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REPORT

The effects of parasites on the early life stages of a damselfish

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Abstract Early life history traits, such as larval growth, influence the success of coral reef fish in the transition from the larval to the juvenile life phase. Few studies, however, have examined the relationship between parasites and growth in the early life history stages of such fishes. This study examined how parasite prevalence (% infected) and load, and the relationship between parasite presence and fish growth, differed among life stages of the damselfish *Pomacentrus amboinensis*. Parasite prevalence decreased significantly between the larval stage, which was sampled immediately before settlement on the reef (97 %) and recently settled juveniles (60 %); prevalence was also high for 4-month-old juveniles (90 %), 7-month-old juveniles (100 %) and adult fish (100 %). Total numbers of parasites per fish decreased dramatically (fourfold) between larval and recently settled fish, and then increased in the older stages to levels similar to those observed in larvae, but they did so more gradually than did prevalence. One explanation for these patterns is that heavily infected larvae were preferentially removed from the population during or soon after settlement. Daily fish growth, determined from otolith increments, revealed that growth did not differ between parasitised and non-parasitised larval fish, whereas recently settled fish that were parasitised had faster growth; these parasitised recently settled fish also displayed faster growth prior to settlement. These data provide evidence that parasites may explain some of the variation in growth and survival observed among coral reef fishes after settlement and thereby have a greater impact on population dynamics than previously understood.

Keywords Parasites · Otolith growth · Fish larvae · Settlement · Coral reef ecology

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Introduction

Nearly all coral reef fishes have a complex life cycle consisting of a pelagic larval stage followed by a relatively sedentary reef-associated juvenile/adult stage (Leis and Carson-Ewart 1998). The pelagic larval phase and the period immediately after settlement are the times at which the greatest mortality occurs. Survival during the larval stage and at settlement is extremely low for marine fish (Leis 1991), and it has been estimated that as many as 55.7 % of juvenile reef fish die with the 1–2 days of settlement (Almany and Webster 2006). Despite this, little is known of the relative importance of the processes that influence survival. Early life history traits not only influence larval survival during settlement, but can also influence performance and survival in later life stages (e.g., Hoey and McCormick 2004; Gagliano et al. 2007; Grorud-Colvert and Sponaugle 2011).

Although predation is regarded as the major cause of mortality before and after settlement (Holmes and McCormick 2006), it is apparent that many factors contribute to predation susceptibility by affecting larval traits. These factors may include maternal effects such as variation in endogenous reserves (Gagliano and McCormick 2007a) and

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environmental factors such as food availability, temperature and water currents (Green and Fisher 2004; Gagliano and McCormick 2007b), all of which are known to also affect growth. For fishes, variable larval growth has the potential to drive not only the age and size at settlement, but also the number of settling fish and the rate of survival (McCormick and Molony 1992). Although several studies have examined the effects of coral reef fish larval history on survival during and after settlement (McCormick and Hoey 2004; Bergenius et al. 2005; Gagliano et al. 2007), studies on the effects of parasites during these life stages are rare.

Although parasites play important roles in coral reef ecosystems, they have long been regarded as being a neglected group when it comes to quantifying biodiversity. The neglect is explained by their small size, the fact that they are hidden on or within a host, and the need for more complex preparation than is required for many other groups of animals (Justine et al. 2010). There is abundant evidence that parasites are highly abundant in the animals of coral reefs (e.g., Rigby et al. 1999; Cribb et al. 2000; Justine 2010; Miller et al. 2011) but most species have not been studied beyond establishing their basic taxonomy and host distribution. Coral reef fishes from the Great Barrier Reef have been known to be infected with the following groups of metazoan parasites: Turbellaria, Monopisthocotylea, Polyopisthocotylea, Digenea, Cestoda, Nematoda, Copepoda, Isopoda, Acanthocephala and Hirudinea (Grutter 1994; Miller et al. 2011). It is well known that the community and abundance of parasites on hosts can vary according to the habitat (Marcogliese 2002). Because larvae and recently settled coral reef fishes inhabit such different environments, it is also likely that their parasites also vary. Such information is important for understanding the ecology of parasites and young fishes as this can provide insights into the different habitats that parasites may occupy, as well as how fish at different developmental stages might be affected differently by the parasites.

Despite what is known for adult reef fish, few studies have examined the parasite fauna of larval and juvenile coral reef fishes (Rigby and Dufour 1996; Cribb et al. 2000; Penfold et al. 2008; Grutter et al. 2010). Even less is known about their ecological role (Finley and Forrester 2003; Forrester and Finley 2006), with the exceptions of some gnathiid and cymothoid isopods (Adlard and Lester 1995; Grutter 1999; Fogelman and Grutter 2008). Although gnathiids clearly have the potential to play a role in the ecology of young fishes, studies on the effects of other, and possibly more common, parasites are also needed. Parasites of fishes are known to impair performance, affect reproduction and behaviour, increase predation risk and sometimes cause direct mortality (Seppälä et al. 2004; Macnab et al. 2009). Parasites thus have the potential to regulate host populations (Scott and Dobson 1989).

Little is known of the ecological impacts of parasites on fishes around the time of settlement, apart from those of haematophagous gnathiid isopods on damselfishes. Settling *Neopomacentrus azysron* infected with one or three gnathiids had a mortality of 12 and 16 %, respectively (Grutter et al. 2008). Gnathiid isopods killed *A. polyacanthus* juveniles in 6.3 % of infections, but only in fish <10 mm standard length (SL) (Penfold et al. 2008). Settling *P. amboinensis* previously infected with a gnathiid swam more slowly, had higher oxygen consumption and had lower survival in the field than uninfected individuals (Grutter et al. 2011). Only 3.5 % of juvenile *P. amboinensis* were infected with gnathiids in the wild at any one time (Grutter et al. 2011). However, like mosquitoes on land, their presence on fish is ephemeral, as they drop off quickly after feeding on blood, only to be replaced by new ones. Thus, their ecological impact may be considerably greater than suggested by prevalence alone.

The effects of other more common parasites, including endoparasitic trematode worms, on young fishes are poorly understood. Parasite prevalence (mostly trematodes) increased with age in *P. moluccensis*, with the greatest increase occurring immediately after settlement (Grutter et al. 2010). In one of three cohorts, fish with endoparasites had lower growth from time of hatching and settled about 2 days later than non-parasitised fish. There have been no analyses of the passive role and impact of other kinds of endoparasites, especially larval trematodes, nematodes and cestodes.

This study examines the role of parasites in influencing the vulnerable but critical life stage immediately before and after settlement to the reef population. The aim of this study was to a) identify and quantify the parasites of fish in the early life history stages and b) determine the relationship between parasite presence and fish growth in the early life stages of the damselfish *Pomacentrus amboinensis.*

Materials and methods

Host collection

Pomacentrus amboinensis were collected from Lizard Island, GBR, Australia (14°40′S, 145°28′E), between November 2004 and April 2009. Larval fish ($n = 203$) were collected between 9 November and 23 December 2004 using light traps following Meekan et al. (2001). Traps were moored off the reef edge, about 500 m from the shore, and between 1800 h and 0700 h each day. Recently settled fish were collected between 24 December 2004 and 18 January 2005, and juvenile fish were collected approximately 4 months later, between 30 March to 1 April, and 7 months later, between 26 and 29 July 2005. Recently

settled and juvenile fish were captured with a hand net, immediately placed in individual quick-sealing plastic bags measuring 15 cm × 9 cm and transported in a slurry of ice and water (which euthanised the fish) to the research station. Specimens were preserved in 5-mL vials containing 80 % ethanol. Fish and the contents of the plastic bag were examined under a dissecting microscope (70–100X) for parasites.

To identify parasites using DNA analyses, a further 14 juveniles and 28 adult fish were collected from Lizard Island between 14 and 24 April 2009. These fish were caught using hand nets, barrier nets or spears. Fish caught using nets were placed individually into quick-sealing plastic bags and were transported as above. Some individuals were kept alive until dissection by holding them in aquaria with running seawater. The fish were dissected on the island in order to retrieve fresh parasite samples for DNA analysis. Parasites were either placed directly into 100 % ethanol for DNA analysis or were fixed for morphological identification by placing them in vertebrate saline just off the boil and then transferring them to Eppendorf tubes containing 10 % formalin.

Parasite identification

Each fish was examined for ecto- and endoparasites. Fish were placed in vertebrate saline. Standard length (SL) and total length (TL), both measured to the nearest 0.5 mm, and fish weight, measured to the nearest 0.001 g were recorded. The body surface of the fish was scanned at 20× magnification. The fins were removed and scanned. The gills were examined for parasites or cysts embedded in the gill lamellae. The contents of the gut cavity were removed and examined. For the older juveniles and adult fish, a "gut wash" (Cribb and Bray 2010) was conducted in an attempt to dislodge attached internal parasites. The body flesh was then blended using a handheld blender in order to release metazoan tissue parasites such as metacercariae (larval trematodes). Parasites were identified to species level where possible; when this was not feasible, they were placed in individual vials according to morphology, containing either 100 % ethanol for DNA analysis or 10 % formalin for morphological identification.

For morphological identification, preserved digenean specimens were stained in Mayer's haematoxylin, destained in 1 % HCl, neutralised with 1 % ammonia, dehydrated through a graded series of ethanols, cleared in methyl salicylate and then mounted on slides in Canada balsam. Copepod specimens were cleared in lactic acid for 24 h before being stained using Chlorazol Black and viewed under a compound microscope in glycerol.

DNA analysis

Parasite samples were removed from the ethanol and then re-suspended in a TE buffer overnight at 39 °C. DNA was extracted from single specimens using the phenol–chloroform procedure following Sambrook et al. (1989). The ITS2 rDNA region was amplified in a 20 µl reaction; for adult parasites, this consisted of 1.6 µl $MgCl_2$ (25 mM), 2 µl of PCR buffer (Promega) (10×), 0.8 µl dNTPs (5 mM), 0.75 µl of each primer (forward and reverse) (10 pmol), 0.25 µl of Taq polymerase (Promega) (5 units/µl) and 2 µl of DNA template (5–100 ng), and the remaining volume of distilled (DNA and RNA free) water. The same amount of solution was used for larval worms; however, 5 µl of DNA template was added. The forward primer used was "3S" (5′-GGT ACC GGT GGA TCA CGT GGC TAG TG-3′) (Anderson and Barker 1993) and the reverse primer was "ITS2.2" (5′-CCT GGT TAG TTT CTT TTC CTC CGC-3′) (Cribb et al. 1998). The amplification reactions were run using a MJ Researcher thermal minicycler (Bresatec) using the following temperatures and cycles: 95 °C for 3 min, 45 °C for 3 min and 72 °C for 90 s; 4 cycles of 95 °C for 45 s, 50 °C for 45 s and 72 °C for 90 s; 8 cycles of 95 °C for 20 s, 52 °C for 20 s and 72 °C for 90 s; and a final extension period at 72 °C for 5 min followed by a final holding temperature of 4 °C. Amplified DNA was purified using a QIAquick™ PCR purification kit (Qiagen) according to the manufacturer's protocol (Miller and Cribb 2007), before they were sequenced using ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction kit. Consensus sequences were constructed using the program *Sequencher*™ version 4.5 (Gene Codes Corp). Sequence alignments were performed using Clustal W™. Comparisons of parasite sequences obtained from adult and larval fish were performed using a range of clustering techniques including neighbour joining in Mega4 (Tamura et al. 2007).

Otolith analysis

Sagittal otoliths were processed following Wilson and McCormick (1997). Images of otoliths were taken using a SPOT Insight™ digital camera (Diagnostic Instruments, Inc. Michigan, USA) mounted on an Olympus BH-2 compound microscope using SPOT Advance ™ imaging software. Fish age (days) was estimated by counting the number of daily increments, starting from the nucleus to the margin. The width of each increment (growth) from the nucleus along the longest axis of the otolith to the margin was measured from images using the software Optimas version 6.5 (Media Cybernetics, Silver Spring, MD, USA). The settlement date of recently settled fish was determined by calculating the difference in growth from one increment

to the next and assuming the greatest decrease was the day fish had settled onto the reef (Wilson and McCormick 1999).

Statistical analyses

A Pearson Chi-square goodness-of-fit test was used to determine the differences in parasite and bucephalid metacercariae prevalence among fish groups. Statistical analyses involving ontogenetic stages were only conducted for larval, recently settled, 4- and 7-month-old fish, but not adults, as these stages were caught in the same year. As there was no significant difference in parasite prevalence between larval fish collected in November (98.2 %) and December (97.8 %) ($\chi^2 = 0.416$, $df = 1$, $P = 0.5196$), these fish were pooled for analyses. Due to the expected values in more than 20 % of cells being fewer than 5 when comparing total parasite prevalence among the four ontogenetic stages, which violated the assumptions of the Pearson Chi-square goodness-of-fit test (Zar 1999), the total parasite prevalence of 4- and 7-month-old fish were pooled. Generalised Linear Models (GLMs) assuming either a normal or a Poisson error distribution were used to test for differences in the six responses measured: otolith increment at hatching, cumulative otolith increment at settlement, age at settlement, SL at capture (Normal distributions), total number of parasites and number of metacercariae (Poisson distribution), among the ontogenetic stages (fixed factor). *Kudoa* sp. was excluded from analysis as it is a spore-forming parasite; therefore, it cannot be counted as being one parasite. The assumptions of normality and homogeneity of variance were examined using the Shapiro–Wilk test and the O'Brien test, respectively. These statistical analyses were conducted using JMP IN version 9 (SAS Institute Inc, USA).

Growth curves were created for larval and recently settled fish up to settlement and at capture, to test for the effect of parasitism on the growth of early life history stages. Only larval and recently settled fish had sufficient parasitised and non-parasitised fish to allow a formal test of the effect of parasite presence on growth-related attributes. As the relationship between otolith increment number (i.e., days since hatching) and parasitism was nonlinear for all these stages, nonlinear mixed effect models were fitted, with a three-parameter logistic model found to have the best fit (other models tested included a Generalised Linear Mixed Model, Gompertz Growth Curve and a Four-Parameter Logistic Model). For recently settled fish, growth was analysed from time of hatching to the time of settlement on the reef (for comparison with larval fish sampled at time of settlement) and from time of hatching to time of capture. We assumed that otolith growth corresponds closely to somatic growth (Campana 2011). The analyses involved comparing the asymptote (maximum growth reached) and x-mid [the x value at the inflection point or tangent of this point (half way) and represents the maximal growth rate] of the curves between parasitised and non-parasitised fish for each ontogenetic stage. The three-parameter logistic models were fitted to the otolith growth data using the functions "SSlogis" and "nlme" in R version 2.9.2 (R Development Core Team 2009). The function for the three-parameter logistic model is shown in Eq. 1, where y (x) is the cumulative otolith width at time x. ϕ_1 is the asymptotic height, ϕ_2 is the x-mid and ϕ_3 is the scale parameter that represents the distance on the x-axis between the x-mid and the response (Pinheiro and Bates 2000).

$$y(x) = \frac{\phi_1}{1 + \exp[(\phi_2 - x)/\phi_3]} \quad (1)$$

Growth curves were visualised by plotting the mean (±SE) of each cumulative increment (day) for all individuals.

Results

Ontogenetic changes in parasite assemblage

The parasites encountered belonged to five classes: Maxillopoda (copepods), Cestoda, Turbellaria, Trematoda and Monogenea (Table 1). Additionally, a myxozoan infection of a *Kudoa* sp. was found in the heart of a single adult *P. amboinensis* (SL 55 mm). Worm length, oral sucker size and the terminal genitalia of the parasites were used to identify the worms in the gut. Gut worms found in the larval fish were all immature and could only be identified as hemiurid metacercariae (distinct from other species) by the presence of an ecsoma (Table 1).

In all fish stages, cestodes found in the gut were metacestodes; however, they could only be identified to Order Tetraphyllidea. Monogeneans were found attached to the gills of all fish stages. These were not identified to species due to lack of morphological features. Turbellarians were also not identified further due to a lack of any distinct internal features. Two copepod species found on 4- and 7-month juvenile fish (SL 25.5 and 42.3 mm, respectively) were identified as *Hatschekia crenulata* (Hatschekiidae) and *Peniculisa bellwoodi* (Pennellidae). A third copepod type, also found on juvenile fish, could not be identified as all specimens collected were immature (G. A. Boxshall, pers. comm.).

ITS2 rDNA sequences that were obtained from the five trematode metacercariae showed that they all belonged to the Bucephalidae. One sequence (in an adult fish, SL 62 mm) was identified as relating to *Prosorhynchoides lamprelli,* the adult of which infects carangid fishes (Bott

Table 1 Host size, various measures of the parasites and fish sample size of larval, recently settled, juvenile and adult stage *Pomacentrus amboinensis* from Lizard Island

	Larval fish		Recently settled		4-month Juvenile fish		7-month Juvenile fish		Juvenile fish[a]		Adult fish[a]		
SL (Mean ± SE)	11.35 ± 0.04		14.28 ± 0.26		25.49 ± 0.69		27.67 ± 0.97		42.27 ± 1.84		65.13 ± 1.37		
Total parasites (Mean ± SE)	8.22 ± 0.28		2.0 ± 0.40		3.61 ± 0.73		9.03 ± 0.75		6.07 ± 1.08		7.54 ± 0.83		
Total parasite prevalence (%)	97 %		60 %		90 %		100 %		92 %		100 %		
	$n = 203$		$n = 100$		$n = 31$		$n = 29$		$n = 14$		$n = 24$		
Parasite category	% (Range)	Mean ± SE	% (Range)	Mean ± SE	% (Range)	Mean ± SE	% (Range)	Mean ± SE	% (Range)	Mean ± SE	% (Range)	Mean ± SE	Total fish infected
COP: *Hatschekia crenulata*							24.1 (1.2)	1.6 ± 0.7			8.3 (1)		6
COP: *Peniculisa bellwoodi*					19.3 (1–4)	1.6 ± 1.1	10.3 (1–2)	1.5 ± 0.8					9
COP: Unidentified			3 (1)		6.5 (1)		7 (1)						4
COP: Copepodite					9.7 (1)				28.6 (1–2)	1.5 ± 0.8			10
ISO: Cymothoidae	0.5 (1)												1
ISO: Gnathiidae			3 (1)										2
MYX.: *Kudoa* sp.											4.2[b]		1
CES: Tetraphyllidea metacestode	2.5 (1)						3.4 (1)		28.6 (1)		17 (1)		15
TUR: Turbellarian sp. unid.	2.5 (1–2)	2 ± 0.6	25 (1–7)	1.8 ± 0.3	3.2 (1)		3.4 (1)		14.3 (1)				45
TRE: *Aponurus laguncula* (A)[c]											8.3 (1)		2
TRE: *Hysterolecitha heronensis* (A)													2
TRE: *Hysterolecitha nahaensis* (A)[c]					6.5 (1)				14.3 (1–2)	1.5 ± 0.5	4.2 (1)		3
TRE: *Lecithaster stellatus* (A)[c]									7.1 (1)		8.3 (1)		3
TRE: *Thulinia microrchis* (A)											8.3 (1)		2
TRE: *Derogenes pearsoni* (A)[c]											4.2 (1)		1
TRE: *Lepotrema monile* (A)[c]											4.2 (1)		1
TRE: Bivesiculidae sp. unid. (J)											4.2 (1)		1
TRE: Lecithasteridae sp. unid. (J)					22.6 (1–2)	1.1 ± 0.1	41.4 (1–4)	2.2 ± 0.5	21.4 (1–3)	1.7 ± 0.7			25
TRE: Hemiurid metacercariae (J)	22.8 (1–7)	1.3 ± 0.1	6 (1)										52
TRE: Bucephalid metacercariae (M)	89.7 (1–13)	6.3 ± 0.2	23 (1–11)	4.6 ± 0.6	29 (1–7)	3.2 ± 0.8	66 (1–13)	5.7 ± 0.9	64.3 (1–5)	3.8 ± 0.6	41.7 (1–7)	3.3 ± 0.8	242
TRE: Flesh metacercariae (M)			6 (1–4)	1.5 ± 0.5	19.4 (1–7)	2.5 ± 1	55.2 (1–9)	3.2 ± 0.7	7.1 (1)		41.7 (1–8)	2.4 ± 0.7	34
MON: sp. unid.	3.4 (1–2)	1.1 ± 0.1	13 (1–13)	2.3 ± 0.9	22.6 (1–2)	1.3 ± 0.2	6.9 (1–3)	2.5 ± 0.5	36 (1–3)	1.8 ± 0.5	54.2 (1–19)	4.8 ± 1.3	47

Measures for each parasite category are total parasite prevalence (%), range of parasites per infected host and mean (standard error or SE) number of parasites per infected fish (given only for parasite categories with relatively high infection prevalence)

COP copepods, *ISO* isopods, *MXY* myxozoa, *CES* cestodes, *TUR* turbellarian, *TRE* trematode, *MON* monogenean, *A* adult, *J* juvenile, *M* metacercariae

[a] Juvenile and adult fish were collected from Lizard Island in April 2009

[b] Not possible to give an accurate range, as it is a spore-forming parasite

[c] New host record

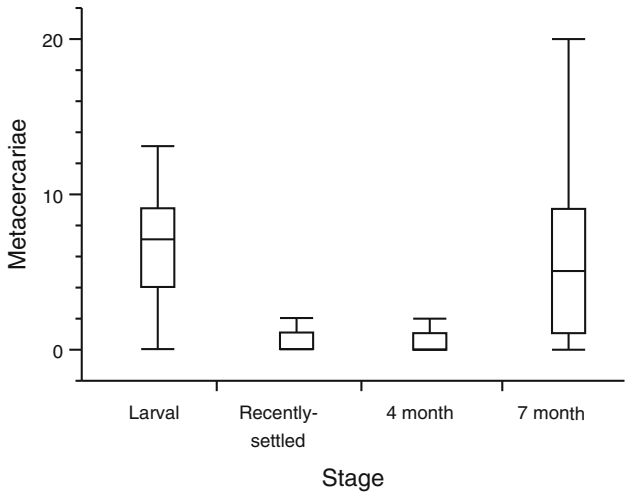

Fig. 1 The *box* whisker plots: *boxes* indicate 25th and 75th quantiles, *central line* in *box* is the median, whiskers are the minimum and maximum distribution values of the number of metacercariae per *Pomacentrus amboinensis* for four fish ontogenetic stages. See "Materials and methods" for details on stages

and Cribb 2005). Two sequences (in a juvenile and adult fish, SL 47 and 67 mm) were identical and matched an unidentified *Dollfustrema* found in a muraenid as an adult and from a range of fish families as metacercariae. One sequence (adult fish, SL 65 mm) matched a second unidentified *Dollfustrema* species, which infect muraenids as an adult with infective metacercariae stages found in several fish species. The last sequence (adult fish, SL 60 mm) had not been encountered previously, but could be identified as a bucephaline. As the sample of five metacercariae revealed four different species, it is likely that the metacercarial fauna comprises a diverse range of meta cercariae.

Ontogenetic changes in parasite infection

Total parasite prevalence differed significantly among the three ontogenetic stages ($\chi^2 = 82.38$, $df = 2$, $P = < 0.0001$). This was largely due to the low prevalence of parasites in recently settled fish compared with the other fish groups (Table 1). Bucephalid metacercarial prevalence differed among larval, recently settled, 4- and 7-month fish stages ($\chi^2 = 147.76$, $df = 3$, $P = < 0.0001$) with the prevalence highest in larval fish and the lowest in recently settled fish (Table 1).

The total number of parasites per fish also differed among the four ontogenetic stages for which statistical comparisons could be made (GLM, $\chi^2 = 1015.68$, $df = 361$, $P = < 0.0001$) (Table 1).

The number of metacercariae per fish differed among the four ontogenetic stages of fish (GLM, $\chi^2 = 1{,}086.66$, $df = 360$, $P < 0.0001$). Larvae had a relatively high mean abundance of metacercariae per fish, which decreased dramatically in recently settled (Fig. 1).

Effect of parasites on growth-related attributes

Otolith analysis revealed that larval fish hatched in the period 22–24 October 2004, whereas the recently settled fish hatched in the period 9–11 December 2004. There were no significant differences between parasitised and non-parasitised fish in growth-related attributes (Tables 2, 3).

Growth rates of larval and recently settled parasitised and non-parasitised fish

For larval fish, a three-parameter logistic model (Eq. 1) of the cumulative otolith increment width (a proxy for fish size) over time (days since hatching) revealed no significant differences in the maximal otolith growth rate (see Fig. 2; dotted line) ($t_{1,60} = -0.70$, $P > 0.48$), nor in the maximum growth reached (see Fig. 2; long-dashed line) ($t_{1,60} = 0.27$, $P < 0.78$) between parasitised and non-parasitised larval fish. By contrast, the maximal otolith growth rate for recently settled fish differed significantly between parasitised and non-parasitised fish (see Fig. 3; dotted line) ($t_{1,40} = -9.42$, $P < 0.0001$), whereas, similarly to the larval fish, the maximum growth reached (see Fig. 3; long-

Table 2 The means (±SE) of several measures of fish growth, age and standard length for parasitised and non-parasitised larval and recently settled *Pomacentrus amboinensis*

	Larval		*P*	Recently settled		*P*
	Parasitised	Non-parasitised		Parasitised	Non-parasitised	
Otolith increment at hatching (μm)	4.86 ± 0.18	4.41 ± 0.26	0.8318	4.51 ± 0.19	4.06 ± 0.2	0.2740
Cumulative otolith increment at settlement (μm)	227 ± 1.11	218 ± 4.06	0.3079	225 ± 4.60	220 ± 7.08	0.2351
Age at settlement (days)	21 ± 0.15	21 ± 0	0.8458	21 ± 0.38	22 ± 0.31	0.5095
Age at capture (days)	Same as above	Same as above		31 ± 1.17	31 ± 1.60	0.9685
SL at capture (mm)	11.4 ± 0.05	12 ± 1	0.7719	14.2 ± 0.43	14.2 ± 0.7	0.9609
Cumulative otolith increment at capture (μm)	Not applicable	Not applicable		312.94 ± 8.5	290.72 ± 13.4	0.1491

Table 3 The means (±SE) of several measures of fish growth, age and standard length for all ontogenetic stages, *Pomacentrus amboinensis*

	Larval	Recently settled	4 month	7 month
Otolith increment at hatching (μm)	4.84 ± 0.17	4.35 ± 0.14	8.35 ± 0.24	8.77 ± 0.44
Cumulative otolith increment at settlement (μm)	227 ± 1.1	224 ± 3.86	190 ± 7.77	191 ± 9.34
Age at settlement (days)	21 ± 0.15	21 ± 0.28	20 ± 0.31	21 ± 0.45
SL at capture (mm)	11.3 ± 0.77	14.2 ± 1.61	25.4 ± 1.96	27.6 ± 2.26

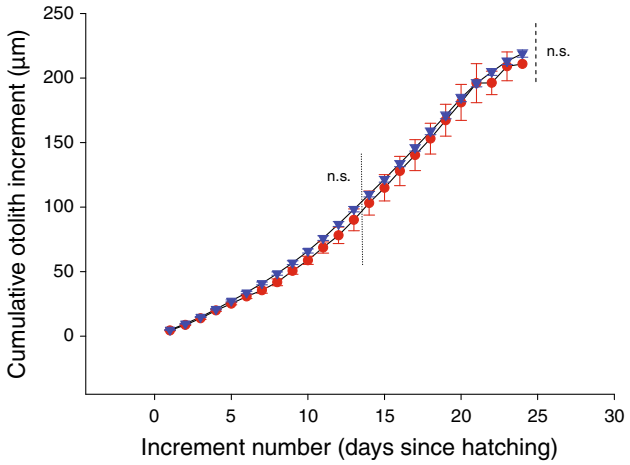

Fig. 2 The cumulative otolith increment growth of larval *Pomacentrus amboinensis* (mean ± SE) for parasitised (*blue triangles*) and non-parasitised (*red circles*) fish. *Dotted line* = the maximal growth rate, *long-dashed line* = maximum growth rate, n.s. = $P > 0.05$

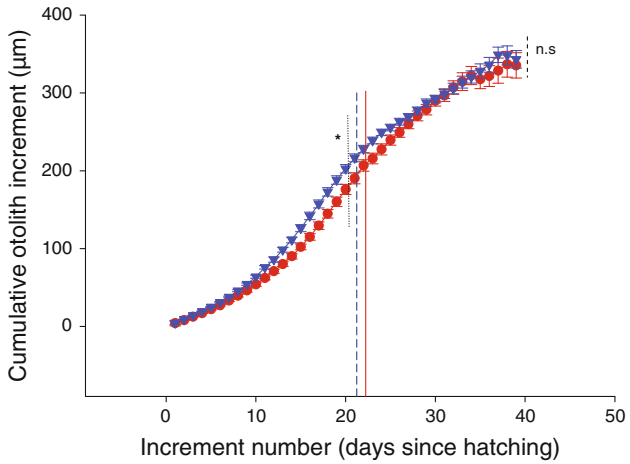

Fig. 3 The cumulative otolith increment growth of recently settled *Pomacentrus amboinensis* (mean ± SE) for parasitised (*blue triangles*) and non-parasitised (*red circles*) fish, up to capture. *Vertical lines* represent the mean age at settlement for parasitised (*red solid line*) and non-parasitised (*blue dashed line*) recently settled fish. *Dotted line* = the maximal growth rate, *significance of maximal growth rate at $P < 0.05$, *long-dashed line* = maximum growth rate, n.s. = $P > 0.05$

dashed line) did not differ ($t_{1,40} = 1.27$, $P > 0.203$) between parasitised and non-parasitised fish. This indicated that recently settled parasitised fish had faster growth than non-parasitised fish in the early period of their lives but that this difference had disappeared by the time the fish were captured.

Discussion

This study demonstrates that parasites may explain some of the variation in growth in the early life stages of a coral reef fish. These data suggest that parasite interaction is more complex and is of greater ecological importance than presently understood.

Ontogenetic changes in parasite loads

The parasite assemblage and prevalence of parasites in *P. amboinensis* at Lizard Island changed markedly through ontogeny in that parasite richness first declined and then increased over time. This pattern was repeated in the total number of metacercariae (high in larvae then reduced in recently settled fish). Metacercariae are larval stages of worms, living encysted in fish until the host is consumed and the worm is transmitted to the definitive host (Lafferty 1999). These patterns are in contrast to those of *P. moluccensis* sampled at the same location in two lunar pulses; larval fish had low parasite prevalence when sampled in December 2003 and January 2004 (4 and 0 %), which increased dramatically to 34 and 56 % in recently settled fish, then 42 and 49 % for juvenile fish (Grutter et al. 2010). The difference in parasite prevalence with ontogeny, in particular the low prevalence prior to settlement in *P. moluccensis*, may explain the differences in the relationship between growth and parasites between recently settled *P. moluccensis* and *P. amboinensis* (Grutter et al. 2010).

The high parasite prevalence reported here for larval fish is also at variance with the few other previous studies. In French Polynesia, Rigby and Dufour (1996) found just 4 % of settling groupers (Epinephelinae) were infected with trypanorhynch and phyllobothriid metacestodes. In New Caledonia, Cribb et al. (2000) found a combined parasite

prevalence of 23 % for 13 parasite species in a wide range of recruiting coral reef fish species. Although fluctuations in metacercarial prevalence have been demonstrated in freshwater systems (Chubb 1979), this has yet to be investigated in the marine environment. Overall it appears that the total prevalence of 95 % recorded for this cohort of larval fishes is exceptional.

Although metacercarial intensity was high within larval fish (range 1–13 parasites), the richness and intensity of all other types of parasites was low. In particular, no reef-based parasites (e.g., gnathiid isopods) were observed, except for a single cymothoid isopod whose reef-association is unknown. These results are thus consistent with the hypothesis that parasitism of fish at the larval stage facilitated the evolution of the pelagic larval phase (Strathmann et al. 2002). By having a pelagic phase, larval fish have less exposure to parasites with benthic connections than if they remained resident on reefs for their entire lives.

A possible explanation for the decrease in the parasite prevalence seen among larval and recently settled fish at settlement is increased mortality of infected fish. Fish parasites can cause host mortality, either directly by killing the host or indirectly by affecting its performance, for example, swimming ability and metabolism (Grutter et al. 2011)) and behaviour, and thus increasing the risk of predation (Barber et al. 2000; Grutter et al. 2011). Mortality may also occur when infestation is exceedingly high. The effects of parasites on their host depend on the type of parasites. Little is known about the effects of endoparasitic worms found in the gut compared to ectoparasites. However, parasites found in the gut are known to compete with their host for nutrients (Rosenthal 1967) thus causing damage to the gut mucosa through attachment and feeding (Ivanchenko and Grozdilova 1981), which may reduce survival. Sirios and Dodson (2000) showed that larval rainbow smelt *Osmerus mordax* infected with cestodes worms found in the gut, ingested less food and were smaller than non-parasitised individuals. These cestode worms are able to occupy up to 60 % of the digestive tract. Many studies show that parasite-mediated alteration of the behaviour or appearance of the intermediate host enhances transmission by increasing the susceptibility of the host to predation by the next host in the life cycle (Lafferty 1999). Indeed, trematode metacercariae are notorious for affecting fish behaviour (Shirakashi and Goater 2005; Seppälä et al. 2008). A plausible interpretation of the change in metacercarial prevalence between the larval and recently settled fish seen here is thus that parasitised fish were selectively removed from the population during or soon after settlement, as a result of altered behaviour that increased predation. Metacercariae located in the flesh or fins are thought to use host-derived energy in order to remain functional (Ondračková et al. 2004). Infected hosts may therefore need increased foraging to compensate for energy loss. Thus, the low prevalence of metacercariae seen in recently settled fish could be due to heavily infected individuals having been removed by predation. Notably, if increased predation of infected fishes does occur, it may not enhance parasite transmission in this system because the species involved may have their adults in pelagic fishes whereas it is presumably resident reef piscivores that eat settling fish larvae.

Growth rate of parasitised and non-parasitised larval and recently settled fish

The growth of larval *P. amboinensis* did not vary between parasitised and unparasitised fish. Neither maximal nor maximum growth at time of capture differed between parasitised and non-parasitised individuals. Because larval fish were captured with light traps just prior to settlement, and overwhelmingly (203 of 204 individuals) did not have parasites that could have infected them whilst in the light traps (Jones et al. 2007), it is inferred that they became infected with parasites while in the pelagic environment. This suggests that the growth of *P. amboinensis* is not affected by "pelagic" parasites. By contrast, the maximal growth rate for parasitised recently settled *P. amboinensis* was higher than for non-parasitised fish. These fish had settled on the reef $\sim$10 days prior to collection. Although this result is counterintuitive, other studies have reported an increase in host growth correlated with parasite infection in juvenile fish. For example, juvenile sticklebacks (*Gasterosteus aculeatus*) parasitised with *Schistocephalus solidus* metacestodes have faster growth than non-parasitised individuals (Arnott et al. 2000). Notably, however, these parasites are relatively enormous compared to the size of their hosts. Also, juveniles of three species of freshwater fish infected with metacercariae of *Posthodiplostomum cuticola* (Diplostomidae) had faster growth than non-parasitised fish; this increase is possibly due to parasite manipulation of host behaviour resulting in increased host foraging (Ondračková et al. 2004).

When the growth of the recently settled fish was analysed only up to the time of settlement, we found that the effect of parasites on maximal growth rate was already present. This indicates that this variation in settler growth in relation to parasites was present prior to settlement. In turn, this implies that parasitised, recently settled fish were growing more rapidly than non-parasitised fish before settlement. In contrast, the larval fish samples showed no effect of parasitism on growth. This raises the question of why the effect of parasites on growth differed in these two fish stages.

One explanation is that the larval and recently settled fish groups belonged to different cohorts. Backward

calculations of the settlement date of recently settled fish revealed that they hatched in early December, whereas larval fish collected in light traps hatched in the last week of October, indicating the two groups were not from the same cohort. However, due to the two fish groups belonging to different cohorts, the variation in space and time that would have occurred may have affected the composition and parasite load seen in these groups, thus affecting fish growth. Metacercariae were the commonest parasites, but their identity could not be determined due to their immaturity. It is therefore unclear whether different species of metacercariae could have different effects on host fish. Molecular analyses are required to identify and differentiate these species.

An alternative explanation for the ontogenetic difference in the relationship between growth and parasitism is that the negative effects of parasitism manifest only in juvenile fish. Mortality immediately after settlement can be high and strongly selective for many life history attributes (Searcy and Sponaugle 2001; Holmes and McCormick 2009). Additionally, slow-growers typically suffer higher mortality than faster growing individuals (Vigliola and Meekan 2002). Therefore, a possible explanation for the pattern observed is that slow-growing parasitised fish may have been selectively removed, leaving behind the fast-growing parasitised individuals, which are able to sustain the parasite load. Thus, the combination of slow growth and parasitises would be disadvantageous for a young fish during the early stages of benthic life. Selection profiles imposed on recently settled juveniles are in part due to the predator assemblage in the vicinity of the settlement site (Holmes and McCormick 2010) and differences in the physiology of fish during the metamorphosis between larval and juvenile life stages have also been observed (McCormick et al. 2002; Nilsson et al. 2007). For example, water temperature is known to affect fish physiology such as early larval growth, size at settlement and growth during early juvenile life (Green and Fisher 2004; Rankin and Sponaugle 2011), and so these may also play a role in altering the impact of a parasite load on the host.

Other measures of growth, size and age were not affected by parasitism in the larval and recently settled stages. Otolith increment width at hatching did not differ between parasitised and non-parasitised fish in larval and recently settled fish. Maternal condition is known to affect the size of offspring at hatching, with parents in good condition producing larger young (McCormick 2006). Grutter et al. (2010), however, found that parasitised recently settled *P. moluccensis* had smaller otoliths at hatching than non-parasitised fish, although only one of three monthly cohorts showed this relationship. They suggested the relationship between parasites and otolith size in *P. moluccensis* could be due to individuals that were faster growing at the time of hatching being more likely to be removed, slower-growing fish being more likely to be infected or that infection at hatching reduced their size.

Size and age at settlement of *P. amboinensis* did not differ with parasite infection. These findings again contrast with those of Grutter et al. (2010), who found that parasitised recently settled *P. moluccensis* were on average two days older than non-parasitised fish, despite being the same size as non-parasitised fish at the time of settlement. They suggested that the slower-growing parasitised fish required more time to grow to the same size as non-parasitised fish at settlement.

Overall, this study supports the recent findings that parasites may influence the condition and growth rates of young fishes (Sirios and Dodson 2000; Fogelman and Grutter 2008; Jones and Grutter 2008) and suggests that they explain some of the variation in growth and survival of young *P. amboinensis* reported previously (McCormick and Hoey 2004; Gagliano and McCormick 2007b; Gagliano et al. 2007). The evidence presented here, however, suggests that some of the effects may be counterintuitive, that effects are species and possibly even cohort specific, and that much more research in the field is necessary.

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