

NOTE

Spatial variation in selective mortality on larval traits in the coral reef fish *Chromis viridis*

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ABSTRACT: Larval traits in reef fishes influence their probability of surviving after settling, but few studies have explored the extent to which selective mortality varies from site to site. We examined the effects of larval traits on the survival of *Chromis viridis* at 2 sites in Moorea, French Polynesia. We compared average traits of recently settled fish with those of 1 mo old survivors from the same cohort. At both study sites, there was selection for larger size at settlement. A commonly used statistical approach that does not account for correlations among larval traits (ANOVA) indicated that selection on planktonic larval duration (PLD) and larval growth rate was inconsistent between the 2 sites, with selection for PLD at one site and larval growth rate at the other. Larval growth rate and PLD, however, were strongly correlated, such that faster-growing larvae settled at younger ages at both sites. Selection gradient analysis, which accounts for correlations among larval traits, revealed that selection for longer PLD and faster larval growth rates occurred at both study sites but was stronger at one site than the other. We detected no significant differences in habitat characteristics or predator assemblages between the 2 sites. Our findings highlight the need to statistically control for correlations among larval traits when measuring selection, as well as the need to explore spatial variation in selection on larval traits.

KEY WORDS: Larval growth · Planktonic larval duration · Size at settlement · Moorea · Otolith · Correlated larval traits

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INTRODUCTION

Larval traits affect the probability that fish survive after settling from the plankton (reviewed by Sponaugle 2010, Johnson et al. 2010, Perez & Munch 2010). Relatively few studies, however, have evaluated the spatial consistency of selection on larval traits. Most studies have either focused on selection at single sites or have pooled samples across sites to paint a general picture of selection.

While valuable approaches, they cannot reveal potentially important spatial variation in selection. Selective mortality might differ among sites as a result of differences in predator abundance or type, competitor density, or habitat quality. For example,

Holmes & McCormick (2006) found that size-selective mortality differed among sites with different predator assemblages. Samhuri et al. (2009) found that the direction of selection was affected by the density of adult conspecifics.

In this study, we explored whether selection on 3 larval traits of a coral reef fish was consistent between 2 similar sites in the same lagoon. We evaluated selection over the first month after settlement on the size at settlement, planktonic larval duration (PLD), and larval growth rates. We chose size at settlement and larval growth rates to evaluate the 'growth–mortality' hypothesis (Anderson 1988), to determine whether a larger size at settlement and faster growth rates are selected for as predicted. We

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examined PLD to determine whether there was selection on the duration of the larval period and to test whether larval growth rates influenced the duration of the larval period in a manner that was consistent among sites.

MATERIALS AND METHODS

Study sites and species

We studied *Chromis viridis*, a small planktivorous damselfish, at 2 sites in the back reef along the north shore of Moorea, French Polynesia. This species settles to corals inhabited by conspecifics, creating large aggregations, primarily in branching corals, such as *Porites rus* (Lecchini et al. 2007). Our study sites were 2 km apart, separated by a deep (>30 m) pass through the barrier reef, and located near the reef crest at the mouth of Cook's Bay, one on the east side (Melissa's red buoy [MRB]: 17° 28' 38.01" S, 149° 49' 2.35" W) and the other on the west side (West Cook's Bay [WCB]: 17° 28' 47.55" S, 149° 50' 6.31" W). The sites had reefs <4 m deep interspersed with sandy patches. We selected these sites because recent recruits of *C. viridis* were abundant at these sites and they had similar habitat.

Collection techniques

Collections were completed between late January and early March of 2010. To test for selection occurring within the first month after settlement, we collected individuals in 2 age groups from a single cohort: new settlers (0 to 7 d post settlement; n = 33 and 52 at MRB and WCB, respectively) and, 1 mo later, young juveniles (30 to 37 d post settlement; n = 25 and 11 at MRB and WCB, respectively). It is likely that some selective mortality had already occurred by the time we made our collections of new settlers, which averaged 2.9 d post-settlement, but our comparison should provide an unbiased estimate of selection over the following month because the post-settlement age of new settlers, as well as 1 mo olds, did not differ between sites ($t_{83} = 0.52$, $p = 0.60$; $t_{34} = 1.00$, $p = 0.33$, respectively). All collections were made by SCUBA divers using hand nets and clove oil as an anesthetic. Fish were collected haphazardly and roughly evenly across each site. After preservation in 95 % ethanol, the fish were measured to 0.1 mm standard length.

Otolith analysis

Sagittal otoliths were used to determine the PLD, size at settlement, and larval growth rate. Otoliths exhibited a settlement mark, distinguished by a change in ring width and a dark band (Bay et al. 2006). Standard length was tightly linked to otolith diameter in fish, ranging in size from 8.3 to 30.0 mm ($r^2 = 0.98$, $n = 121$), so we used otolith traits as proxies for somatic traits to avoid errors associated with back calculation. PLD was determined by counting the number of rings from the core of the otolith to the settlement mark. Rings were assumed to be formed daily, as other studies on this species have assumed (Wellington & Victor 1989, Bay et al. 2006, Mellin et al. 2009) and as is generally the case in fishes (Pitcher 1988, Campana 1990). The width of otolith daily bands was used to describe daily growth rate in micrometers per day. Size at settlement was estimated as the radius of the otolith from the core to the settlement mark. We estimated daily larval growth rate over the full larval duration by dividing the radius of the otolith to the settlement mark by the pre-settlement age. All measurements were made along the longest axis of the otolith (the rostrum), using a compound microscope with an attached camera and Image-Pro Plus imaging software. Otoliths were read twice, and if these readings were not within 10 % of each other, they were read a third time. All third otolith reads were within 10 % of 1 of the first 2 reads, so all of the 121 otoliths were used in the study. The mean of the 2 reads within 10 % of each other was used to estimate larval traits.

Predator density and habitat surveys

We sampled predatory fishes and habitat attributes along 9 transects of 30 × 2 m at each site; 3 replicate transects were done in each of 3 habitat zones: the sand and rubble, the sand-coral ecotone, and the main reef. Transects were separated from one another by at least 30 m and were placed systematically to cover the majority of each site. Potential predators of young damselfishes were defined based on Schmitt & Holbrook (2007) and our own observations, and counted within a 2 m wide by 1 m high window along each transect. Substrate composition was quantified using random-point-contacts in every meter along each transect. The substrate was defined as live coral, dead coral, macroalgae, sand, or 'other'.

Data analysis

We used 2 statistical approaches to test for selection on larval traits: one that provided explicit tests of differences between the 2 study sites but that failed to account for correlations among the larval traits; and one that accounted for correlations among traits but did not explicitly test for differences between sites. ANOVA, using SYSTAT 13, was used to test for differences in selection between sites by comparing the mean larval traits of new settlers with those of 1 mo old survivors. A separate ANOVA was used for each of the 3 larval traits. These models had 2 fixed factors, Site and Age (new settler and 1 mo old juvenile), and their interaction. Significant Age or Site \times Age terms indicate selection. A significant Age effect indicates a difference between juvenile and settler group means. A significant Site \times Age interaction indicates a difference in selection between sites.

To account for the effects of correlations among larval traits on estimates of selection, we calculated selection gradients using the approach introduced by Johnson et al. (2012). This approach is analogous to multiple regression in that correlations among predictor variables are statistically controlled for. It is also analogous in that multicollinearity of predictor variables can make the results unreliable. The 3 larval traits we measured were highly multicollinear. We chose to remove size at settlement from the analysis because it is determined by PLD and larval growth rate, and we were interested in the mechanisms that lead to different sizes at settlement. To evaluate how correlations between the 2 larval traits altered estimates of selection, we calculated both selection gradients, which account for correlations, and selection differentials, which do not. Linear selection coefficients as well as nonlinear selection

coefficients were initially explored, but the estimates of nonlinear selection appeared unreliable due to low sample sizes (Johnson et al. 2012), so we do not include them here. Selection differentials, selection gradients, and associated significance tests were completed in R (v. 3.0.3) using a resampling program provided by Johnson et al. (2012).

To test if there was a larval growth vs. PLD tradeoff that differed between the 2 study sites, we used ANCOVA in SYSTAT 13. In this analysis, we used only the recent settlers (i.e. excluding 1 mo old juveniles), so that we could minimize the effect of post-settlement mortality on differences between the 2 sites. PLD was the dependent variable, and Site and Larval growth rate were the independent categorical and continuous independent variables, respectively. A significant Site \times Larval growth rate interaction indicates that the slope of the relationship between PLD and larval growth differed between sites.

Tests for differences between sites in either substrate composition or predator assemblage were done with a permutational analysis of variance (PERMANOVA); using PRIMER v. 6 with PERMANOVA+. Separate tests were done for habitat and predators, both using a 2-way model with Site and reef Zone treated as fixed factors.

RESULTS

ANOVA revealed that there was selection for larger size at settlement, which was consistent between the 2 sites (Age: $F_{1,117} = 26.7$, $p < 0.001$; Site \times Age: $F_{1,117} = 1.9$, $p = 0.17$; Fig. 1a). Also, at settlement, fish at WCB were larger than those at MRB (Site: $F_{1,117} = 20.5$, $p < 0.001$). In contrast to size at settlement, selection on pre-settlement growth rate and PLD

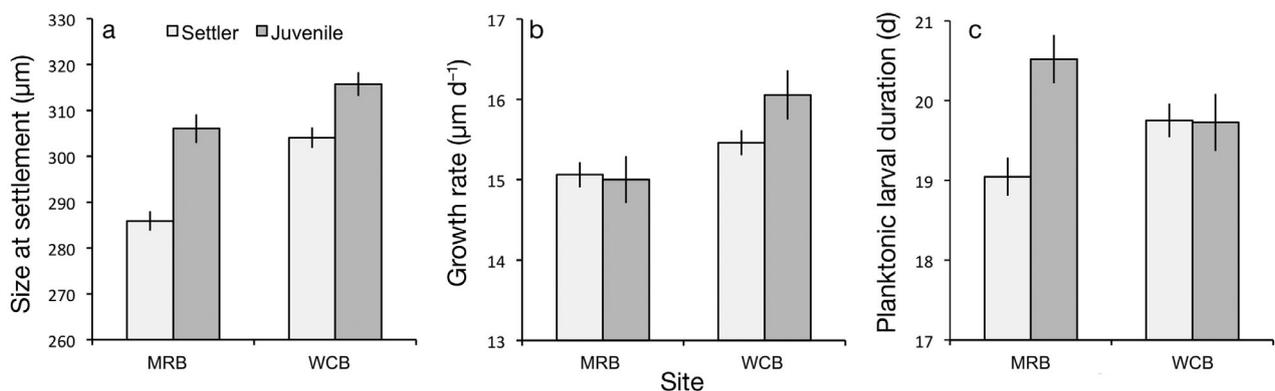


Fig. 1. Larval traits of *Chromis viridis* at 2 sites (MRB and WCB) and 2 ages (settler or juvenile). Data are mean \pm 1 SE from $n = 33$ and 52 recent settlers, and 25 and 11 juveniles (1 mo old) from MRB and WCB, respectively

appeared to be inconsistent between the 2 study sites. ANOVA indicated that there was selection for longer PLDs at MRB but not at WCB (Site \times Age: $F_{1,117} = 5.8$, $p = 0.017$; Age: $F_{1,117} = 5.5$, $p = 0.021$; Site: $F_{1,117} = 0.02$, $p = 0.89$; Fig. 1c). ANOVA indicated that there was no selection on larval growth rates, though larval growth of fish settling at WCB was faster than at MRB (Site \times Age: $F_{1,117} = 1.8$, $p = 0.178$; Age: $F_{1,117} = 1.2$, $p = 0.269$; Site: $F_{1,117} = 9.0$, $p = 0.003$; Fig. 1b).

The analysis of selection gradients, which accounted for correlations between the larval traits PLD and growth rate (Fig. 2), gave a different perspective on differences in selection between sites. The selection gradients analysis provided compelling evidence of selection for longer PLD and faster larval growth rates at both sites (Table 1). Selection on both of these traits appeared to be about 2- to 3-fold stronger at the MRB site than the WCB site. Selection differentials, which do not account for correlations between traits, produced the same results as those from ANOVA.

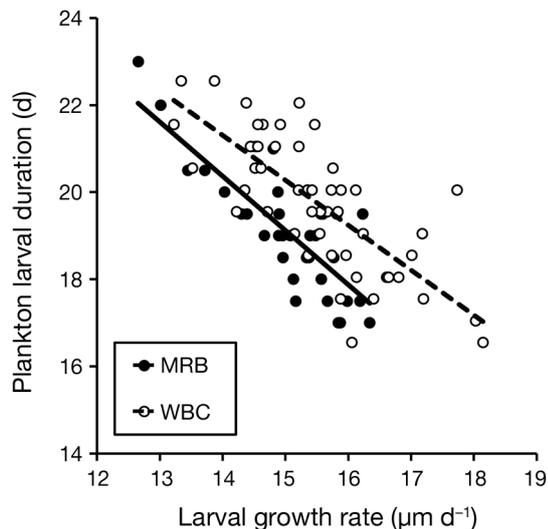


Fig. 2. Relationships between planktonic larval duration and daily growth rate for *Chromis viridis* at 2 sites: MRB ($n = 33$) and WCB ($n = 52$)

Table 1. Selection coefficients for planktonic larval duration (PLD) and mean growth rate at 2 sites (MRB and WCB). Linear selection differentials (S), and linear selection gradients (β) are shown. Significant values ($p < 0.05$) are shown in **bold**

Site	Trait	Linear selection differentials			Linear selection gradients		
		S	SE	p	β	SE	p
MRB	PLD	1.066	0.384	0.0002	2.960	0.720	<0.0001
	Larval growth	-0.066	0.397	0.85	2.335	0.740	0.001
WCB	PLD	-0.015	0.261	0.96	0.915	0.332	0.007
	Larval growth	0.530	0.307	0.07	1.225	0.413	0.0006

Neither the habitat attributes measured nor the predatory fish assemblage differed significantly between the 2 study sites. Substrate composition did not differ between the 2 study sites (PERMANOVA: Site: $F_{1,12} = 1.43$, $p = 0.29$), though there were differences among the 3 habitat Zones (sand-rubble, sand-coral ecotone, and reef; $F_{2,12} = 8.38$, $p = 0.003$), which were consistent between Sites (Site \times Zone: $F_{2,12} = 0.42$, $p = 0.75$). The assemblage of predators of recently settled fishes included 2 wrasses (*Thalassoma hardwicke* and *Halichoeres trimaculatus*), 2 triggerfishes (*Rhinecanthus aculeatus* and *Melichthys vidua*), one grouper *Epinephelus merra*, one emperor *Lethrinus olivaceus*, and one jack *Caranx melampygus*. This assemblage did not differ significantly between Sites ($F_{1,12} = 1.64$, $p = 0.23$) or among habitat Zones ($F_{2,12} = 2.53$, $p = 0.07$), nor was there an interaction between Site and Zone ($F_{2,12} = 0.63$, $p = 0.70$).

PLD ranged from 17 to 23 d with an average of 19.7 d ($n = 121$). Among recent settlers ($n = 85$), PLD declined with larval growth rate equally at both sites (Site \times Larval growth rate: $F_{1,81} = 1.0$, $p = 0.33$; larval growth rate: $F_{1,82} = 122.6$, $p < 0.0001$; Fig. 2). For any given larval growth rate, recent settlers at WCB had remained in the plankton 1.1 d longer than those at MRB (Site: $F_{1,82} = 28.7$, $p < 0.0001$).

DISCUSSION

Our results generally support the 'growth–mortality' hypothesis (Anderson 1988) and the 'bigger-is-better' hypothesis (Miller et al. 1988) in that individuals that settled at larger sizes had faster growth rates, and longer PLD survived at higher rates. The results of the most commonly used approach for evaluating selection on larval traits, comparing mean trait values between settlers and survivors from the same cohort without accounting for correlations among traits, suggested that selection for larger size at settlement was achieved in different ways at the 2 sites: longer PLD at one site, but faster larval growth at the other. By accounting for the strong correlation between growth rate and PLD with selection gradient analysis (Johnson et al. 2012), it was revealed that there was selection for longer PLD and faster growth rates at both sites. In fact, accounting for this correlation

between the 2 traits even changed the apparent direction of selection, from negative to positive in some cases. These findings highlight the value of using an approach that takes into account highly correlated larval traits.

Selection tended to be more intense at one site than the other. The cause of this difference is unclear. The predator assemblage and substrate composition did not differ significantly between the 2 sites. However, more detailed analysis of the habitat (e.g. abundance of different coral species) and predator assemblages (e.g. size distributions) might have revealed important differences between the 2 sites. Alternatively, population density, which we did not measure, might have explained the differences (e.g. Samhuri et al. 2009).

We found that longer PLD was selected for, which is counter to the predictions of the 'stage-duration' hypothesis (Leggett & Deblois 1994). Selection after settlement for individuals with longer PLD probably occurs because larger size is advantageous no matter how it is achieved (e.g. faster larval growth or longer PLD). D'Alessandro et al. (2013) demonstrated that there can be different selective pressures acting during the larval and juvenile life stages, resulting in changes in selective mortality. In our study species, it is likely that selection on PLD acts in opposite directions during the larval and early juvenile phases.

At both of our study sites, larvae with faster growth rates had shorter PLDs, a pattern that has been noted in other species (Cowen 1991, Sponaugle & Grorud-Colvert 2006, Grorud-Colvert & Sponaugle 2011). This flexibility in PLD suggests that individuals may have a specific size range at which they are capable of settling, and PLD depends on how quickly an individual reaches settlement-competent size. At one of our sites, recent settlers had spent one more day in the plankton before settling. With the extremely high rates of mortality that larvae experience (Leggett & Deblois 1994), a 1 d difference (~5% of average PLD in our study species) in the time spent in the plankton could significantly affect the probability of surviving the larval period (e.g. reduce it by 20%; D'Alessandro et al. 2011).

Our study reveals that the intensity of selection on larval traits can vary at small scales, e.g. between 2 reefs <2 km apart. Considering small-scale spatial variation (e.g. Holmes & McCormick 2006) as well as temporal variation in selective mortality (Grorud-Colvert & Sponaugle 2011, Rankin & Sponaugle 2011), it is likely that spatio-temporal variation in selective mortality may be widespread. Studies that test for and evaluate the causes of spatial and tempo-

ral variation in selective mortality on larval traits will help to reveal why variation in these traits is maintained, and predict the direction and strength of selective mortality under novel circumstances.

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LITERATURE CITED

- Anderson JT (1988) A review of size dependent survival during pre-recruit stages of fishes in relation to recruitment. *J Northwest Atl Fish Sci* 8:55–66
- Bay LK, Buechler K, Gagliano M (2006) Intraspecific variation in the pelagic larval duration of tropical reef fishes. *J Fish Biol* 68:1206–1214
- Campana SE (1990) How reliable are growth back-calculations based on otoliths? *Can J Fish Aquat Sci* 47: 2219–2227
- Cowen RK (1991) Variation in the planktonic larval duration of the temperate wrasse *Semicossyphus pulcher*. *Mar Ecol Prog Ser* 69:9–15
- D'Alessandro EK, Sponaugle S, Llopiz JK, Cowen RK (2011) Larval ecology of the great barracuda, *Sphyræna barracuda*, and other sphyrænids in the Straits of Florida. *Mar Biol* 158:2625–2638
- D'Alessandro EK, Sponaugle S, Cowen RK (2013) Selective mortality during the larval and juvenile stages of snappers (Lutjanidae) and great barracuda *Sphyræna barracuda*. *Mar Ecol Prog Ser* 474:227–242
- Grorud-Colvert K, Sponaugle S (2011) Variability in water temperature affects trait-mediated survival of a newly settled coral reef fish. *Oecologia* 165:675–686
- Holmes TH, McCormick MI (2006) Location influences size-selective predation on newly settled reef fish. *Mar Ecol Prog Ser* 317:203–209
- Johnson DW, Christie MR, Moye J (2010) Quantifying evolutionary potential of marine fish larvae: heritability, selection, and evolutionary constraints. *Evolution* 64: 2614–2628
- Johnson DW, Grorud-Colvert K, Rankin TL, Sponaugle S (2012) Measuring selective mortality from otoliths and similar structures: a practical guide for describing multivariate selection from cross-sectional data. *Mar Ecol Prog Ser* 471:151–163
- Lecchini D, Planes S, Galzin R (2007) The influence of habitat characteristics and conspecifics on attraction and survival of coral reef fish juveniles. *J Exp Mar Biol Ecol* 341:85–90
- Leggett WC, Deblois E (1994) Recruitment in marine fishes: Is it regulated by starvation and predation in the egg and larval stages? *Neth J Sea Res* 32:119–134
- Mellin C, Galzin R, Ponton D, Vigliola L (2009) Detecting age-structured effects in growth performance of coral reef fish juveniles. *Aquat Biol* 6:31–39
- Miller TJ, Crowder LB, Rice JA, Marschall EA (1988) Larval

- size and recruitment mechanisms in fishes: toward a conceptual framework. *Can J Fish Aquat Sci* 45:1657–1670
- Perez KO, Munch SB (2010) Extreme selection on size in the early lives of fish. *Evolution* 64:2450–2457
- Pitcher CR (1988) Validation of a technique for reconstructing daily patterns in the recruitment of coral reef damselfish. *Coral Reefs* 7:105–111
- Rankin TL, Sponaugle S (2011) Temperature influences selective mortality during the early life stages of a coral reef fish. *PLoS ONE* 6:e16814
- Samhuri JF, Steele MA, Forrester GE (2009) Inter-cohort competition drives density dependence and selective mortality in a marine fish. *Ecology* 90:1009–1020
- Schmitt RJ, Holbrook SJ (2007) The scale and cause of spatial heterogeneity in strength of temporal density dependence. *Ecology* 88:1241–1249
- Sponaugle S (2010) Otolith microstructure reveals ecological and oceanographic processes important to ecosystem-based management. *Environ Biol Fishes* 89:221–238
- Sponaugle S, Grorud-Colvert K (2006) Environmental variability, early life-history traits, and survival of new coral reef fish recruits. *Integr Comp Biol* 46:623–633
- Wellington GM, Victor BC (1989) Planktonic larval duration of one hundred species of Pacific and Atlantic damselfishes (Pomacentridae). *Mar Biol* 101:557–567

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