since the *P. amboinensis* profile represents an average of fish collected over 2 lunar pulses of recruitment, this may suggest that water masses with characteristics that favor otolith growth were consistently present around the Lizard Island fringing reef during the collection period (November–December 1994). Although recruits from December were growing slower than the November cohort, they still possessed a faster growth rate than the pre-settlement growth at other locations.

Secondly, if the otolith curves can be loosely interpreted as representing somatic growth, the difference between pre- and post-settlement trends has interesting ramifications for the notion of events in the larval phase biasing or influencing post-settlement events. The curves suggest that, in general, the growth rate prior to settlement was markedly higher than just after settlement. Furthermore, larval growth rates can have a direct bearing on the subsequent average growth rate once the fish had settled to the reef. These differences in the otolith growth are often subtle, but may correspond directly to differences in somatic growth rates leading to size advantages later in life. For instance, *Pomacentrus amboinensis* at Lizard Island during the November recruitment suggests that larval stage growth advantages are not always lost after settlement, and may be spatially-, temporally- and species-dependent.

The 'transition-centered' method of plotting otolith increments is useful for displaying trends in otolith growth that occur around settlement. This means of display avoids the masking effect of variable planktonic larval durations within species (typical of the traditional method), while still maintaining the differences in increment widths among individuals. The traditional method of plotting otolith increment profiles is most problematic in those species with highly variable planktonic larval durations. In these species the transition may vary by more than 10 d, resulting in a masking of the true trend around settlement when increment profiles are plotted chronologically from the

nucleus (authors' unpubl. data). If the consistent identification of the start of the transition zone can be achieved using the overall structure of the otolith, then by using the 'transition-centered' method, differences in increment widths among individuals of a species will be reflected in the variance around the mean pre- and post-settlement increment traces, thereby pointing to an interesting process occurring amongst individuals germane to the settlement process. By standardising in this way, researchers may attain greater resolution in determining the trends in increment widths around the settlement event, and the levels of variation around this important transition period.

The 'transition-centered' method showed that there is a tendency within a species for increments to converge to a common width immediately at settlement. The commonality of this width suggests that this convergence may be genetically determined. This width may represent the minimal growth of the otolith possible at this stage of development (metamorphosis), and may equate to an adaption to the fishes new benthic lifestyle requirements. Although few studies have made detailed measurements of size or somatic growth over metamorphosis, many of these studies show that fish have a period of non-linear growth during this developmental transition (e.g. Fukuhara 1988). It is likely that the link between otolith-fish growth and somatic growth (e.g. biological intercept method of Campana 1990) will change at developmental transitions such as metamorphosis. This will complicate the back-calculation of changes in growth over this important ecological transition.

The use of settlement-marks to calculate larval duration and back-calculated recruitment patterns offers an efficient method of revealing the temporal patterns of recruitment to many localities, on spatial scales larger than would be logistically possible using traditional methods (e.g. visual censuses). The results of this study have validated a key assumption regarding the use of otoliths for calculating larval durations and recruitment histories for a number of reef fish species. Available evidence suggests that the change in increment widths over settlement, whilst varying in magnitude, are species-specific and are remarkably consistent among locations and through time. These increment signatures of the settlement event may provide a much needed insight into the growth changes that occur during the intricate process of metamorphosis.

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