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RESEARCH ARTICLE

Behaviour that influences dispersal and connectivity in the small, young larvae of a reef fish

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Abstract Determining the scale of larval dispersal and population connectivity in demersal fishes is a major challenge in marine ecology. Historically, considerations of larval dispersal have ignored the possible contributions of larval behaviour, but we show here that even young, small larvae have swimming, orientation and vertical positioning capabilities that can strongly influence dispersal outcomes. Using young (11–15 days), relatively poorly developed (8–10 mm), larvae of the pomacentrid damselfish, *Amblyglyphidodon curacao* (identified using mitochondrial DNA), we studied behaviour relevant to dispersal in the laboratory and sea on windward and leeward sides of Lizard Island, Great Barrier Reef. Behaviour varied little with size over the narrow size range examined. Critical speed was 27.5 ± 1.0 cm s^{-1} (30.9 BL s^{-1}), and in situ speed was 13.6 ± 0.6 cm s^{-1}. Fastest individuals were 44.6 and 25.0 cm s^{-1}, for critical and in situ speeds, respectively. In situ speed was about 50% of critical speed and equalled mean current speed. Unfed larvae swam 172 ± 29 h at 8–10 cm s^{-1} (52.0 ± 8.6 km), and lost 25% wet weight over that time. Vertical distribution differed between locations: modal depth was 2.5–5.0 and 10.0–12.5 m at leeward and windward sites, respectively. Over 80% of 71 larvae observed in situ had directional swimming trajectories. Larvae avoided NW bearings, with an overall mean SE swimming direction, regardless of the direction to nearest settlement habitat. Larvae made smaller changes between sequential bearings of swimming direction when swimming SE than in other directions, making it more likely they would continue to swim SE. When swimming NW, 62% of turns were left (more than in other directions), which would quickly result in swimming direction changing away from NW. This demonstrates the larvae knew the direction in which they were swimming and provides insight into how they achieved SE swimming direction. Although the cues used for orientation are unclear, some possibilities seemingly can be eliminated. Thus, *A. curacao* larvae near Lizard Island, on average swam into the average current at a speed equivalent to it, could do this for many hours, and chose different depths in different locations. These behaviours will strongly influence dispersal, and are similar to behaviour of other settlement-stage pomacentrid larvae that are older and larger.

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Introduction

Most fishes that live on coral reefs, like most marine animals, have pelagic larvae (Leis and Carson-Ewart 2004) that have the potential to disperse long distances during their pelagic larval duration (PLD) of several days to several weeks. In fact, recent research indicates that dispersal of reef-fish larvae in demographically meaningful numbers seldom achieves such distances, and may be considerably more constrained than would be predicted by passive drift in currents alone (Swearer et al. 2002; Taylor

and Hellberg 2003; Jones et al. 2005; Almany et al. 2007; Gerlach et al. 2007). A likely contributor to this apparently limited dispersal is non-passive behaviour by the larvae, a possibility frequently ignored in studies and models of larval dispersal. Determination of the extent to which behaviour of larvae can influence dispersal trajectories must start with information on dispersal-relevant larval behaviour, and how this develops over the PLD. Although information on behaviour of moderately large settlement-stage larvae of some taxa is becoming available (Leis 2006), information on smaller, less well-developed larvae is rare.

One fish species with small, relatively undeveloped larvae is *Amblyglyphidodon curacao* (Bloch), a small (to 110 mm SL), but prominent and abundant Indo-Pacific damselfish (Pomacentridae). Adults of this species typically live in lagoons and other sheltered areas, often associated with staghorn corals of the genus *Acropora*: hence, the common name Staghorn Damselfish. This species is widespread, occurring from the NW Indian Ocean to southern Japan and eastward into the Pacific Ocean to Micronesia. In Australia, *A. curacao* is found along both coasts south to the Tropic of Capricorn (Hoese et al. 2006). It occurs in depths of 1–40 m, and eats both zooplankton and benthic algae (Randall 2005).

Very little is known of the early-life history of *A. curacao*. Like most, if not all, pomacentrids, *A. curacao* spawns oblong demersal eggs that are guarded by the male. The egg is about 1.4 × 0.5 mm and hatches in 4.5 days as a yolk-sac larva of 2.9 mm SL (based on Japanese specimens: Tanaka and Mori 1989). Tanaka and Mori's (1989) reared larvae survived for 9 days, but at that time were only 3.8 mm SL and had not developed past the preflexion stage. Larval development is otherwise undescribed. Ten recently settled individuals of 7.0–7.4 mm SL from Palau had 12–15 (mean 13.1) "pre-transition" otolith rings that were interpreted as daily growth increments (Wellington and Victor 1989). A PLD of 15–22 days (mean 17) was reported from the Great Barrier Reef for this species (Bay et al. 2006), suggesting that regional differences may exist in PLD, and by implication, in size at settlement.

In November–December 2005, we captured in light traps at Lizard Island, on the Great Barrier Reef, moderate numbers of pomacentrid larvae that were small (8–10 mm), relatively poorly developed, almost entirely transparent and without scales. Given their state of development, we initially took them to be the middle developmental stages of one of the pomacentrids of the genus *Pomacentrus* or *Chrysiptera* that typically settle at 12–15 mm SL, and that are extremely common in light-trap catches at Lizard Island. These larvae were similar in morphology to reared 16-day old, 9 mm SL, larvae of *Pomacentrus amboinensis*, a species that settles at about 23 days old and 12 mm SL (Murphy et al. 2007; Figs. 1, 2). Aside from differences in pigment, the unidentified larvae differed obviously from 16-day old *P. amboinensis* only in lacking scales (Figs. 1, 2). The unidentified light-trap larvae did not settle when placed in aquaria, nor did they transform in spite of being maintained in aquaria for a few days while being fed newly hatched brine shrimp. Subsequently, we identified these small larvae as *Amblyglyphidodon curacao* (see "Results"). Larvae of this species had not previously been reported from light traps at Lizard Island, most likely because the larvae were overlooked due to their small size and transparency. Although we cannot be certain that these larvae were competent to settle, the otolith ages (PLD) and sizes we obtained, match or exceed those reported for settled *A. curacao* (Wellington and Victor 1989; Bay et al. 2006), so we assume they were competent to settle.

Capture of these small, relatively poorly-developed larvae in good condition provided the opportunity to study dispersal-relevant behaviour of larvae that were younger and morphologically less developed than those typically captured in light traps (Choat et al. 1993). They were much more 'larval' in appearance than any pomacentrid species we had studied previously (Leis and Carson-Ewart 1997, 2002, 2003; Leis and Fisher 2006; Fisher et al. 2005). We measured potential swimming speed (critical speed, U_{crit}) and unfed swimming endurance in the laboratory, and in the field, measured in situ swimming speed, orientation and vertical distribution. In spite of their small size, and poorly

Fig. 1 *Amblyglyphidodon curacao* larva (*bottom*, AMS I.43781-044, 9 mm) compared at the same scale to *Pomacentrus amboinensis* of similar size (*middle*, AMS I. 38139-017, 16 dah, 9 mm) and *Pomacentrus amboinensis* approaching settlement-stage (*top* AMS I.38139-041, 21dah, 10.5 mm). The *P. amboinensis* larvae were reared (Murphy et al. 2007), whereas the *A. curacao* larva was a wild specimen captured by light trap. Differences in ground colour are due to differences in handling and preservation

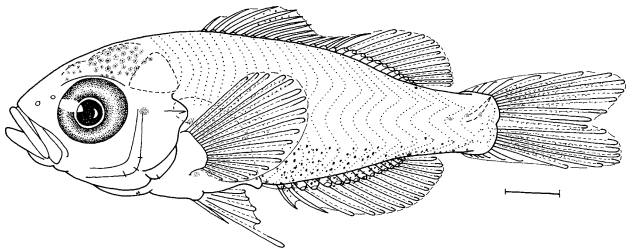

Fig. 2 Illustration of 9 mm SL *Amblyglyphidodon curacao* larva (AMS I.43781-043) showing distinctive melanophores. The specimen lacked scales. Scale bar is 1 mm

developed morphology, these *A. curacao* larvae had behaviours comparable to settlement-stage larvae of other pomacentrid species that were older and larger: they had better swimming performance than many species, and similar orientation and vertical distribution behaviour. This has implications for dispersal-relevant behaviour of the smaller larval stages of other species.

Our goal was to investigate dispersal-relevant behaviour in *A. curacao*, to compare this with behaviour of larvae of related species, and to briefly describe the larvae to facilitate future research on this species.

Materials and methods

Larvae and their identification

Larvae were captured using light traps deployed 100–200 m seaward of the reefs off the west coast of Lizard Island, Great Barrier Reef, Australia (14°40′S, 145°27′E) during November and December 2005. Light traps were emptied in the morning, and all observations were conducted with larvae on the day of their capture. All larvae retained for identification and measurement were euthanized and preserved in 70% EtOH. Length is reported as standard length (SL) in mm of preserved larvae. Wet weight of preserved larvae was measured after specimens were blotted dry with tissue in a standard manner. Variation of mean values is given as standard error (±SE). Each larva was used for only one type of observation.

In order to identify the larvae, fin-ray counts were made on several specimens: the counts were dorsal XIII,12, anal II,14, pectoral 17. These were compared to pomacentrid species known to occur on the Great Barrier Reef (GBR) (Randall et al. 1997). This comparison eliminated all but 8 of the 104 GBR species: *Amblyglyphidodon aureus*, *A. curacao*, *A. leucogaster*, *Chrysiptera biocellata*, *C. cyanea*, *C. taupou, Pristotis obtusirostris* and *Pomacentrus pavo*. Of these, only the larvae of *P. pavo* have been described (Tanaka et al. 2004): these differ in morphology and pigmentation from the unidentified larvae, thus eliminating *P. pavo* as a possibility. Larvae of *Chrysiptera hemicyanea* and *C. parasema* have been described (Tanaka and Nitta 1997; Tanaka and Yamada 2001). Although neither is one of the three candidate *Chrysiptera* spp, they also differ from the unidentified larvae, thus making it unlikely that the unidentified larvae are a species of *Chrysiptera*. Except for the just hatched larvae of *A. curacao* (Tanaka and Mori 1989), no larvae of any *Amblyglyphidodon* or *Pristotis* species have been described.

Genetic analysis of mitochondrial DNA (mtDNA) was carried out to determine to which of these species the larvae belonged. DNA was extracted (see below) from EtOH-fixed or frozen material from eight specimens from the GBR held in the Australian Museum (AMS) fish collection: two of the unidentified larvae (AMS I.43781-012 & -013), identified adult or juvenile specimens of *Amblyglyphidodon aureus* (AMS I. 33717-001), *A. curacao* (AMS I.33699-006), *A. leucogaster* (AMS I.33701-009), *Pomacentrus moluccensis* (AMS I.43781-005: no *P. pavo* material was available) and *Chrysiptera rollandi* (AMS I. 43781-006: no material of the three candidate *Chrysiptera* spp was available) and *Pristotis obtusirostris* (AMS I.38635-016). In addition, GenBank data on species of *Pomacentrus* and *Chrysiptera* were included. DNA-based species identification of the two unidentified larvae was carried out using regions from two mitochondrial genes: 12S rDNA and 16S rDNA.

DNA was extracted from 0.01–0.05 g of tissue using CTAB and phenol–chloroform according to methods modified from Saghaimaroof et al. (1984). After ethanol precipitation, DNA was resuspended in 50–200 µl sterile MQ-H_2O. Two mitochondrial genes were amplified, 12S rDNA and 16S r DNA with the primers 12S rDNA f (AAA GCT TCA AAC TGC GAT TAG ATA CCC CAC TAT), 12S rDNA r (TGA CTG CAG AGG GTG ACG GGC GGT GTG T) (Kocher et al. 1989) and 16SF (CGC CTG TTT AAC AAA AAC AT) and 16 SR (CCG GTC TGA ACT CAG ATC ACG T) (Kocher and White 1989), respectively. PCR amplifications were carried out in total volumes of 50 µl containing final concentrations of: 5 µl 10× reaction buffer (Bioline), 4 mM $MgCl_2$, 0.05 mM of each dNTP, 12.5 pmol of each primer, 0.1 units Taq polymerase (Bioline) and 1–100 ng of template genomic DNA. Cycling conditions were carried out at an annealing temperature of 50°C (initial denaturation at 94°C for 1 min), then 30 cycles of: 94°C (20 s), 50°C (45 s), 72°C (1 min), and a final extension at 72°C for 5 min. Successful amplification was checked by running 5 µL (or 20%) of the reaction on a 1.5% agarose gel. Purification of the PCR products was then carried out using AMPURE PCR purification system available from Agencourt Bioscience Corporation (distributed by GeneWorks Pty Ltd in Australia) according to the manufacturer's instructions. Cycle

sequencing was done on the purified DNA sample using ½ volume DYEnamic ET terminator premix (distributed by Amersham Biosciences for GE Healthcare) with the same primers used for PCR using 4 µl of ET-mix, 5 µM of primer, and 40–100 ng PCR product template. Cycle sequencing was done using 35 cycles of 95°C (20 s), 50°C (15 s) and 60°C (2 min). The samples were purified using ethanol and ammonium acetate purification run on a MegaBACE 500 Genetic Analyser (Amersham Biosciences). Forward and reverse strands are combined and sequences checked for errors using the computer program Sequencer (Genecodes). Only samples that provided a clear and unambiguous sequence were subject to further analyses.

Multiple alignments were performed on the combined (12S + 16S data set) using ClustalX Thompson et al. (1997) using a gap opening of 10, a gap extension of 0.05 and a gap separation distance of 8. Maximum parsimony (MP) and maximum likelihood (ML) analyses were carried out on the combined nucleotide dataset using PAUP*, version 4.0b10 (PPC) (Swofford 1998). This involved the use of an heuristic search with random sequence addition (10 replicates each) using accelerated transformation of character states and the TBR branch swapping algorithm, 100 bootstrap replicates each.

Laboratory observations

A multilane swimming chamber was used to measure both critical speed and swimming endurance (Clark et al. 2005). We used a chamber of clear perspex with six lanes, each 30 mm wide, 50 mm high and 180 mm in length, identical to that of Stobutzki and Bellwood (1997), except that 0.5 mm mesh was used to delimit the ends of each lane due to the small size of the larvae. A 40 mm long section of flow straighteners at the start of each lane minimized turbulence and boundary layers. The flow rate within the chamber was adjusted using a calibrated valve. Calibration of flow rates (Clark et al. 2005) was done each day the chamber was in use. The chamber used unfiltered seawater from the seawater system of the Lizard Island Research Station (LIRS).

Larvae were placed in the swim chamber and allowed to acclimate to a flow rate of 0.5 cm s^{-1} for a period of 5 min before any measurements of swimming ability were taken. Water temperature in the chamber ranged from 29–32.6°C. Two measurements of swimming performance were made: critical speed (U_{crit}) (Brett 1964), which measures maximum swimming speed over periods of minutes, and endurance, which measures how long larvae can swim without food or rest at a fixed speed (Stobutzki and Bellwood 1997).

The flow rate for U_{crit} tests started at 0.5 cm s^{-1} and was increased by approximately 4.2 cm s^{-1} every 5 min, until the larvae were unable to swim against the flow. Critical speed of larvae was calculated using the equation of Brett (1964):

$$U_{cirt} = U + (t/t_i \times U_i)$$

where

U penultimate speed
U_i speed increment (4.2 cm s^{-1} in the present study)
t time swum in the final speed increment
t_i the time interval for each velocity increment (5 min)

The total time for a critical speed measurement was proportional to the U_{crit} achieved, and varied from 35 min for the slowest individual to 62 min for the fastest. Given the endurance of which these larvae are capable (see "Results"), it is unlikely that the larvae would have become fatigued over such a short period of time.

For the endurance test, six larvae were made to swim at a fixed speed until they became fatigued (i.e., could no longer maintain position against the current and drifted onto the downstream mesh). Larvae were observed regularly, and the time of fatigue (=swimming duration) was recorded. If the moment of ultimate fatigue was not observed, it was estimated as the midpoint between the time when the larva was last observed swimming and the time when it was found no longer swimming. Chambers were outdoors but shaded during the day and illuminated by an overhead fluorescent light at night. The initial flow rate was 10 cm s^{-1}, but this gradually slowed to 8 cm s^{-1}—most likely due to clogging of swim chamber meshes by material in the unfiltered seawater—by the time the last larva became exhausted. Endurance in units of swimming time was converted to distance by using the daily measurements of flow speed, and is reported in kilometres. For the endurance test, 15 larvae were drawn haphazardly from a 12 l container holding several dozen larvae. Six were used for measurements of endurance, and the rest were preserved to provide an estimate of size and weight at the start of the run. As the larvae became fatigued, they were removed from the chamber and preserved, but two of the specimens could not be recovered intact.

Study area

Two sites off Lizard Island were used for in situ observations of larval behaviour—Watsons Bay on the leeward (NW) side of the island and Coconut Beach on the windward (SE) side, about 5 km apart (see map in Leis and Carson-Ewart 2003). Larvae were released ca 500 m from the reef edge as determined by GPS. The water was 14–19 m deep in Watsons Bay over largely bare sand bottom

and 32–33 m deep off Coconut Beach over nearly continuous, dark algae (*Halimeda*) on sand. The bottom was frequently visible to the divers during the Watsons Bay dives, but was not visible to them off Coconut Beach.

In situ observations

In situ observations off the windward side of the island could be made only during calm conditions that were available on only 3, 4 and 5 December. In situ observations off the leeward side of the island were made on 9 days between 23 November and 12 December. In situ observations were made between 08:00 and 14:00 local time.

Larvae were transported to field sites in covered buckets fitted with battery powered aerators. Once at the site, acclimation to ambient water conditions was achieved by gradual addition of fresh seawater into the buckets. Approximately 50% of the water in a bucket was replaced hourly. In situ observation of larvae followed the methods of Leis et al. (1996) and Leis and Carson-Ewart (1997, 1998). Briefly, two divers descended to a depth of 5 m, where the observer diver released a larva from a small container (the direction faced by the observer diver when releasing a larva was randomised). The larva was allowed to choose its initial trajectory and was then followed by the two divers. The observer diver observed the larva, concentrating on keeping it in view and maintaining a position directly behind it. The second diver followed the observer diver and recorded data. Each larva was only followed once, and if possible, recaptured at the end of the observation period for preservation. If a larva was not recaptured, it was excluded from analyses that required size information. A total of 74 larvae were observed of which 57 (77%) were recaptured.

Swimming speed, depth and swimming direction were measured in situ. An attempt was made to observe larvae for a 10-min period, taking measurements of swimming direction and depth using a dive compass and computer respectively, every 30 s. Direction was recorded to the nearest 5° (in degrees magnetic which is 7° east of degrees true), and depth to the nearest 0.1 m. Speed was calculated from distance travelled as measured by a calibrated flowmeter (Leis and Carson-Ewart 1997) over the full period of observation. Stops, pauses or hovering were incorporated into the speed measurements. Reported speeds are over the full period of observation unless noted otherwise. Although 88% (n = 65) of larvae were observed for a full 10 min, some individuals were observed for less time either because the larva swam deeper than our safety depth (normally 15 m), was lost by the observer, or was eaten by a predator. Three larvae swam rapidly downward and were either lost or went deeper than our safety depth in less than 4 min and were used only for analysis of vertical distribution. The other six larvae for which we had fewer than 10 min of observations were included in speed and orientation observations. These include 5 min of observations on three larvae, 6 min on two, and 8 min on one.

Amplitude is the difference between greatest and least depth for each individual. A change in vertical direction from ascent to descent (or the opposite) was defined as changes in depth of at least 1 m.

Age estimates

Sagittal otoliths were removed from the left side of six individuals ranging in size from 8.7 to 9 mm SL. Each otolith was mounted in resin on a slide and the surface of the otolith was polished. The resin was then reheated and the otolith turned over, so the underside could also be polished. Counts by three observers of what we assume to be daily rings were then made using a microscope fitted with a high-resolution video system.

Data analysis

The 21 observations of vertical distribution over 10 min for each larva may be autocorrelated. To test for this, autocorrelation charts were generated using Statistix software (NH Analytical Software, St Paul, MN, USA) for each individual trajectory. Nine larvae (12%) had no autocorrelation at any lag, 56 (76%) had a significant autocorrelation only at a lag of one observation, nine (12%) had significant autocorrelation at both lags of 1 and 2, and no larvae had significant autocorrelation at lags of 3 or greater. Therefore, analyses of vertical distribution were performed as follows: for the 12% of larvae with no autocorrelation using all data, for the 76% of larvae with a significant lag of 1 using only every second observation, and for 12% of larvae with a significant lag of 2 using, only every third observation. This resulted in 840 usable observations of depth for the 74 larvae. Autocorrelation charts confirmed that reduced data sets lacked significant autocorrelation. Depth–frequency distributions with the data pooled for all individuals were compared using 2-tailed 2-sample Kolmogorov–Smirnov (KS) tests. The null hypotheses were that these distributions did not differ between locations (Watsons Bay versus Coconut Beach), or within locations between two size classes (<9 mm and ≥9 mm).

Circular statistics were used to analyse most of the orientation data (Batschelet 1981; Zar 1996) using Oriana software (version 2, Kovach Computing Services, Pentraeth, Wales, UK). Mean vector length (r), is a measure of angular dispersion, or precision, ranging from 0 (maximum

dispersion) to 1 (lack of dispersion). Values of r were arc sine transformed prior to statistical analysis. The Rayleigh test was used for single-sample hypotheses about directional swimming, and the Watson–Williams F test was used to test hypotheses about directional swimming involving more than one sample.

For all statistical tests, we report actual P values whenever possible, but consider $P < 0.05$ to constitute a "significant" difference.

Results

Identification

The use of molecular genetic techniques confirmed that the pomacentrid larvae were of *A. curacao*. The ML and MP analyses of the combined 12S and 16S dataset from 10 pomacentrid species differed only in the position of *Pristotis obtusirostris* and show that the two larvae AMS I.43781-012 (GenBank EF419263, EF419271) and AMS I.43781-013 (EF419264, EF419272) unequivocally group (bootstrap value 100) with the *Amblyglyphidodon curacao* adult (AMS I.33699-006, EF419266, EF419274) (Fig. 3). (GenBank Accession numbers for other taxa included are: AMS I.33717-001 EF419265, EF419273; AMS I.33701-009 EF419267, EF419275; AMS I.43781-005 EF419268, EF419276; AMS I.43781-006 EF419269, EF419277; AMS I.38635-016 EF419270, EF419278 from this study, and previously published sequences; AF285922, AF285944, AY279568, AY279671, AF081236, AY365122, AF285934, AF285956, AF081238, AY365123, AF285935, AF285957, AY098624, AY098629). Thus, the larvae are clearly identifiable as *Amblyglyphidodon curacao.*

The preserved *A. curacao* larvae ranged from 8.0–10.5 mm SL (the 57 larvae recaptured following in situ observation ranged in size from 8 to 9.75 mm SL with an average of 8.9 ± 0.07 mm SL). All larvae have fully-formed fin rays (DXIII,12; AII,14; $P_1$17; P_2I,5) and two nares, but none have any scales. They are of moderate depth and lack obvious head spination or other obvious larval specializations (Figs. 1, 2). There is no obvious change in morphological development over the available size range. Larvae are characterized by a dense covering of melanophores on the dorsal surface of the fore and mid brains, a single dense melanophore at the posterior end of both dorsal and anal fins (the dorsal melanophore absent in some individuals), at least one (usually two) melanophores just anterior to the symphysis of the cleithra, and most distinctively, many very fine melanophores scattered along the base of the anal fin (but only a few on the rays or membrane) and extending dorsally, but not reaching the lateral midline. The number and extent of these 'peppery' melanophores varies, but the melanophores occur at least along the base of the anal fin in all individuals. In addition, the caudal and pectoral fins have even finer melanophores outlining some of the fin rays: the extent of this pigment also varies. Some individuals have a few fine melanophores on the membrane of the central portion of the spiny dorsal fin. Limited internal melanophores are present on the gut and gas bladder, and under the hindbrain and in the branchial region, and a few small melanophores occur on the urostyle and penultimate vertebral centrum (Fig. 2). In life, the larvae are transparent, with a silvery gut and eyes and a slightly yellowish cast to the brain. None of the other pigment is visible to the naked eye. The general morphology, state of development and body proportions of these larvae are very similar to those of 14–15-day-old (9 mm) *Pomacentrus amboinensis* larvae (Fig. 1). Other than pigment, the major differences are that *P. amboinensis* is fully scaled at this age and size, but has a smaller pectoral fin (Murphy et al. 2007). Settlement-stage *P. amboinensis*, in contrast, are considerably larger (Fig. 1).

Age and growth

The six larvae for which otolith counts were obtained had estimated ages of 11–15 days. Although the mean size did increase with age, age and size were not significantly correlated ($P = 0.26$). Age was, therefore, not a good predictor of size for these larvae over the narrow size range examined, explaining only 30% of the variation in size (Fig. 4), however, this conclusion requires further testing based on more individuals. Over the size range studied here, growth was only 0.06 mm day^{-1}. In contrast, using a size of 2.9 mm at hatching (Tanaka and Mori 1989), and the size and ages we found (Fig. 4), growth over the whole PLD would be 0.2–0.6 mm day^{-1}.

Critical speed

Critical speed was measured in 31 individuals from five batches of six individuals over 2 days (two fish were inadvertently included in one lane on one occasion, hence 31 individuals). Critical speed was related to size (SL) with a significant linear relationship ($U_{crit} = 4.47SL - 12.29$, $P = 0.04$, $r^2 = 0.14$, $n = 31$) (Fig. 5), although the relationship had relatively little predictive value, explaining only 14% of the variation, probably due to the narrow size range of the available larvae. The overall average U_{crit} of the 31 larvae (which had an average SL of 8.9 mm) was 27.5 ± 1.0 cm s^{-1}. The fastest individual (9 mm SL), had U_{crit} = 44.6 cm s^{-1}, which was 17 cm s^{-1} faster than average. Mean scaled critical speed was 30.9 ± 1.0 body

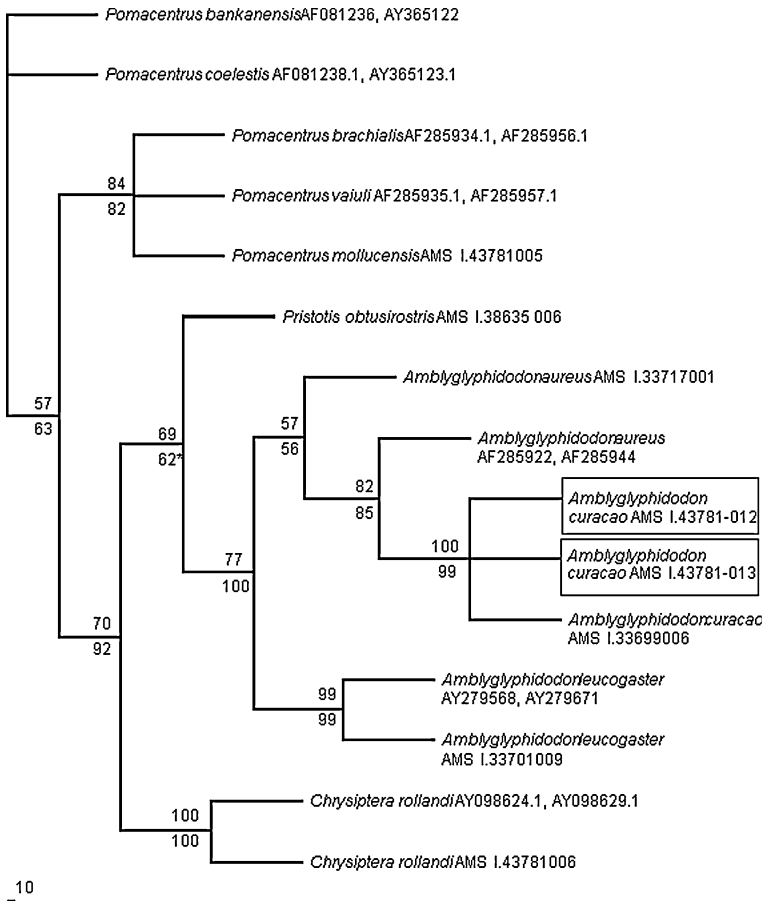

Fig. 3 The 50% Majority rule consensus tree from Maximum Likelihood (bootstrap values > 50% above branches) and maximum parsimony (bootstrap values > 50% below branches) phylogenetic trees inferred using combined 12S rDNA and 16S rDNA data. Codes of AF or AY following species names indicate Genbank submissions for 12S and 16S data, respectively. AMS codes refer to Australian Museum registration numbers. *Boxes* indicate the two larvae identified to species by these analyses. Asterisk indicates position that differed in the MP analysis, which placed *Pristotis obtusirostris* sister to *Chrysiptera rollandi* (bootstrap value 62)

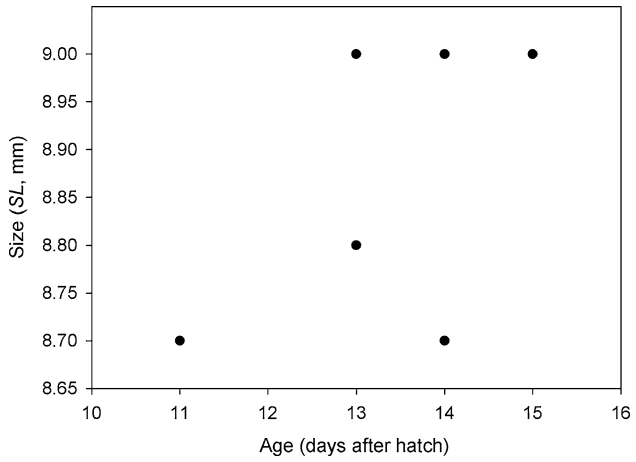

Fig. 4 Age (based on otolith rings) of *Amblyglyphidodon curacao* larvae of different sizes

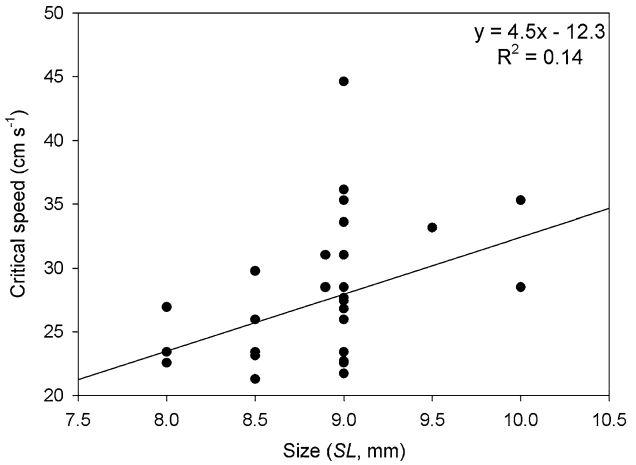

Fig. 5 Relationship between critical speed and size of *Amblyglyphidodon curacao* larvae. The linear relationship was significant ($P = 0.04$)

lengths per second (BL s^{-1}), with a maximum value of 49.6 BL s^{-1}.

In situ speed

In situ speeds averaged 13.6 ± 0.6 cm s^{-1} ($n = 71$) over the full observation period. The best performing individual (9.2 mm SL) swam at 25 cm s^{-1}. Average scaled in situ speed was 14.8 ± 0.8 BL s^{-1} with a maximum of 22.9 BL s^{-1}. In situ speed was not significantly correlated with body size ($P = 0.20$, $r^2 = 0.03$, $n = 54$). Significantly more larvae swam faster in the first half of the observation period than in the second (Sign test, $P < 0.001$, $n = 57$

of 71 faster in first), but the average difference in speed was small (2.4 cm s^{-1}, or 16%). Average in situ speed (13.6 cm s^{-1}; 14.8 BL s^{-1}) was almost exactly half of average U_{crit} (27.5 cm s^{-1}; 30.9 BL s^{-1}).

Endurance in the laboratory

Endurance swimming times ranged from 33 to 231 h with an average time of 172 ± 29 h (n = 6). During these periods, the average distance swum was 52.0 ± 8.6 km, with a range of 10.8–69 km.

The larvae did not undergo a significant change in length during the period of endurance swimming, but they did become significantly lighter, losing an average of 25% of their initial wet weight. Average size was 9.07 ± 0.09 mm SL at the start of the trial and 9.13 ± 0.13 mm SL at the end ($P > 0.20$, T test). Average weight was significantly greater (T test, $P = 0.003$) at the start of the endurance trial (15.9 ± 0.6 mg) than at the end (11.9 ± 0.7 mg).

Vertical distribution in situ

The overall mean amplitude (i.e., the difference between the deepest and shallowest depth for each individual larva) was 5.2 ± 0.3 m (n = 74) (Table 1). Mean amplitude of small larvae (<9 mm) was not significantly different from that of large larvae (≥9 mm) (Table 1). In contrast, mean amplitude at Coconut Beach was significantly greater than mean amplitude at Watsons Bay (Table 1) by 1.4 m. Five of the larvae swam below the safety depth limit, four of them at Coconut Beach, and two did so monotonically downward. Individual larvae changed from ascent to descent or the opposite an average of 2.5 (SE = 0.2) times during the 10 min of observations. This did not differ between locations or size of larvae. One or two changes in vertical direction were most common, occurring in 27 and 20% of individuals, respectively, whereas either no change or three were each made by 13% of individuals, and at the extreme, three larvae (4%) changed vertical direction seven times.

Vertical distribution behaviour differed between locations, but only to a limited extent between size classes of larvae. Figure 6 provides examples of actual vertical profiles in the two study locations. In Watson's Bay, modal depth was 2.6–5.0 m, whereas at Coconut Beach, modal depth was 10.1–12.5 m (Fig. 7). The depth–frequency distribution differed significantly between the two sites, with larvae swimming deeper at Coconut Beach (K-S test, $P < 0.0001$, n = 677 at WB, 163 at CB).

Vertical distribution behaviour did not differ consistently between large (≥9 mm) and small (<9 mm) larvae.

Table 1 Depth amplitude in larvae of *Amblyglyphidodon curacao*

	n	Mean (m)	Range (m)	P (T test)
Overall	76	5.2 (±0.3)	1.1–12.6	–
Site				
Watsons Bay	60	4.7 (±0.3)	1.1–10.3	< 0.01
Coconut Beach	16	6.9 (±0.6)	3.2–12.6	
Size				
<9 mm	24	5.3 (±0.4)	2.0–9.0	> 0.05
≥9 mm	44	4.8 (±0.4)	1.1–10.7	

Values in brackets are standard errors

At Watsons Bay, small larvae had a significantly different depth–frequency distribution than did large larvae. Although both size classes had the same modal depth of 2.6–5.0 m (Fig. 8), large larvae spent more time in the upper 5 m, and less time deeper than 7.6 m than did the small larvae (K-S test, $P < 0.01$, n = 190 for small larvae, 435 for large larvae). In contrast, at Coconut Beach, although large larvae tended to occur deeper than small larvae (Fig. 9), the depth–frequency distributions of large and small larvae were not significantly different (K-S test, $P > 0.05$, n = 68 for small larvae, 76 for large larvae).

Orientation

At both Watsons Bay and Coconut Beach, a large majority of individual larvae swam directionally (87.7 and 76.5%, respectively), and this did not differ between site or size class (G-test, $P > 0.05$, n = 71, 53, respectively). The precision of directionality (measured as r, the length of the mean vector) did not differ significantly between the two sites, or between large and small larvae (Table 2), although there was a non-significant tendency for larger larvae to have more precise directional swimming.

Overall, the larvae tended to swim southeast, regardless of the direction to the nearest reef habitat, during the 10-min periods when they were under observation. The mean bearings of the 63 individuals that had directional trajectories were used to determine if there was an overall—i.e., population—swimming orientation. This comprised 13 larvae from Coconut Beach and 50 from Watsons Bay. The distributions of the mean bearings from the two locations did not differ significantly (Watson–Williams test, $P = 0.64$), and had similar overall mean bearings (147° and 162°, respectively). When combined, the resultant distribution of 63 mean individual bearings was significantly different from uniform (Rayleigh test, $P = 0.035$), with an overall mean bearing of 151° (i.e., SE, Fig. 10) (Rao's spacing test returned $P > 0.10$, which indicates there was not significant bimodality in the distribution of these data).

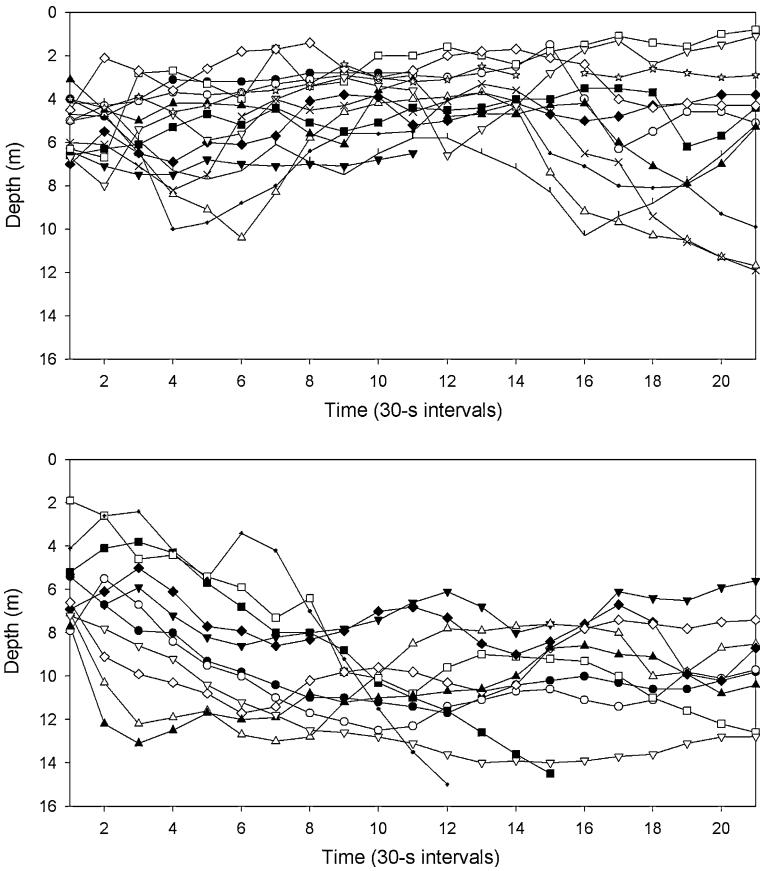

Fig. 6 Examples of vertical distribution behaviour of *Amblyglyphidodon curacao* larvae at top Watsons Bay, 2 December 2005 and bottom Coconut Beach, 3 December 2005. Each *line* represents the vertical trajectory of an individual larva

The departure from uniformity was principally due to an under representation of individual mean bearings to the NW (Fig. 10): only 6 larvae had mean bearings between 270° and 360°, whereas a uniform distribution would be expected to have 15.8. Numbers of mean individual bearings in the other quadrants were 17 (NE), 19 (SE) and 21 (SW).

The 71 larvae we observed made a total of 1,148 changes in swimming direction between the sequential 30-s bearings (in 242 instances, no change in direction took

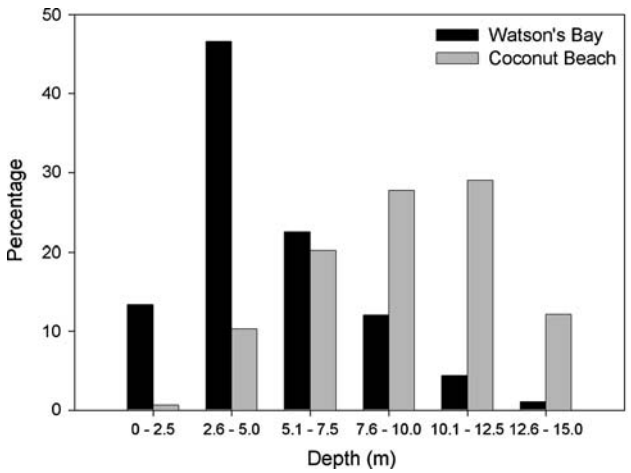

Fig. 7 Depth–frequency distribution of *Amblyglyphidodon curacao* larvae at Watsons Bay (*black bars*) and Coconut Beach (*grey bars*). These distributions were significantly different (K-S test, $P = 0.00001$, see text)

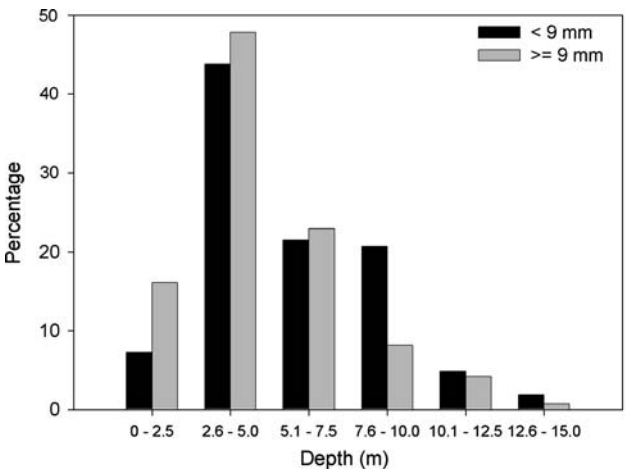

Fig. 8 Depth–frequency distribution of *Amblyglyphidodon curacao* larvae at Watsons Bay according to size (*black bars* are <9 mm SL, *grey bars* are ≥9 mm SL). These distributions were significantly different (K-S test, $P < 0.01$, see text)

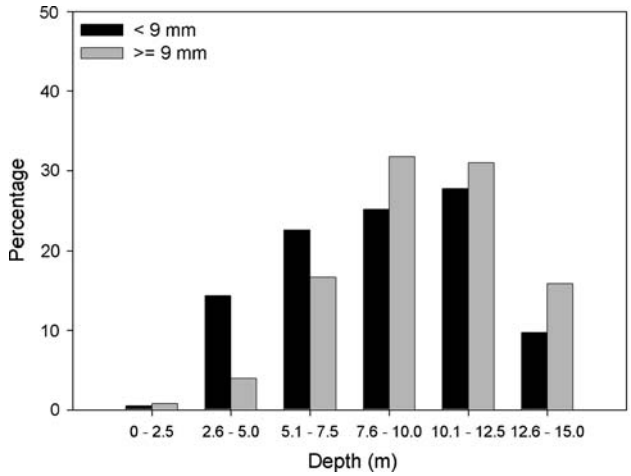

Fig. 9 Depth–frequency distribution of *Amblyglyphidodon curacao* larvae at Coconut Beach according to size (*black bars* are <9 mm SL, *grey bars* are ≥9 mm SL). These distributions were not significantly different (K-S test, $P > 0.05$, see text)

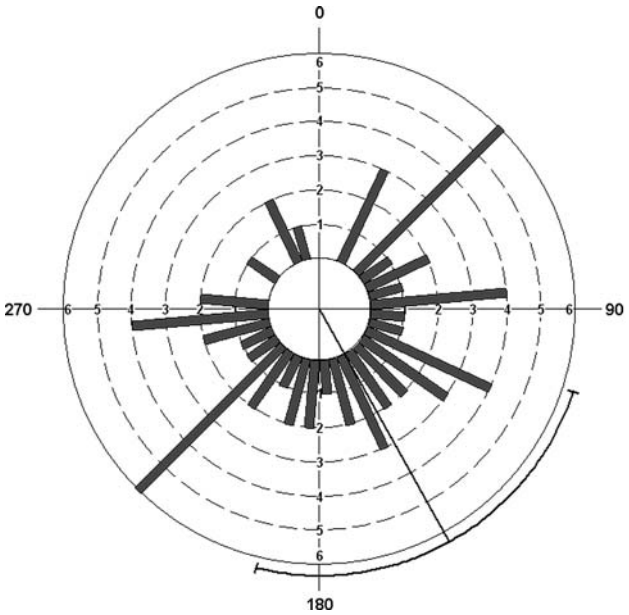

Fig. 10 Frequency distribution of mean in situ swimming directions for *Amblyglyphidodon curacao* larvae at Watsons Bay and Coconut Beach combined. *Thick lines* represent the mean values for individuals, the thin radius that penetrates the outer, *circle* represents the overall mean bearing, and the external arc centred on that radius is the 95% confidence interval of the mean. Mean bearing = 151°, $r = 0.23$, $P = 0.035$. r is the length of the mean vector, P is for the Rayleigh test

place). Of the changes in direction, 57.8% (663) were left turns, whereas 42.2% (485) were right turns: 47 individuals made more left turns than right, 20 more right turns than left turns, and 4 had an equal number of left and right turns ($P < 0.01$, two-tailed Sign Test). Overall, larvae made 2.5 more left turns than right turns on average during the observation period, and the average change in direction between 30-s bearings was 4° more for left than for right turns. Therefore, assuming a uniform radial distribution of turning frequency and magnitude, the average individual would complete a full 360° counter-clockwise change in swimming direction every 40.4 min. However, both the frequency of left and right turns and the size of turns varied with swimming direction.

Information derived from the changes between successive swimming direction bearings for each individual was examined to shed light on the results of the analysis of mean bearings. Swimming directions were divided into four quadrants (NE, SE, SW, NW). Regardless of the direction of swimming, larvae either made more left than right turns, or made larger left turns, or both. While swimming to the SE, SW and NW larvae made significantly more left than right turns, and the greatest disparity occurred while swimming to the NW (Table 3). The difference (about 2°) between the size of left and right turns made while swimming SE and SW was not significant, although the difference (5.9°) for larvae swimming NW approached significance (Table 3). In contrast, while swimming NE, larvae did not have a significant difference in the proportion of turns, but the left turns were significantly larger (6°) than the right turns. Overall, larvae swimming to the south made smaller turns that larvae swimming north. This would be expected to result in more swimming to the south than to the north, which is what was observed.

Because most larvae did not swim randomly, the sequential bearings of most individual larvae cannot be considered to be independent, and cannot be analysed by most statistical methods. So, we simply note that there were 67% more swimming bearings to the SE than to the NW (Table 3). Larvae swimming to the SE had a smaller average change in direction between 30-s bearings than larvae swimming in other directions (about a 3° difference, Table 3). Larvae swimming NW had the greatest tendency to turn left (62.6% of all turns were to the left, Table 3).

Table 2 Precision of directionality in swimming in larvae of *Amblyglyphidodon curacao*. Swimming precision is measured as '*r*' (length of the mean vector), which can reach a maximum of 1.0

Site or size class	*n*	mean *r*	*P* (T test)
Watsons Bay	57	0.73 (±0.03)	0.20
Coconut Beach	17	0.64 (±0.06)	
<9 mm	20	0.61 (±0.06)	0.07
≥9 mm	33	0.73 (±0.04)	

There was no significant difference in precision between locations or size class, however, the difference in precision between small and large larvae approached significance. Values in brackets are standard errors

Table 3 Differences between consecutive bearings taken every 30 s for larvae of *Amblyglyphidodon curacao*

Swim direction	*n*	Average change in direction	Average left turn	Average right turn (*T* test)	Turn direction left:right (%) (G test)
NE	359	19.3° (1.0)	25.9° (1.7)	20.0° (1.4) $P = 0.007$	52.8:47.2 $P > 0.10$
SE	408	16.9° (0.9)*	21.7° (1.3)	19.5° (1.6) $P = 0.27$	58.3:41.7 $P < 0.005$
SW	382	19.9° (1.2)	24.6° (1.6)	22.4° (2.0) $P = 0.40$	57.9:42.1 $P < 0.005$
NW	244	20.3° (1.5)	27.4° (2.4)	21.5° (2.3) $P = 0.09$	62.6:37.4 $P < 0.001$

N is the number of differences between consecutive bearings (in some cases the difference was zero, and only those in which there was a difference are called turns). Values in brackets are standard errors. * Significantly different from the other three directions (*T* test, $P = 0.018$). Under "Average right turn", *T* test refers to the difference in the magnitude between left and right turns. Under "Turn direction", G test refers to whether there was a significant difference from 50:50

Discussion

The larvae of *A. curacao* studied here are morphologically typical of pomacentrids of similar size (Murphy et al. 2007), yet, they are smaller and less well developed than most other settlement-stage pomacentrids (Table 4). These larvae are shorter (SL) at settlement than all but one of the 28 pomacentrid species considered by Fisher et al. (2005), and that single species, *Dascyllus aruanus*, is considerably deeper bodied than is *A. curacao*, so is, in fact, larger if not longer. For example, *A. curacao* larvae are 25% smaller in SL, 44% smaller in total lateral area and about 1–2 weeks younger than settlement-stage *P. amboinensis*, perhaps the most studied pomacentrid in the Indo-Pacific (Murphy et al. 2007). Instead, they are within 10% of the length and lateral area of similarly aged *P. amboinensis*, and both have fully developed fins. Therefore, it is of interest to determine if their behavioural performance is more similar to that of pomacentrids at settlement-stage, or at smaller, less-well developed stages. Although size had little influence on behaviour of *A. curacao* larvae we studied, this is probably to be expected with such a small range in size (8–10 mm) and stage of development.

The U_{crit} of *A. curacao* was essentially the same as that predicted by the U_{crit} versus size relationship for settlement-stage larvae found by Fisher et al. (2005): predicted 28.8 cm s^{-1} and observed 27.5. Mean U_{crit} of 8–10 mm SL *A. curacao* was about 7 cm s^{-1} faster than that of similar-sized *P. amboinensis* (Fisher et al. 2000): the latter was somewhat below the predicted speed for larvae of that size. Whether this difference is due to the developmental state of 8–10 mm *P. amboinensis* (i.e., they were not settlement stage) or to the fact that the data were from laboratory-reared, rather than wild, larvae cannot be ascertained. For settlement-stage pomacentrid larvae, Leis and Carson-Ewart (1997) did not find a significant relationship between in situ speed and size, and this is reflected in the performance of *A. curacao*—it is the smallest species in Table 4, but it has an in situ speed in the middle of the range. Nevertheless, the in situ speed of *A. curacao* is equivalent to average current speeds in the Lizard Island area (Frith et al. 1986; Leis and Carson-Ewart 2003), meaning that *A. curacao* larvae are effective swimmers sensu Leis and Stobutzki (1999). In *A. curacao*, in situ speed was 50% of U_{crit}, and a similar relationship has been found in both settlement-stage and younger larvae of most species examined to date (Leis and Fisher 2006; Leis et al. 2006a, b). The fastest individuals were 60–90% faster than average (depending on method), indicating the best performers may be able to exert considerably more influence on dispersal than average performers.

The mean speeds of which *A. curacao* larvae are capable are well above the range of speeds (0.5–5 cm s^{-1}) that heuristic models indicate can influence dispersal outcomes (Leis 2006), and they are equivalent to ambient current speeds in the Lizard Island region. Other pomacentrid larvae of similar size have similar swimming capabilities. In contrast, in other families larvae of similar sizes and state of development vary in swimming ability (see Leis 2006), but none reported thus far are as fast as pomacentrids. For larvae of 8–10 mm, for example, two sparids and a carangid have relatively fast mean speeds with U_{crit} 10–20 cm s^{-1} and in situ speed 6–11 cm s^{-1}. Slower are a sciaenid with means of U_{crit} 5–8 cm s^{-1} and in situ speed 3–4 cm s^{-1}, and an apogonid with U_{crit} of 7–10 cm s^{-1}.

Larvae of *A. curacao* have exceptional endurance, particularly considering their small size. Stobutzki (1998) found a positive correlation between size and swimming endurance in pomacentrid larvae, but the endurance we measured in *A. curacao* was as great as any pomacentrid studied by Stobutzki even though the *A. curacao* larvae were lighter than other pomacentrids at settlement, and are among the shortest pomacentrids at settlement. This is in part due to differences in methodology: our endurance tests took place at 8–10 cm^{-1}, whereas Stobutzki used 13.5 cm s^{-1}. Fish larvae can swim further if they swim slower (Fisher and Bellwood 2002), but applying the speed versus duration correction derived by Fisher and Bellwood (doubled speed cuts distance swum in half), results in a 'corrected endurance' in *A. curacao* much greater than

Table 4 Comparison of morphological and behavioural characteristics of *A. curacao* larvae with *P. amboinensis* of similar size (mid-development) and settlement-stage larvae of seven pomacentrid species (arranged by size)

Species	SL (mm)	TLA (mm^2)	PLD (days)	U_{crit} (cm s^{-1})	In situ speed (cm s^{-1})	Endurance (km)	Modal depth (WB) (m)	Modal depth (CB) (m)	Orientation (WB)	Orientation (CB)
Amblyglyphidodon curacao	9.1 (0.1)	28.9 (0.7)	11–16	27.5 (1.0)	13.1 (0.7)	52 (8.6)	2.6–5.0	7.6–12.5	SE	SE
Pomacentrus amboinensis (mid-development)	9.5 (0.4)	26.8	14–15	20.7 (3.4)	–	12 (3.5)	–	–	–	–
Chromis atripectoralis	10.3 (0.3)	32.2 (1.2)	20–21	34.7 (3.1)	25.8 (0.4)	15.4 (1.9)	2.6–7.5	5.1–7.5	SE	SE
Dischistodus prosopotaenia	10.7 (0.3)	35.3 (0.7)	17	26.9 (2.8)	10.8 (0.8)	7.3 (2.3)	–	–	–	–
Chrysiptera rollandi	11.1 (0.3)	37.1 (2.5)	12–23	24.3 (1.4)	11.7 (0.5)	18.1 (5.4)	2.6–5.0	2.6–5.0	SW (ns)	SW (ns)
Pomacentrus amboinensis	12.1 (0.2)	51.8 (1.4)	17–30	34.6 (4.4)	10.8 (1.5)	22.5 (3.6)	–	–	–	–
Pomacentrus lepidogenys	12 (–)	–	20	40.4 (3.3)	18.1 (0.9)	–	0–5.0	–	SE	–
Pomacentrus nagasakiensis	13.3 (0.2)	62.8 (1.7)	23	49.1 (4.9)	–	36.0 (10.7)	–	–	–	–
Neopomacentrus cyanomos	14.0 (0.1)	64.0 (0.9)	17–18	36.1 (2.1)	22.6 (1.1)	31.4 (6.3)	0–2.5	0–5.0	E (ns)	S

Field behaviour from the Lizard Island area: Watsons Bay (WB) and Coconut Beach (CB). Values are means (SE), except range for PLD. Standard Length (*SL*) and Total Lateral Area (*TLA*) from Leis and Clark (2005). PLD from Thresher et al. (1989), Wellington and Victor (1989), Stobutzki (1998), Bay et al. (2006). U_{crit} from Fisher et al. (2005). In situ speed from Leis and Fisher (2006). Endurance from Stobutzki (1998). Depth from Leis (2004): WB and CB had significantly different depth–frequency distributions in all cases, even where modes do not differ. Orientation from Leis and Carson-Ewart (2003): all are mean bearings and are significantly different from uniform unless marked ns. For mid-development *P. amboinensis*, U_{crit} and Endurance for larvae of 8–10 mm SL from Fisher et al. (2000); TLA for a 9.3 mm SL larva from the present study; and PLD for reared larvae of size similar to *A. curacao* from Murphy et al. (2007). Not all data are available for all species

expected for its size. The ecological meaning of swimming endurance measured in the laboratory without food or rest (Stobutzki and Bellwood 1997) is unclear, because larvae in situ do feed, and are unlikely to swim non-stop to exhaustion. So, the endurances measured this way cannot be directly applied to field situations (see Leis 2006), but it is clear the endurance of *A. curacao* of 8–10 mm SL is considerable and is apparently much greater that other pomacentrids of similar size, and many that are larger. During the endurance trial, *A. curacao* larvae lost an average of 25% of their wet weight. This is similar to the loss in dry weight found by Stobutzki (1997) in larvae of five other pomacentrid species (5–33%, mean 17%), in spite of the fact that pre-swim wet weight of *A. curacao* larvae was only 33–50% that of the other pomacentrids. In contrast, 8–10 mm SL larvae of other families (Apogonidae, Percichthyidae, Sparidae, Sciaenidae) swimming at 10 cm s^{-1} had endurance of 0–10 km (Leis 2006), whereas 8–10 mm SL larvae of a carangid had endurance of 5–17 km (Leis et al. 2006a).

Orientation in *A. curacao* larvae was similar to that of other pomacentrids. In common with other studies of orientation by fish larvae (Leis 2006), a large majority of individual *A. curacao* larvae swam directionally. Larvae of *A. curacao* had an overall mean swimming direction to the SE. Similarly, the four other pomacentrid species studied at Lizard Island also had a southerly swimming direction (Table 4), although this varied somewhat among species and location. Like *A. curacao*, both *Chrysiptera rollandi* and *Chromis atripectoralis* larvae lacked a difference in swimming direction between windward and leeward sides of Lizard Island. *Neopomacentrus cyanomos* larvae, in contrast, did swim in significantly different directions on the two sides of Lizard Island. One possible explanation for the southerly swimming direction of pomacentrid larvae at Lizard Island is that this is a response to the regional current which flows predominately from the south (Frith et al. 1986; Leis and Carson-Ewart 2003), and is an attempt to counteract current advection. Because mean in situ swimming speed was the same as mean current speed, *A. curacao* larvae of 8–10 mm SL would suffer little net displacement by currents given the mean swimming direction we observed.

Two turning-related behaviours contributed to the tendency of larvae to swim to the SE and not to the NW, as reflected both in the distribution of mean vectors and in the number of 30-s swimming directions partitioned among four quadrants. The fact that turns by larvae swimming SE were smaller than turns made when swimming in other directions means larvae already swimming SE would be more likely to continue in their original direction than would larvae swimming in any other direction. Further, because larvae swimming NW had the greatest bias in

turning direction this would result in a greater tendency for larvae swimming NW to change swimming direction than for larvae swimming in other directions. Larvae initially swimming NW would soon be swimming W to SW due to the imbalance in turns to the left. These turning behaviours help explain how a SE average swimming direction can be maintained, and because turning behaviour differs with swimming direction they further indicate that the larvae can determine and control their direction of swimming. It is unclear what the general bias toward left turns indicates, or why this should happen, but such turning may facilitate detection of and orientation by certain sensory cues, particularly if polarized light or directional sound is involved.

Although analysis of turning behaviour does not clearly implicate the sensory cues the larvae used to accomplish directional swimming, some possibilities can be eliminated from consideration. Because swimming directionality was the same on both sides of the island, island-derived cues, such as reef sound and smell, or differences in wave pattern can be eliminated from consideration (Kingsford et al. 2002), unless these cues can somehow provide a fixed reference point that would enable a larva to detect its movement in the prevailing current (see below). This leaves magnetic cues or visual cues such as a solar compass. Although the capacity to detect magnetic cues is not known in larvae of marine fishes, it has been demonstrated in juvenile salmon and adults of several other marine fish species (Kingsford et al. 2002) and thus remains a possibility. In larvae of other pomacentrid species, observation of more westerly swimming directions in the late afternoon than in the morning implied that a solar compass was involved in orientation (Leis and Carson-Ewart 2003), and use of solar compass by juvenile fishes is documented (Kingsford et al. 2002). Unfortunately, we do not have information on orientation of *A. curacao* larvae late in the day to test this hypothesis. Because the larvae swam into the prevailing current, it is conceivable that they detected this current, and were responding to it. However, small, pelagic animals swimming in a moving water column would not be expected to be able to detect a current (Kingsford et al. 2002) without a fixed reference point (e.g., a view of the bottom, or a directional sound cue). Although fish larvae can hear and can localize a sound source over modest spatial scales (e.g., Leis et al. 2002; Simpson et al. 2005; Wright et al. 2005), there are no data on whether fish larvae can detect a remote sound source, and use this as a fixed reference to sense a current. Clearly, the sensory abilities and cues used for orientation by larval marine fishes require further investigation.

In common with larvae of most other species studied at Lizard Island, larvae of *A. curacao* swam deeper off Coconut Beach than in Watsons Bay (Leis 2004). Modal depth was about 10 m deeper off Coconut Beach than in Watsons Bay. This is consistent with results from other locations: larvae swim deeper where the water column is deeper, even though they do not closely approach the bottom in more shallow water (Leis and Carson-Ewart 2001). Presumably, a view of the bottom or a difference in the quality or quantity of light reflected off the bottom was involved in this behaviour. Individuals differed in the way they moved vertically (amplitude, changes in vertical direction, etc), and there was not a consistent influence of size of the larvae on vertical distribution behaviour. Ontogenetic differences in vertical distribution are known in a number of species (see review in Leis 2006), but the fact that the effect of size was not consistent at the two locations in this study means that such differences could vary spatially. On the other hand, the narrow size range of the larvae we studied would make detection of ontogenetic differences in vertical distribution difficult. In Watsons Bay, divers observing *A. curacao* larvae frequently had the impression that the larvae descended until the bottom was visible, and then either levelled off or ascended, whereas, off Coconut Beach the bottom was never clearly visible to the divers. Although this anecdotal information must be treated cautiously, particularly as it is not known how the visual capabilities of larvae compare with those of divers, it is possible that *A. curacao* larvae have a preferred depth, but they may not descend to it once the bottom is visible. If so, the preferred depth might be achieved off Coconut Beach, but not in the somewhat shallower Watsons Bay.

The behaviour of *A. curacao* larvae would have a strong influence on their distribution and dispersal. The larvae swam at speeds equivalent to average local currents in an orientated way. In fact, they avoided swimming with the mean flow, rather swimming either into it or across the flow. Our laboratory measurements show these larvae have the ability to swim about twice as fast as they do swim in the sea, and have the capacity to swim at similar speeds for many hours covering tens of kilometres.

In many situations, current speed and direction differ with depth, so vertical distribution behaviour can have a strong indirect influence on dispersal. The currents in the immediate vicinity of Lizard Island are more vertically structured off the leeward (i.e., Watsons Bay) side. In Watsons Bay surface flows are faster and more downwind (i.e., away from the island) than are deeper flows, whereas off Coconut Beach, there is little difference in current speed or direction with depth (Leis 1986). These spatial differences in the vertical structure of currents, interacting with the spatial differences in vertical distribution behaviour documented here have the potential to influence dispersal outcomes in different ways on the two sides of the island. Further, the bottom was frequently visible (to the divers) from the depths occupied by the larvae at Watsons

Bay, but not off Coconut Beach, and this opens the possibility that the larvae might use the bottom as a point of reference for vertical or horizontal orientation, but only at Watsons Bay.

As they approach settlement, larvae of *A. curacao* are morphologically less developed and younger than are larvae of many other pomacentrids, but they seem behaviourally equivalent to the older, larger larvae. Compared to the older, larger larvae of other pomacentrids, larval *A. curacao* can swim nearly as fast and possibly further, and orientate as well both vertically and horizontally. Therefore, they seem to have equivalent ability to influence dispersal trajectories. Given that *A. curacao* we studied were essentially morphologically equivalent to the presettlement larvae of the other species of similar age and size, it seems likely that the presettlement larvae of these other species will also have strong swimming and orientation abilities, and therefore, similar ability to influence their dispersal. Our work based on wild *A. curacao* larvae corroborates the relatively small amount of information available on the swimming abilities of young, small pomacentrid larvae reared in the laboratory (Fisher et al. 2000; Fisher and Bellwood 2001), and it provides a first insight into other behavioural abilities (orientation and vertical distribution) of younger, smaller pre-settlement pomacentrid larvae.

A final point concerns the fact that our still pelagic larvae of *A. curacao* from the GBR were both larger and older than settled individuals of *A. curacao* from Palau (Wellington and Victor 1989). Within-species regional differences in PLD have been reported in other pomacentrids (Bay et al. 2006), and as size is usually correlated with age, one would also expect to find regional differences in size at settlement.

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