# Chapter 4: Intraspecific variation in the pelagic larval duration.

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## Abstract

The pelagic larval duration (PLD) of coral reef fishes is an important life-history trait that can both affect and record the ecology and evolution of these species. Although the importance of PLD has been previously recognized, PLDs are available from only a handful of papers and, as a result, a typological view of the PLD of species has developed. Emerging evidence, however, suggests considerable intraspecific variation of PLDs. Here I present additional estimates of PLD for ten species of Pomacentridae and two species of Gobiidae, and coupled with previously published estimates, examine spatial and temporal variation of PLDs within these species. In eight of the twelve species examined here, within-population mean PLDs differed between sampling times, locations within regions and among regions. In contrast, the range of these same PLD estimates overlapped at all spatial and temporal scales examined in eleven of the twelve species, but not between regions in one species (*Amphiprion melanopus*). Therefore, despite tight error estimates typically associated with estimates of PLD taken from a particular population at a particular time in some taxa, the overlapping ranges in PLD reported here indicate that the length of the pelagic larval phase is a much more plastic trait than previously appreciated. Improved understanding of within-species variation in PLD has considerable potential to provide further insights into the ecology and evolution of tropical reef fishes.

## Introduction

The life cycle of nearly every species of coral reef teleost fish includes a pelagic larval phase and a benthic reef-associated one (Leis 1991; Leis and Carson-Ewart 2000). During the past few decades the importance of the dispersive larval phase for understanding aspects of these species' ecology (e.g., Swearer et al. 2002; Sponaugle et al. 2002) and evolution (e.g., Shulman 1998; Bonhomme and Planes 2000) has begun to be appreciated. For example, processes acting on pelagic larvae may affect recruitment rates (Caley et al. 1996), biogeographical distributions (Thresher et al. 1989; Wellington and Victor 1989; Victor and Wellington 2000; Zapata and Herron 2002; Robertson et al. 2004), connectivity among populations (Doherty et al. 1995; Shulman and Bermingham 1995; Shulman, 1998; Riginos and Victor 2001), individual condition (McCormick 1998a; Searcy and Sponaugle 2000), post-recruitment growth (McCormick and Hoey 2004) and survival (Shima and Findlay 2002). Because of these important links between the pelagic and benthic phases, it is important to have reliable estimates of the spatial and temporal variation in pelagic larval durations (PLD) (Leis 1991; Victor 1991; Cowen and Sponaugle 1997; Victor and Wellington 2000). For example, understanding interspecific spatial variation in PLDs may provide insights into processes that vary at geographical scales. Species recruiting to remote locations may display longer PLDs than those recruiting to well connected ones (e.g., Brothers and Thresher 1985; Victor 1986a), if selection favours connectivity, or shorter PLDs if self-recruitment is favoured. Similarly, geographical patterns in larval growth and dispersal may be informed by inter- and intraspecific spatial patterns of PLDs (Cowen and Sponaugle 1997). Temporal variation in PLDs may provide insight into environmental effects on larval duration and recruit quality (e.g., Searcy and Sponaugle 2000; Shima and Findlay 2002; Sponaugle and Pinkard 2004).

PLD can be reliably estimated using pre-settlement counts of daily rings deposited in otoliths (Pannella 1971; Victor 1982; Pitcher 1988). Estimates of PLDs are now available for a large number of species (Brothers et al. 1983; Brothers and Thresher 1985; Thresher and Brothers 1985; Victor 1986a; Thresher and Brothers 1989; Thresher et al. 1989; Wellington and Victor 1989), but little attention has been paid to the possible implications of variation in PLDs either within or among populations. Where variation in PLDs has been reported, this has typically been a subsidiary outcome of research studying other phenomena, not its primary focus (e.g., Murdoch and Doherty 1997; Kerrigan 1996, Sponaugle and Cowen 1997; Wilson and McCormick 1997;

Searcy and Sponaugle 2000; Sponaugle and Pinkard 2004; but see Thorrold and Milicich 1990; Radtke et al. 2001; Wellington and Victor 1992; McCormick 1994). Because the estimates of PLD are being used in a variety of applications such as predicting genetic differentiation among populations (e.g., Doherty et al. 1995; Shulman and Bermingham 1995) and explaining the evolution of larval durations (Bonhomme and Planes 2000), it is imperative to know the spatial and temporal variation of such estimates.

Sources of intraspecific variation in PLDs are potentially diverse. For any species, a range of PLDs might be expected with the lower limit of this range indicating the minimum time taken to attain competency to settle (Searcy and Sponaugle 2000) and the upper limit reflecting the maximum period of survival in the pelagic environment. Variation in PLDs within species should, therefore, reflect genotype X environment interactions influenced by the conditions encountered by larvae in the plankton such as food availability and temperature (e.g., McCormick and Molony 1992 1995; Meekan et al. 2003), plus any maternal effects (Kerrigan 1997; McCormick 1998b), and innate physiological capacities for growth and development (Victor 1986b; Cowen 1991; McCormick 1999). Alternatively, differences in PLDs in allopatric populations of the same species may indicate evolved mean differences in pelagic duration.

Here I document intraspecific variation in the means and ranges of PLDs estimated for 12 species of tropical reef fishes, both among populations separated in space, and within populations through time. The species compared include ten species of damselfishes (Family Pomacentridae) and 2 species of gobies (Family Gobiidae). Presented comparisons are based on combinations of new estimates of PLDs and previously published ones. These comparisons are interpreted as to whether the variation observed most likely reflects plasticity of PLDs, or evolved differences among populations.

## Materials and methods

The PLDs of ten species of damselfishes (i.e., *Amphiprion melanopus* Bleeker, *Amphiprion akindynos* Allen, *Chrysiptera rollandi* (Whitley), *Chrysiptera rex* (Snyder), *Chromis viridis* (Cuvier), *Pomacentrus wardi* Whitley, *Pomacentrus moluccensis* Bleeker, *Pomacentrus amboinensis* Bleeker, *Amblyglyphidodon curacao* (Bloch), *Amblyglyphidodon aureus* (Cuvier)) and two species of gobies (i.e., *Amblygobius* *rainfordi* (Whitley), *Amblygobius phalaena* (Valenciennes)) were estimated in individuals collected from populations in the south (i.e., One Tree Island 23$^{o}$30S; 152$^{o}$05E) and/or north (e.g., Lizard Island 14$^{o}$40S; 145$^{o}$28E) of the Great Barrier Reef (GBR) during 2000. Fishes were collected using a range of methods. Fishes were measured (SL ± 0.1 mm) and then frozen. Otoliths were later removed (sagittae only), cleaned and stored and sagittal transverse sections were obtained following Wilson and McCormick (1997). Otoliths were read using a high-powered microscope (x40 magnification) and polarised transmitted light. In all species, a settlement mark was apparent as a dark ring followed by a marked decrease in increment width (i.e., Type 1, following Wilson and McCormick 1999). The pre-settlement rings were counted from the nucleus to the settlement mark along one axis. Three blind counts were done on consecutive days and percentage error (PE) estimates were calculated following Beamish and Fournier (1981):

$$\mathrm{PE} = \frac{1}{N}\sum_{j=1}^{N}\left[\frac{1}{R}\sum_{i=1}^{R}\frac{\left|Xij - Xj\right|}{Xj}\right]\times 100 \qquad \text{Eq. 1}$$

where $N$ is the number of fish aged, $R$ is the number of times increments on each otolith were counted, $Xij$ is $i$th age determination for the $j$th fish and $Xj$ is the average age estimated for the $j$th fish.

For all the species examined here, published estimates of mean PLD, and their associated error and range, where available, were used in combination with my data to sample variation in PLDs among times and locations. Because total ring counts of newly settled individuals tend to overestimate PLD by a few days (Wellington and Victor 1989), only estimates based on pre-transitional daily ring counts were used in the comparisons reported here. The techniques to quantify the pre-transitional phase are well established and these counts have been used most widely, and were therefore preferable for the purposes of this investigation. Where possible, mean, error (95% CI) and range were compared among studies on the same species. When 95% CIs could be calculated and did not overlap, means were deemed to be significantly different. In a number of cases from the literature, error estimates were not reported. In such cases, mean estimates were deemed to differ if they were not encompassed by the 95% confidence intervals calculated for other mean estimates.

## Results

Sequential daily ring counts were very consistent with percentage error estimates of less than 4 % (range 1.47 – 3.35 rings) in the majority of species but 13.1 % in *P. wardi*. The mean larval duration displayed significant spatial and temporal variation in eight of the twelve species and these differences ranged from just over half a day (0.63 *A. aureus*) to more than 5 days in *P. wardi* (5.14) (Fig. 1). These differences were primarily associated with differences among locations, although *P. wardi* displayed significant temporal variation in PLD estimates.

The majority of species displayed a range of PLDs of approximately 5 – 6 days (mean 5.47 days ± SE 0.85) although this was much greater in the gobies (15.25 ± SE 2.50) than in the pomacentrids (4.17 ± SE 0.58). The range of larval durations among times and locations overlapped in all but one of the species examined. In *Amphiprion melanopus* the GBR locations ranged from 8 – 14 days whereas the Palauan population ranged from 15 – 22 days. In *P. wardi* the ranges of previously published PLD estimates (from the same location) were non-overlapping before the addition of our estimate.

## Discussion

Previous authors have argued for the consideration of variation in PLDs in trying to understand the importance of the pre-settlement life-history stage of fishes to other aspects of their ecology and evolution (Leis 1991; Victor 1991; Wellington and Victor 1992; Cowen and Sponaugle 1997; Leis 2002), but their calls, to a certain extent, have been ignored. This study confirms significant intraspecific variation in PLDs in eight out of twelve species. Despite the high spatial and temporal variation in PLDs, variability at specific times and places (when it could be estimated) was generally low (Fig. 1). This indicates that although the processes affecting the length of larval life are complex, they tend to produce PLDs that vary little at specific times and places. Therefore single point estimates can underestimate considerably the capacity for intraspecific PLD variation.

The intraspecific range of larval durations observed here were about 5.5 days for pomacentrids and 15 days for gobies. This data confirm that the potential for variation in PLDs varies among taxonomic groups with pomacentrids being less variable than gobies (Thresher et al. 1989; Wellington and Victor 1989; Sponaugle and Cowen 1994;

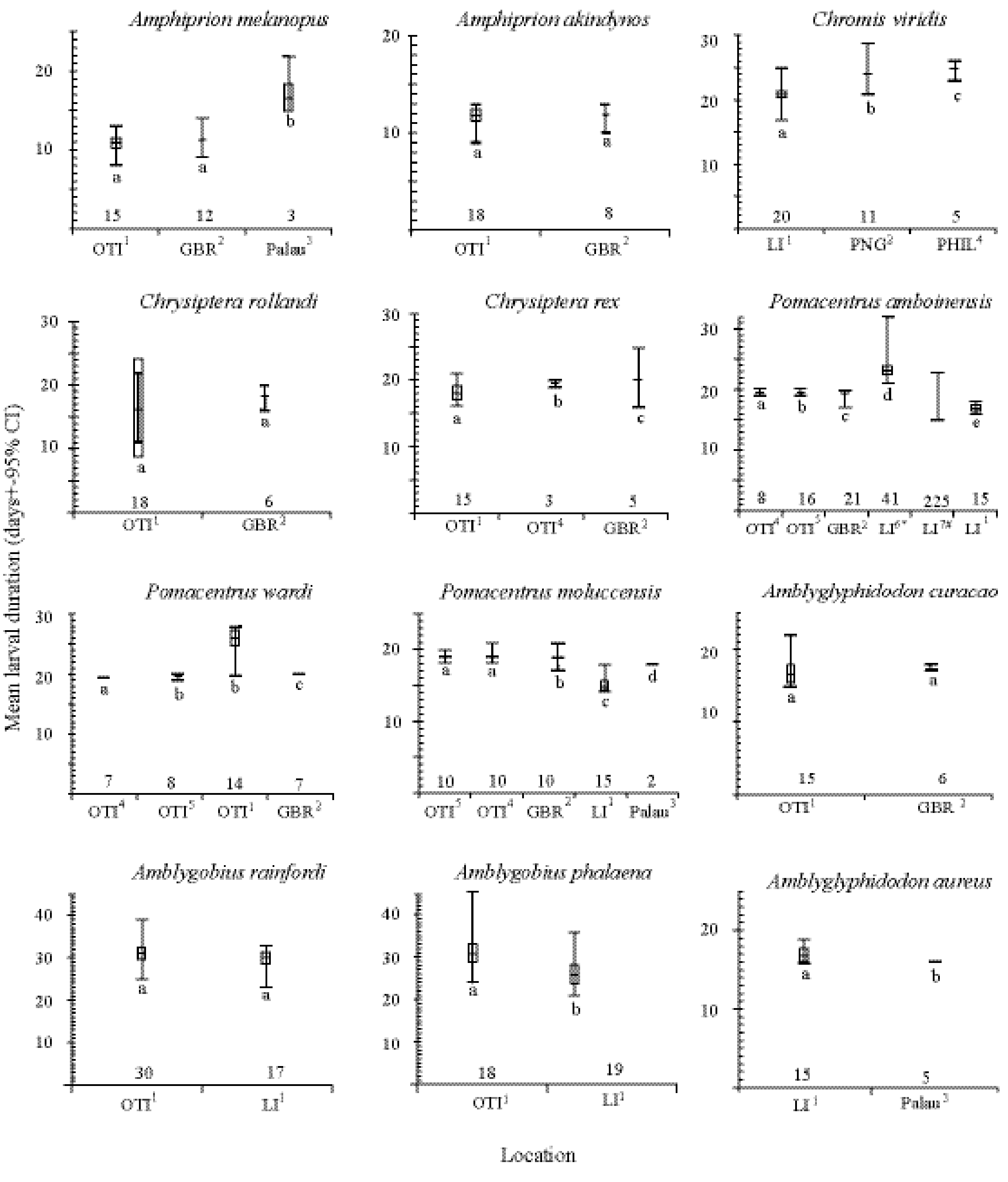

**Fig. 1:** Mean larval duration (horizontal bar) ± 95% confidence intervals (where available) (box) and range (whiskers) in twelve species of coral reef fish. Statistical significance and sample sizes are indicated below data points. OTI = One Tree Island, LI = Lizard Island, GBR = Great Barrier Reef (specific location not given), PHIL = Philippines, PNG = Papua New Guinea, Palau = Palau. Sources (year of sampling): [1]This study (2000), [2]Thresher et al. 1989 and Thresher and Brothers 1989 (1983), [3]Wellington and Victor 1989 (1987), [4]Brothers and Thresher 1985 (not indicated), [5]Brothers et al. 1983 (1976-77, 1978-99), [6]Wilson and McCormick 1997 (1994), [7]Kerrigan 1996 (1990-93). *1 Wilson and McCormick (1997) found no statistical difference in PLDs among 5 locations and for simplicity only one representative

location has been shown here. # Kerrigan (1996) found significant differences among seasons and season by pulses and only the overall range of PLDs has been indicated.

Cowen and Sponaugle 1997). The ranges of PLDs overlapped in all species examined except *A. melanopus* (Fig. 1).

The overlapping ranges of these PLDs may reflect environmentally mediated variation in growth rates during the pre-competent phase, and/or potentially behaviourally controlled delays in settlement (Cowen and Sponaugle 1997). Whatever the proximate cause of this observed variation in larval lifespan, the selective forces maintaining this plasticity remain to be understood.

*Amphiprion melanopus*, on the other hand, represents the first published example (to my knowledge) of intraspecific divergence of PLDs in coral reef fishes. This divergence suggests that local adaptation has occurred between Palauan and GBR populations of this species and that a longer larval duration has evolved in the apparently more isolated location (i.e., Palau) or shorter PLDs, among the well-connected locations (i.e., GBR). This conclusion, however, is based on a very small sample size from Palau (n = 3) and must remain tentative until more data are available. Similarly, it should be noted the non-overlapping pelagic larval durations are not necessary evidence of evolved differences in PLDs among populations. More subtle shifts in the distributions of PLDs among populations could also indicate evolved differences, but reliable estimations of such differences will require considerable additional sampling effort. These examples illustrate that conclusions derived from single point estimates of PLDs may fail to incorporate considerable intercohort variation in PLDs. Future investigations will need to consider this variation.

Geographical patterns in the length of larval life have primarily been investigated by interspecific or intergeneric comparisons (Brothers and Thresher 1985; Victor 1986a; Thresher et al. 1989; Wellington and Victor 1989; Victor and Wellington 2000) and several patterns have emerged from these analyses. Species, or genera, with shorter larval durations may have more localised populations (defined on the basis of colour pattern variation, Thresher et al. 1989), smaller ranges (Wellington and Victor 1989) and may also be characterised by considerable local genetic structure (Doherty et al. 1995; Nelson et al. 2000). Conversely, species at geographically isolated locations may have significantly longer PLDs compared to congeneric species at more central locations (Brothers and Thresher 1985; Victor 1986a). *A. melanopus* appears to have sufficiently restricted gene flow to allow for local adaptation in traits such as mean larval duration. Further investigations incorporating intraspecific variation in PLD among well-connected and isolated populations may provide new insights into the relationship between larval duration and geographical isolation. Pelagic larval duration has also been used as a proxy for dispersal ability (Victor and Wellington 2000) and, in turn, used to predict genetic differentiation among populations (Waples 1987; Doherty et al. 1995; Shulman and Bermingham 1995; Shulman 1998; Riginos and Victor 2001). Typically, studies of the relationship between PLD and genetic differentiation have used mean larval durations estimated at few times or places (Waples 1987; Doherty et al. 1995; Shulman and Bermingham 1995; Shulman 1998; Riginos and Victor 2001). Mean PLDs however, are clearly spatially and temporally variable (Cowen and Sponaugle 1997; this study). Because very low levels of migration can prevent genetic divergence through drift among locations (Wright, 1943), exploration of the relationship between PLD and genetic structuring of populations may be best done using maximum, rather than mean, larval durations (Leis 1991; Victor 1991), even if PLDs at a species' upper limit are only rarely expressed.

## Conclusion

Variations in pelagic larval durations of coral reef fishes may be important in ecological and evolutionary contexts and provide significant information about many pre- and post-settlement processes that may be otherwise logistically difficult to document. By re-examining PLD in a range of coral reef fish species, this investigation has been able to provide three tangible examples of this. First, intracohort variability in PLDs can substantially underestimate intraspecific variability in this trait. Second, additional information presented here for one species, *P. wardi*, show that previously divergent ranges in PLDs at opposite ends of the GBR now appear to be overlapping at these locations. Third, with the addition of further estimates of PLD for *A. melanopus* on the GBR, the GBR and Palau populations of this species appear to have diverged. Understanding variation in larval duration is a worthwhile pursuit that has already provided many new insights, but one that can still yield more.