

# Journal of Animal Ecology

MR JOSHUA THIA (Orcid ID : 0000-0001-9084-0959)

Article type : Research Article

Handling Editor: Ron Bassar

Larval traits show temporally consistent constraints, but are decoupled from post-settlement juvenile growth, in an intertidal fish

Joshua A. Thia<sup>★1</sup>, Cynthia Riginos<sup>1</sup>, Libby Liggins<sup>2</sup>, Will F. Figueira<sup>3</sup>, Katrina McGuigan<sup>1</sup>

★ Corresponding author: [josh.thia@live.com](mailto:josh.thia@live.com)

1 School of Biological Sciences, The University of Queensland, Queensland 4072, Australia.

2 Institute of Natural and Mathematical Sciences, Massey University, Auckland 1045, New Zealand.

3 School of Life and Environmental Sciences, University of Sydney, Sydney, NSW, Australia.

**Key-words:** *Bathygobius cocosensis*, complex life-cycles, early life-history traits, ontogenetic trajectory, otolith analysis, path analysis, planktonic larvae, trait covariance.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/1365-2656.12844

This article is protected by copyright. All rights reserved.

Accepted Article

## Abstract

1. Complex life-cycles may evolve to dissociate distinct developmental phases in an organism's lifetime. However, genetic or environmental factors may restrict trait independence across life stages, constraining ontogenetic trajectories. Quantifying covariance across life-stages and their temporal variability is fundamental in understanding life-history phenotypes and potential distributions and consequences for selection.
2. We studied developmental constraints in an intertidal fish (*Bathygobius cocosensis*: Gobiidae) with a discrete pelagic larval phase and benthic juvenile phase. We tested whether traits occurring earlier in life affected those expressed later, and whether larval traits were decoupled from post-settlement juvenile traits. Sampling distinct cohorts from three annual breeding seasons afforded tests of temporally variability in trait covariance.
3. From otoliths (fish ear stones), we measured hatch size, larval duration, pelagic growth (larval traits) and early post-settlement growth (juvenile trait) in 124 juvenile *B. cocosensis*. We used path analyses to model trait relationships with respect to their chronological expression, comparing models among seasons. We also modelled the effect of season and hatch date on each individual trait to quantify their inherent variability.
4. Our path analyses demonstrated a decoupling of larval traits on juvenile growth. Within the larval phase, longer larval durations resulted in greater pelagic growth, and larger size-at-settlement. There was also evidence that larger hatch size might reduce larval durations, but this effect was only marginally significant. Although pelagic and post-settlement growth were decoupled, pelagic growth had post-settlement consequences: individuals with high pelagic growth were among the largest fish at settlement, and remained among the largest early post-settlement. We observed no evidence that trait relationships varied among breeding seasons, but larval duration differed among breeding seasons, and was shorter for larvae hatching later within each season.
5. Overall, we demonstrate mixed support for the expectation that traits in different life-stages are independent. While post-settlement growth was decoupled from larval traits, pelagic

development had consequences for the size of newly settled juveniles. Temporal consistency in trait covariances implies that genetic and/or environmental factors influencing them were stable over our three-year study. Our work highlights the importance of individual developmental experiences and temporal variability in understanding population distributions of life-history traits.

## Introduction

Many organisms possess complex life-cycles whereby the developmental path of an individual is partitioned into distinct morphological and ecological stages. Such complex life-cycles may allow fitness to be maximized at each stage in development (Ebenman 1992; Moran 1994); life-history traits beneficial in one stage may not be beneficial in another, so metamorphosis might serve to partition the life-cycle and allow stage-specific traits to evolve independently (Pechenik *et al.* 1996; Phillips 1998; Campero *et al.* 2008b; Johansson, Lederer & Lind 2010). Independence of developmental phases, therefore, compartmentalises selection in each life-stage (Campero *et al.* 2008b). However, the underlying genetic architecture and physiological processes that govern development may be constrained and unable to fully dissociate trait variation across the life-cycle (Ebenman 1992; Moran 1994; Sponaugle & Grorud-Colvert 2006; Campero *et al.* 2008a). Furthermore, traits expressed at one life-stage can have opposing effects on fitness in subsequent life-stages (Schluter, Price & Rowe 1991; Miles & Wayne 2009). For example, Crean, Monro and Marshall (2011) identified conflicting selection with regard to larval growth rates in an ascidian. Larvae with slower growth rates had greater survival to reproduction, but these individuals attained smaller average size at reproduction (which was expected to influence fecundity) relative to those individuals that grew more rapidly as larvae. Consequently, trait distributions may exist at some intermediate range that optimises total fitness at the expense of maximizing fitness in any one stage (Schluter, Price & Rowe 1991; Marshall & Morgan 2011). Hence, determining developmental

constraints among traits provides insights into how selection shapes life-history phenotype distributions across the life-cycle and the resulting consequences on population dynamics.

An additional layer of complexity comes from the fact that environmental variation may affect the correlations among life-history traits (Schluter, Price & Rowe 1991; Sgrò & Hoffmann 2004; Gutteling *et al.* 2007). For example, De Block and Stoks (2005) demonstrated this in damselflies by manipulating two larval developmental environments: the photoperiod and food availability. Not only did these environmental factors affect traits associated with metamorphosis (age and mass at emergence) but also the way these traits correlated with fitness. Specifically, at a given mass, females fed more in their larval stage had greater lifetime mating success than food-deprived individuals. In other words, the relationship between mass and fitness was modulated by larval environment. Such findings hint at how environmental differences across time may impact the way life-histories are correlated. Few studies, however, explicitly consider how environmental changes through time may lead to temporal variability in trait covariance in wild populations (Pechenik *et al.* 1996; Giménez 2010).

Many marine organisms have complex life-cycles: while adults may be largely—if not entirely—restricted to the benthos, offspring are typically pelagic at their earliest life-stages. For such species, eggs or larvae disperse and develop as part of the plankton. Larvae then undergo metamorphosis to become young juveniles and return to the benthic environment to continue development in their post-settlement phase. Studies of life-history trait distributions between life-stages have revealed that fitness after metamorphosis can depend on trait variation and developmental environments in the larval phase (Marshall & Keough 2003; Raventós & Macpherson 2005; Allen & Marshall 2010; Shima & Swearer 2010), which is contrary to predictions of independent fitness consequences in complex life-cycles. Consequently, the demographic properties of marine populations might be strongly governed by early developmental processes (Searcy & Sponaugle 2001; Bergenius *et al.* 2002; Hamilton, Regetz & Warner 2008; Shima & Swearer 2010; D’Alessandro, Sponaugle & Cowen 2013; Torres *et al.* 2016). For example, Gagliano, McCormick and Meekan (2007) showed that selective mortality in a damselfish—occurring weeks to months after settlement—

Accepted Article

can be traced back to variation in hatch size or pelagic growth. Marine organisms are thus excellent systems to investigate constraints and related fitness consequences in ontogenetic trajectories, but studies to date have primarily focused on comparing age-related shifts in trait distributions. A void exists for research that quantifies the inherent correlations among early life-history traits and their associated trade-offs (Pineda, Hare & Sponaugle 2007; Marshall & Morgan 2011). Moreover, the difficulty in conducting manipulative experiments in organisms with planktonic larvae restricts studying fitness consequences of life-history variation to observational inferences (e.g. Bergenius *et al.* 2002; D'Alessandro, Sponaugle & Cowen 2013; Caie 2016) or to more manageable invertebrate species with absent or very short planktonic phases (e.g. Marshall & Keough 2004; Allen & Marshall 2010; Crean, Monro & Marshall 2011).

Our study focuses on larval and juvenile traits in the intertidal fish, Cocos frillgoby (*Bathygobius cocosensis*: Gobiidae), collected over three annual breeding seasons. As in many marine fish (McCormick 1993; Tanaka *et al.* 1996), transition from the larval to juvenile stage in *B. cocosensis* represents a time of dramatic morphological, physiological, and behavioural change, accompanying occupation of a different habitat (the pelagic versus benthic environment) and niche. Larvae disperse in the open ocean for ~25 days (preliminary data) where coastal and pelagic conditions influence their development. Following metamorphosis, juveniles settle on rocky intertidal shorelines, which are highly variable environments due to daily tidal fluctuations and variability in physical factors (e.g. salinity and temperature) and biological stressors (e.g. predation mode). The distinct biotic and abiotic factors affecting larvae versus juveniles highlight the potential benefits of fully dissociated life-stages. We analysed four early life-history traits (measured from otoliths, or fish ear stones), in the pelagic larval and benthic juvenile stages (Table 1 & Fig. 1), to address two key knowledge gaps regarding trait variation in wild populations of organisms with complex life-cycles.

We characterised the extent of trait covariance—and hence developmental constraints in ontogenetic trajectories—across larval and juvenile life-stages in *B. cocosensis*, with the null hypothesis that traits are uncorrelated. Substantial effort has been invested into understanding how selection reshapes early life-history trait distributions post-settlement in marine organisms, but studies

explicitly quantifying the magnitude of constraint across life-stages are scarce (but see: Pechenik *et al.* 1996; Giménez 2010; Aguirre, Blows & Marshall 2014). Here, we explicitly quantify how much variation in later-expressed traits can be explained by variation in earlier life-history traits. We determined whether patterns of trait covariance were temporally variable, testing the null hypothesis that the direction and magnitude of trait relationships across different life-stages showed no differences among breeding seasons. Traits themselves may also temporally vary due to environmental differences experienced by individuals: different hatch dates within a breeding season may coincide with fine-scale temporal environmental changes, whilst at a broader temporal scale different seasonal cohorts may be affected by year-to-year changes in environmental conditions. We therefore tested the null hypothesis that traits were invariant across breeding seasons and were unrelated to hatch date. Assessing this temporal variability in traits and their covariance is crucial to understanding trait evolution and recruitment dynamics in organisms with complex life-cycles.

## Materials and methods

### Study species and study site

*Bathygobius cocosensis* is a demersal intertidal fish with a wide distribution across the Indo-Pacific region. In Australia, it inhabits rocky coasts throughout tropical and temperate waters and this study focuses on a population at Hastings Point (28°21'45''S 153°34'47''E), New South Wales. Breeding occurs from November to March (personal observations). Eggs are laid and fertilised on the benthos, larvae hatch and undergo a ~25 day pelagic phase (preliminary data) before metamorphosing into juvenile fish, settling in intertidal and shallow subtidal habitat. We collected settled juvenile fish across three breeding seasons: 28 March 2014 ( $n = 26$ ), 17 January 2015 ( $n = 48$ ), and 27 January 2016 ( $n = 50$ ). Juveniles (standard length < 20 mm) were caught using hand nets. Fish were euthanized using eugenol at 100mg/L in seawater before being preserved in 100% ethanol.

## Data collection

Early life-history traits were obtained from otoliths (fish ear stones). Development of these calcified structures is characterised by the deposition of growth increments and analysis of the number and distance between increments affords study of various traits across a fish's ontogenetic trajectory without direct observation (Brothers, Mathews & Lasker 1976; Campana & Neilssra 1985; Sponaugle & Cowen 1997; Shima & Swearer 2010). Otolith extraction and acquisition of otolith-derived traits was conducted by the Barcelona Otolith Reading Services, Blanes, Girona, Spain. Fish have three otolith pairs: the sagittae, lapilli, and asterisci (David, Isley & Grimes 1994). Work by Shafer (2000) in the congener *B. coalitus* suggests that *Bathygobius* species produce reliable daily growth increments and distinct marks at settlement on their sagittae. Our own preliminary investigations indicated that the lapilli provided much clearer daily growth increments relative to the sagittae (see supplementary material for further details of otolith-related methods). Therefore, we conducted all analyses on traits derived from lapilli.

Data manipulation and analyses were conducted in *R* (R Core Team 2016). Initially, linear regressions between all pairwise combinations of estimated age (days), standard length (mm), and maximum lapillus otolith size ( $\mu\text{m}$ ) were conducted to verify that growth was isometric. This is necessary to ensure that measurements of otolith size are proportionally correlated to age and body size for accurate back-calculation of size-at-age and growth estimation (Shafer 2000) and ensuring no bias in otolith readings with age. In all cases, the linear relationship between any two of these variables was highly significant with a large amount of explained variation ( $R^2 > 0.85$ ); scatter plots also provided visual validation of a linear relationship (Fig. S1).

We collected measurements from six early life-history traits (Table 1; Fig. S2) that have demonstrated fitness consequences in other marine studies. Pelagic larval traits were hatch size, larval duration, and pelagic growth. Hatch size was inferred as the distance from the otolith nucleus to hatch check. The hatch check can be a proxy of larval somatic size upon hatching and maternal investment (Gagliano, McCormick & Meekan 2007; Shima & Swearer 2009; D'Alessandro, Sponaugle & Cowen

2013), which might influence predator-mediated mortality or ability to navigate the pelagic environment (Paradis, Pepin & Brown 1996; Allen 2008; Marshall & Steinberg 2014). Larval duration was defined as the number of days (daily increments) from hatching to settlement, including both the hatch check and settlement check. This represents the time individuals spend in the pelagic environment; longer larval durations might reduce fitness because of greater exposure to physical stressors (Pechenik 1999). Finally, we derived pelagic growth as the distance between the hatch and settlement check. Pelagic growth rates can be important determinants of mortality after metamorphosis in marine fish (Searcy & Sponaugle 2001; Gagliano, McCormick & Meekan 2007).

We also measured three traits occurring at, or after, settlement (Table 1; Fig. S2). Settlement size was measured as the distance between the otolith nucleus and the settlement check, a proxy for somatic size-at-settlement. Size can be important in dictating predator-mediated selection on juvenile fish (Holmes & McCormick 2006). We also defined two post-settlement phase traits, growth and size. Both growth rates and size post-settlement have been found to be important targets of selection in juvenile fish (Gagliano, McCormick & Meekan 2007). Post-settlement growth was determined over the first four days post-settlement as the distance between the settlement check and the fourth otolith increment after the settlement check. Lastly, post-settlement size was defined as the distance between the nucleus and the fourth-day-post-settlement increment.

### **Temporal variation in traits**

We tested generally for temporal variability in traits at broad (between seasons) and fine (within seasons over different hatch dates) timescales in *B. cocosensis*. We took the age (days post-hatch) of an individual and subtracted this from the date of collection to get an estimated hatch date. These back-calculated hatch dates produced a spread of values from 16 December through to 20 February across breeding seasons (Fig. S3A). Due to differences in the month of sampling, the distribution of hatch dates differed among seasons: fish collected in 2013/14 generally had later hatch dates than those collected in the 2014/15 and 2015/16 seasons. The average number of days post-



Accepted Article

settlement for collected juveniles was around 23 days, with a minimum and maximum of 5 and 46 days, respectively (Fig. S3B & S3C). We analysed trait variation using individual ANCOVA models, testing the effects of SEASON (categorical factor), HATCH DATE (continuous variable), and their interaction. The interaction term was non-significant for all traits (Table S1), indicating that any hatch date effects were consistent among seasons; models were rerun without the interaction. We represented HATCH DATE as a numerical vector, where 1 = 1 December and 90 = 28 February. Significance was tested using Type II Sum of Squares with the *R* package *car* (Fox *et al.* 2016).

### Trait covariance across life-stages

We considered how earlier occurring traits affect the variation of traits later in the life-cycle of *B. cocosensis* using a path analysis. Because of the way in which we defined settlement size and post-settlement size, these traits contained a measurement dependency on hatch size and on growth (Fig. S2). Therefore, we excluded these two traits from our path analyses, considering only the four traits free of autocorrelation. We subsequently considered the consequences for settlement and post-settlement sizes of variation in the other traits by: (1) determining the correlation between pelagic growth and post-settlement size; and (2) determining the relationship between size and life-stage.

The *R* package *plspm* (Sanchez, Trinchera & Russolillo 2013) was used for path analyses. Path analyses describe the directed dependence among a set of variables, such that the total effect ( $\beta_{TOT}$ ) of one variable on another is the sum of their direct ( $\beta_{DIR}$ ) and indirect ( $\beta_{IND}$ ) effects (Lleras 2005). Each trait was allowed to directly affect any trait that occurred after it in the life-cycle (HATCH SIZE  $\rightarrow$  LARVAL DURATION  $\rightarrow$  PELAGIC GROWTH  $\rightarrow$  POST-SETTLEMENT GROWTH; Fig. 1). Each trait was given its own “block” (latent factor), coded as a “reflective” variable, and standardized (mean = 0, variance = 1) using the *scaled* argument in *plspm*. We constructed four different models: the three seasons (2013/14, 2014/15, and 2015/16) each modelled separately, and the full dataset (seasons pooled). The *boot.val* argument in *plspm* was used to generate 95% confidence intervals (the 2.5% and 97.5% percentiles) for 1,000 bootstrap replicates for trait effects in each model.

To test the null hypothesis that traits non-significantly covaried within a model, we evaluated whether the bootstrap confidence interval around mean direct ( $\beta_{\text{DIR}}$ ) and total ( $\beta_{\text{TOT}}$ ) trait effects overlapped with zero. Furthermore, we tested the null hypothesis that patterns of covariance did not show temporal variation; *i.e.*, that there is no difference in the bootstrap mean of  $\beta_{\text{DIR}}$  or  $\beta_{\text{TOT}}$  between pairs of seasons. To do this, we implemented Welch's *t*-test (Welch 1947; Welch 1951) between pairs of years for each pathway. Welch's *t*-test was deemed appropriate for our analysis because the variances differed among seasons (Ruxton 2006). See the supplementary information for more details on the calculation and significance testing of Welch's *t*.

## Results

### Temporal variation in traits

ANCOVA for each of our measured traits found little evidence for temporal variation, either within or among breeding seasons, in early life-history traits (Table 2). Only LARVAL DURATION exhibited temporal variation, with a significant effect of SEASON ( $F_{2, 120} = 7.156, p = 0.001$ ) and a negative effect of HATCH DATE (standardised  $\beta = -0.094, F_{1, 120} = 17.831, p < 0.001$ ). On average, larvae spent longer in the pelagic in the 2013/2014 breeding season, and, across all three breeding seasons, larvae born later in the season had shorter larval durations.

### Trait covariance across life-stages

The path analyses provided no evidence that covariance patterns varied among breeding seasons, with no significant differences in direct ( $\beta_{\text{DIR}}$ ) or total ( $\beta_{\text{TOT}}$ ) trait effects among seasons (Fig. 2; Tables S2 and S3); therefore, we focus on reporting results for the pooled dataset. Overall, the path analysis revealed little effect of earlier traits on later traits (Fig. 2 & 3). Only the direct relationship of LARVAL DURATION on PELAGIC GROWTH ( $\beta_{\text{DIR}} = 0.669$ ) was significant (Fig. 2). Consistent with this,

PELAGIC GROWTH was also the only trait for which substantial variation was predicted by the path analysis ( $R^2 = 0.445$ ; Table 3). Variation in LARVAL DURATION was poorly predicted by HATCH SIZE ( $R^2 = 0.020$ ; Table 3); however, in two seasons (2014/15 and 2015/16) and in the pooled dataset, the bootstrap 95% CI only just overlapped zero and beta coefficients were moderately strong, suggesting that larger hatch sizes might reduce larval duration (Fig. 2). Variation in POST-SETTLEMENT GROWTH was also poorly explained by pelagic phase traits ( $R^2 = 0.016$ ) (Table 3).

We further considered the consequences of the observed variation in our pelagic traits on size-at-settlement and size-post-settlement, both traits known to have fitness consequences in fish. Consistent with the inference from the path analysis, there was no evidence that variation in HATCH SIZE contributed any significant variation later in life ( $r = 0.005$  and  $-0.007$  for SETTLEMENT SIZE and POST-SETTLEMENT SIZE, respectively). Variation in SETTLEMENT SIZE was entirely driven by PELAGIC GROWTH, which the path analysis indicated was strongly predicted by LARVAL DURATION. That is, irrespective of size-at-hatch, the larger fish at settlement were those that grew the most (and spent the most time) in the plankton. A moderate amount of variation in POST-SETTLEMENT SIZE was explained by POST-SETTLEMENT GROWTH ( $R^2 = 0.272$ ) (Fig. 4A), which the path analysis indicated was independent of PELAGIC GROWTH (Fig. 2 & 3). However, individuals with larger size-at-settlement were still among the largest early post-settlement (Fig. 4B), indicating that post-settlement growth does not overwrite size differences generated by pelagic growth variation.

## Discussion

The partitioning of complex life-cycles into discrete stages creates the potential for selection to maximise fitness in different developmental phases (Pechenik *et al.* 1996; Phillips 1998; Aguirre, Blows & Marshall 2014). Inherent physiological and genetic constraints may, however, limit the degree to which traits important within each life-stage are truly independent (Ebenman 1992; Moran 1994). For the intertidal fish, *Bathygobius cocosensis* (Cocos frillgoby), our path analyses evidenced

Accepted Article

significant trait covariance among larval traits, but independence of post-settlement juvenile growth. However, we note that despite the ability for individuals to decouple post-settlement growth from pelagic phase variation, there appears to be little evidence of compensatory growth to attenuate size differences among individuals in the first few days following settlement. Consequently, individuals that settle small remain small. Trait relationships did not differ significantly across breeding seasons, suggesting that the underlying environmental and genetic processes governing ontogenetic trajectories in our study population were temporally stable. Additionally, there was little evidence that traits themselves exhibited temporal variation. We consider the potential causes and consequences of our observed ontogenetic patterns, their variation, and their impact on demography and trait evolution.

#### **Larval traits covary consistently across seasons and are dissociated from post-settlement growth**

Our path analyses demonstrated a clear disconnect between trait variation in the pelagic larval phase and post-settlement growth in *B. cocosensis*. We note, however, that we have only been able to observe phenotypic patterns of surviving settlers, and are unable to discern whether these covariance patterns are representative of the full distribution of ontogenetic trajectories possible in this species.

The only significant relationship observed among traits was the effect of the larval duration on pelagic growth, which are both pelagic phase traits, indicating that individuals with longer planktonic development attain a greater amount of absolute growth (Fig. 2 & 3) and settle larger (Fig. 4B). Whether larger size-at-settlement is selectively beneficial would require further testing as the direction and magnitude of size-selective mortality in marine organisms is ecologically dependent and variable with ontogeny (Holmes & McCormick 2006; Johnson & Hixon 2010; D'Alessandro, Sponaugle & Cowen 2013; Caie 2016). The effect of hatch size on larval duration of *B. cocosensis* was not statistically significant, but confidence intervals only just included zero in two of the three breeding seasons (2014/15 and 2015/16) and the pooled dataset (Fig. 2 & 3), and a study with greater power (sample size) might have inferred a significant dependency. The moderate negative effect of

hatch size on larval duration (Fig. 2 & 3; Table 3) suggests individuals hatching larger might spend less time in the plankton.

Despite there being potential growth benefits of a longer larval duration in *B. cocosensis*, the planktonic environment can be perilous for small larvae (Gaines & Roughgarden 1987; Paradis, Pepin & Brown 1996); therefore, reducing the larval duration might have fitness benefits (Pepin & Myers 1991; Pechenik 1999). Other marine studies have reported the importance of hatch size on post-settlement mortality (Raventós & Macpherson 2005; Gagliano, McCormick & Meekan 2007; D'Alessandro, Sponaugle & Cowen 2013), suggesting potential carry-over effects of maternal investment. However, in this study, path analyses revealed no effect of hatch size on post-settlement growth (Fig. 2 & 3); size-at-settlement and size-four-days-post-settlement were also uncorrelated with hatch size, despite their measurements containing hatch size variation (Fig. S2), indicating that variable growth mitigates later size consequences of maternal investment.

Although 27% of the variation in post-settlement size was explained by post-settlement growth (Fig. 4A), *B. cocosensis* juveniles that settled large were generally among the largest fish early after settlement (Fig. 4B). That is, growth within the first four days post settlement did not overwrite pelagic effects. A similar effect of decoupled growth rates but persistent size effects has been observed in other studies. For instance, work by Johansson, Lederer and Lind (2010) on the frog *Rana temporaria* demonstrated that though metamorphosis facilitates decoupling of performance measures between life-stages, size as a trait *per se* carried over from tadpoles to froglets, which affected froglet performance traits due to their dependence on body size.

While compensatory growth has been observed in marine fish post-settlement (Gagliano & McCormick 2007), our results suggest that smaller individuals were unable to elevate their growth early post-settlement to attenuate their larval developmental history. An important point of consideration is that the time interval over which we measured post-settlement growth and size was only four days (relative to the 16–31 day range of larval duration over which pelagic growth occurred). However, mortality can be considerable early post-settlement (Holmes & McCormick

2006; Allen & Marshall 2010), and hence, the ability to rapidly compensate for poor larval growth may be important if there is strong size-dependent post-settlement selection. Nonetheless, there can be costs associated with compensation mechanisms (Campero *et al.* 2008a), and conflicting selection across multiple paths to fitness (Crean, Monro & Marshall 2011; Marshall & Morgan 2011) may govern their presence or absence.

### **Birthdays are important for larval duration**

Larval duration was the only trait that exhibited temporal variation in *B. cocosensis*, which differed among our sampling seasons (least squares mean larval duration in 2013/14 was three and two day longer than in 2014/15 and 2015/16, respectively), and later hatching individuals spent less time in the plankton. This temporal variability of larval duration is consistent with other studies in marine fishes that demonstrate within and between season variation in this trait (Radtke, Kinzie & Shafer 2001; Kingsford, Smith & Flood 2011). The lack of an interaction between season and hatch date (Table S1) suggests that hatch dates had similar effects on larval duration in all seasons. We expect that warming waters and greater food availability from early to late summer may promote more rapid development and earlier metamorphosis. Alternatively, fish with later hatch dates may need to reduce their larval durations in order to time their metamorphosis before the end of the settlement season. Indeed, empirical evidence suggests that hatch dates in fish can be under selection (Einum & Fleming 2000; Wright & Gibb 2005) and larval traits may be under pressure to coincide metamorphosis with particular environmental events that favour settlement success (Robertson, Green & Victor 1988; Robertson *et al.* 1999).

## Perspectives and future directions

Understanding how early developmental and environmental experiences shape life-history trait variation, and influence population dynamics, has been the focus of a large number of studies, particularly in marine systems (Pechenik 2006; Sponaugle & Grorud-Colvert 2006; Sponaugle, Grorud-Colvert & Pinkard 2006; Hamilton, Regetz & Warner 2008; Shima & Swearer 2009; Allen & Marshall 2010; Shima, Noonburg & Swearer 2015; Torres *et al.* 2016). Such studies have been concerned with changes in population trait means over life-stages and less well studied is the extent to which traits expressed at different life-stages share variation (covary), the fitness consequences of covariance across stages, and processes that modulate these relationships (Crean, Monro & Marshall 2011; Marshall & Morgan 2011; Aguirre, Blows & Marshall 2014).

Our results provide mixed support for the theoretical expectation that complex life-cycles compartmentalise trait variation (Ebenman 1992; Moran 1994). Although no pelagic phase traits exerted constraints on post-settlement growth in *B. cocossensis*, fish that were relatively large (small) at settlement tended to still be relatively large (small) shortly after settlement. Therefore, while growth itself appears dissociated across early life-stages, growth in the pelagic phase constrains size in the post-settlement phase. Understanding the fitness consequences of this constraint will require assessment of how selection acts on size (Johnson & Hixon 2010; Caie 2016) in settled *B. cocossensis* juveniles.

The general lack of temporal variability exhibited by *B. cocossensis* in individual traits and their covariance patterns suggests a marked amount of stability in the mechanisms that influence these life-history traits. Although few studies have considered trait covariances across life stages, these studies have demonstrated the contrary pattern, where uncharacterised natural variation and known (experimentally manipulated) environmental variation both generated variation in the relationships among early life-history traits (Pechenik *et al.* 1996; Giménez 2010). Life-history trait means have been shown to respond to environmental differences across time and space at small (weeks to months, or kilometres) to large (years, or 100s kilometres) scales (Sponaugle & Cowen 1997; Searcy &

Sponaugle 2001; Sponaugle & Grorud-Colvert 2006; Di Franco & Guidetti 2011; Di Franco *et al.* 2012; Caie 2016). Additionally, spatiotemporal variability in the sources of recruits in different cohorts (Gerlach *et al.* 2007; Kingsford, Smith & Flood 2011; Shima & Swearer 2016) might impact the underlying genetic variation represented in each cohort. The mechanistic causes and long-term consequences of the temporal stability that we have observed require further investigation; we are currently characterising temporal patterns of genetic variation in *B. cocosensis* to determine the potential contribution of recruit source variability.

## **Acknowledgements**

We thank I. Popovic, C. Da Silva, A. Matias, A. Mather, D. Blower, P. Mills, J. Evans and A. Pocock, who have been instrumental in the field and providing feedback on this manuscript. The manuscript was improved in response to comments from an anonymous reviewer, A. Di Franco, and the editor. We thank Nuria Raventós, for preparing our otolith dataset. Fish were collected under NSW DPI Permit P13/0046-1.1 with animal ethics approval from USyd (2015/834) and UQ (SBS.014.12). Funding for this project was provided by The Herman Slade Foundation (HSF 13/14 to CR and LL) and The Ecological Society of Australia (Student Research Award 2015 to JAT).

## **Author contributions**

JAT, CR, LL, and KM were involved in the conceptualisation and design of this experiment. WFF provided support for sampling and critical insights. JAT carried out the analyses and wrote the original draft of this paper. All authors contributed toward revisions and approved the final draft for publication.



## Data accessibility

Data is accessible via figshare, <https://doi.org/10.6084/m9.figshare.6105827> (Thia *et al.* 2018).

## References

- Aguirre, J.D., Blows, M.W. & Marshall, D.J. (2014) The genetic covariance between life cycle stages separated by metamorphosis. *Proceedings of the Royal Society B*, 281, 20141091.
- Allen, J.D. (2008) Size-specific predation on marine invertebrate larvae. *Biological Bulletin*, 214, 42-49.
- Allen, R.M. & Marshall, D.J. (2010) The larval legacy: Cascading effects of recruit phenotype on post-recruitment interactions. *Oikos*, 119, 1977-1983.
- Bergenius, M.A., Meekan, M.G., Robertson, R.D. & McCormick, M.I. (2002) Larval growth predicts the recruitment success of a coral reef fish. *Oecologia*, 131, 521-525.
- Brothers, E.B., Mathews, C.P. & Lasker, R. (1976) Daily growth increments in otoliths from larval and adult fishes. *Fishery Bulletin*, 74, 1-8.
- Caie, K.J. (2016) Selective mortality on early life-history traits of a temperate reef fish. MSc, Victoria University.
- Campana, S.E. & Neilssra, D. (1985) Microstructure of fish otoliths. *Canadian Journal of Fisheries and Aquatic Sciences*, 42, 1014-1032.
- Campero, M., Block, M.D., Ollevier, F. & Stoks, R. (2008a) Correcting the short-term effect of food deprivation in a damselfly: mechanisms and costs. *Journal of Animal Ecology*, 77, 66-73.
- Campero, M., De Block, M., Ollevier, F. & Stoks, R. (2008b) Metamorphosis offsets the link between larval stress, adult asymmetry and individual quality. *Functional Ecology*, 22, 271-277.
- Crean, A.J., Monro, K. & Marshall, D.J. (2011) Fitness consequences of larval traits persist across the metamorphic boundary. *Evolution*, 65, 3079-3089.

- D'Alessandro, E., Sponaugle, S. & Cowen, R. (2013) Selective mortality during the larval and juvenile stages of snappers (Lutjanidae) and great barracuda *Sphyrna barracuda*. *Marine Ecology Progress Series*, 474, 227-242.
- David, A.W., Isley, J.J. & Grimes, C.B. (1994) Differences between the sagitta, lapillus, and asteriscus in estimating age and growth in juvenile red drum, *Sciaenops ocellatus*. *Fishery Bulletin*, 92, 509-515.
- De Block, M. & Stoks, R. (2005) Fitness effects from egg to reproduction: bridging the life history transition. *Ecology*, 86, 185-197.
- Di Franco, A., Gillanders, B.M., De Benedetto, G., Pennetta, A., De Leo, G.A. & Guidetti, P. (2012) Dispersal patterns of coastal fish: Implications for designing networks of marine protected areas. *PLoS ONE*, 7, e31681.
- Di Franco, A. & Guidetti, P. (2011) Patterns of variability in early-life traits of fishes depend on spatial scale of analysis. *Biology Letters*, 7, 454-456.
- Ebenman, B. (1992) Evolution in organisms that change their niches during the life cycle. *The American Naturalist*, 139, 990-1021.
- Einum, S. & Fleming, I.A. (2000) Selection against late emergence and small offspring in Atlantic salmon (*Salmo salar*). *Evolution*, 54, 628-639.
- Fox, J., Weisberg, S., Adler, D., Bates, D., Baud-Bovy, G., Ellison, S., Firth, D., Friendly, M., Gorjanc, G. & Graves, S. (2016) Package 'car'.
- Gagliano, M. & McCormick, M.I. (2007) Compensating in the wild: is flexible growth the key to early juvenile survival? *Oikos*, 116, 111-120.
- Gagliano, M., McCormick, M.I. & Meekan, M.G. (2007) Survival against the odds: ontogenetic changes in selective pressure mediate growth-mortality trade-offs in a marine fish. *Proceedings of the Royal Society B*, 274, 1575-1582.
- Gaines, S.D. & Roughgarden, J. (1987) Fish in offshore kelp forests affect recruitment to intertidal barnacle populations. *Science*, 235, 479-481.
- Gerlach, G., Atema, J., Kingsford, M.J., Black, K.P. & Miller-Sims, V. (2007) Smelling home can prevent dispersal of reef fish larvae. *PNAS*, 104, 858-863.

- Giménez, L. (2010) Relationships between habitat conditions, larval traits, and juvenile performance in a marine invertebrate. *Ecology*, *91*, 1401-1413.
- Gutteling, E., Doroszuk, A., Riksen, J., Prokop, Z., Reszka, J. & Kammenga, J. (2007) Environmental influence on the genetic correlations between life-history traits in *Caenorhabditis elegans*. *Heredity*, *98*, 206-213.
- Hamilton, S.L., Regetz, J. & Warner, R.R. (2008) Postsettlement survival linked to larval life in a marine fish. *Proceedings of the National Academy of Sciences*, *105*, 1561-1566.
- Holmes, T.H. & McCormick, M.I. (2006) Location influences size-selective predation on newly settled reef fish. *Marine Ecology Progress Series*, *317*, 203-209.
- Johansson, F., Lederer, B. & Lind, M.I. (2010) Trait performance correlations across life stages under environmental stress conditions in the common frog, *Rana temporaria*. *PLoS ONE*, *5*, e11680.
- Johnson, D. & Hixon, M. (2010) Ontogenetic and spatial variation in size-selective mortality of a marine fish. *Journal of Evolutionary Biology*, *23*, 724-737.
- Kingsford, M., Smith, F. & Flood, M. (2011) Growth and pelagic larval duration of presettlement and newly settled neon damselfish, *Pomacentrus coelestis*, at multiple spatial scales. *Coral Reefs*, *30*, 203-214.
- Lleras, C. (2005) Path Analysis. *Encyclopedia of Social Measurement*, pp. 25-30.
- Marshall, D.J. & Keough, M.J. (2003) Effects of settler size and density on early post-settlement survival of *Ciona intestinalis* in the field. *Marine Ecology Progress Series*, *259*, 139-144.
- Marshall, D.J. & Keough, M.J. (2004) Variable effects of larval size on post-metamorphic performance in the field. *Marine Ecology Progress Series*, *279*, 73-80.
- Marshall, D.J. & Morgan, S.G. (2011) Ecological and evolutionary consequences of linked life-history stages in the sea. *Current Biology*, *21*, R718-R725.
- Marshall, D.J. & Steinberg, P.D. (2014) Larval size and age affect colonization in a marine invertebrate. *Journal of Experimental Biology*, *217*, 3981-3987.
- McCormick, M.I. (1993) Development and changes at settlement in the barbel structure of the reef fish, *Upeneus tragula* (Mullidae). *Environmental Biology of Fishes*, *37*, 269-282.

- Miles, C.M. & Wayne, M.L. (2009) Life history trade-offs and response to selection on egg size in the polychaete worm *Hydroides elegans*. *Genetica*, 135, 289-298.
- Moran, N.A. (1994) Adaptation and constraint in the complex life cycles of animals. *Annual Review of Ecology and Systematics*, 573-600.
- Paradis, A., Pepin, P. & Brown, J. (1996) Vulnerability of fish eggs and larvae to predation: review of the influence of the relative size of prey and predator. *Canadian Journal of Fisheries and Aquatic Sciences*, 53, 1226-1235.
- Pechenik, J., Hilbish, T., Eyster, L. & Marshall, D. (1996) Relationship between larval and juvenile growth rates in two marine gastropods, *Crepidula plana* and *C. fornicata*. *Marine Biology*, 125, 119-127.
- Pechenik, J.A. (1999) On the advantages and disadvantages of larval stages in benthic marine invertebrate life cycles. *Marine Ecology Progress Series*, 177, 269-297.
- Pechenik, J.A. (2006) Larval experience and latent effects—metamorphosis is not a new beginning. *Integrative and Comparative Biology*, 46, 323-333.
- Pepin, P. & Myers, R.A. (1991) Significance of egg and larval size to recruitment variability of temperate marine fish. *Canadian Journal of Fisheries and Aquatic Sciences*, 48, 1820-1828.
- Phillips, P. (1998) Genetic constraints at the metamorphic boundary: morphological development in the wood frog, *Rana sylvatica*. *Journal of Evolutionary Biology*, 11, 453-463.
- Pineda, J., Hare, J.A. & Sponaugle, S. (2007) Larval transport and dispersal in the coastal ocean and consequences for population connectivity. *Oceanography*, 20, 22-39.
- Radtke, R., Kinzie, R. & Shafer, D. (2001) Temporal and spatial variation in length of larval life and size at settlement of the Hawaiian amphidromous goby *Lentipes concolor*. *Journal of Fish Biology*, 59, 928-938.
- Raventós, N. & Macpherson, E. (2005) Effect of pelagic larval growth and size at hatching on the post-settlement survivorship in two temperate labrid fishes of the genus *Symphodus*. *Marine Ecology Progress Series*.
- R Core Team (2016) *R*: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria.

- Robertson, D.R., Green, D.G. & Victor, B.C. (1988) Temporal coupling of production and recruitment of larvae of a Caribbean reef fish. *Ecology*, *69*, 370-381.
- Robertson, D.R., Swearer, S.E., Kaufmann, K. & Brothers, E.B. (1999) Settlement vs. environmental dynamics in a pelagic-spawning reef fish at Caribbean Panama. *Ecological Monographs*, *69*, 195-218.
- Ruxton, G.D. (2006) The unequal variance *t*-test is an underused alternative to Student's *t*-test and the Mann-Whitney *U* test. *Behavioral Ecology*, *17*, 688-690.
- Sanchez, G., Trinchera, L. & Russolillo, G. (2013) plspm: tools for partial least squares path modeling (PLS-PM). *R Package*, *1*.
- Schluter, D., Price, T.D. & Rowe, L. (1991) Conflicting selection pressures and life history trade-offs. *Proceedings of the Royal Society B*, *246*, 11-17.
- Searcy, S.P. & Sponaugle, S. (2001) Selective mortality during the larval-juvenile transition in two coral reef fishes. *Ecology*, *82*, 2452-2470.
- Sgrò, C. & Hoffmann, A. (2004) Genetic correlations, tradeoffs and environmental variation. *Heredity*, *93*, 241-248.
- Shafer, D. (2000) Evaluation of periodic and aperiodic otolith structure and somatic-otolith scaling for use in retrospective life history analysis of a tropical marine goby, *Bathygobius coalitus*. *Marine Ecology Progress Series*, *199*, 217-229.
- Shima, J.S., Noonburg, E.G. & Swearer, S.E. (2015) Consequences of variable larval dispersal pathways and resulting phenotypic mixtures to the dynamics of marine metapopulations. *Biology Letters*, *11*, 20140778-20140778.
- Shima, J.S. & Swearer, S.E. (2009) Larval quality is shaped by matrix effects: implications for connectivity in a marine metapopulation. *Ecology*, *90*, 1255-1267.
- Shima, J.S. & Swearer, S.E. (2010) The legacy of dispersal: Larval experience shapes persistence later in the life of a reef fish. *Journal of Animal Ecology*, *79*, 1308-1314.
- Shima, J.S. & Swearer, S.E. (2016) Evidence and population consequences of shared larval dispersal histories in a marine fish. *Ecology*.

- Sponaugle, S. & Cowen, R.K. (1997) Early life history traits and recruitment patterns of Caribbean wrasses (Labridae). *Ecological Monographs*, 67, 177-202.
- Sponaugle, S. & Grorud-Colvert, K. (2006) Environmental variability, early life-history traits, and survival of new coral reef fish recruits. *Integrative and Comparative Biology*, 46, 623-633.
- Sponaugle, S., Grorud-Colvert, K. & Pinkard, D. (2006) Temperature-mediated variation in early life history traits and recruitment success of the coral reef fish *Thalassoma bifasciatum* in the Florida Keys. *Marine Ecology Progress Series*, 308, 1-15.
- Tanaka, M., Kawai, S., Seikai, T. & Burke, J. (1996) Development of the digestive organ system in Japanese flounder in relation to metamorphosis and settlement. *Marine & Freshwater Behaviour & Phy*, 28, 19-31.
- Thia, J., Riginos, C., Liggins, L., Figueira, W. & McGuigan, K. (2018) DATASET [Thia et al. 2018 J Anim Ecol]: Larval traits show temporally consistent constraints, but are decoupled from post-settlement juvenile growth, in an intertidal fish. *figshare*. Fileset.  
<https://doi.org/10.6084/m9.figshare.6105827>
- Torres, G., Giménez, L., Pettersen, A.K., Bue, M., Burrows, M.T. & Jenkins, S.R. (2016) Persistent and context-dependent effects of the larval feeding environment on post-metamorphic performance through the adult stage. *Marine Ecology Progress Series*, 545, 147-160.
- Welch, B.L. (1947) The generalization of Student's' problem when several different population variances are involved. *Biometrika*, 34, 28-35.
- Welch, B.L. (1951) On the comparison of several mean values: an alternative approach. *Biometrika*, 38, 330-336.
- Wright, P.J. & Gibb, F.M. (2005) Selection for birth date in North Sea haddock and its relation to maternal age. *Journal of Animal Ecology*, 74, 303-312.

## Figures and Tables

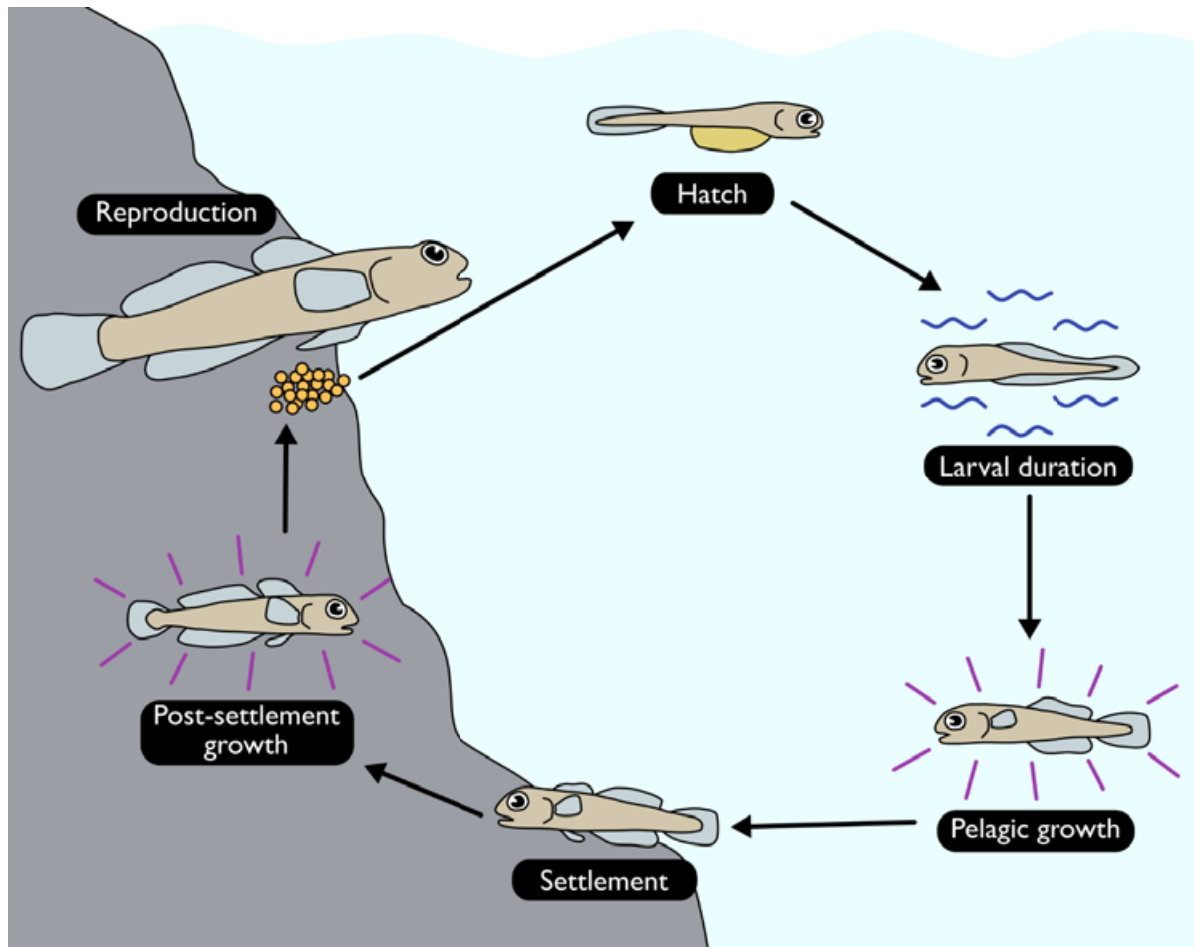


Fig. 1. The life-cycle of *Bathygobius cocosensis*. Reproduction occurs on the benthos. Larvae hatch and undergo a pelagic larval phase. After this period of pelagic development, larvae return to the benthos, where they settle and undergo a metamorphosis into young juveniles. Further growth and development occurs on the benthos, the post-settlement phase, until fish reach adulthood.

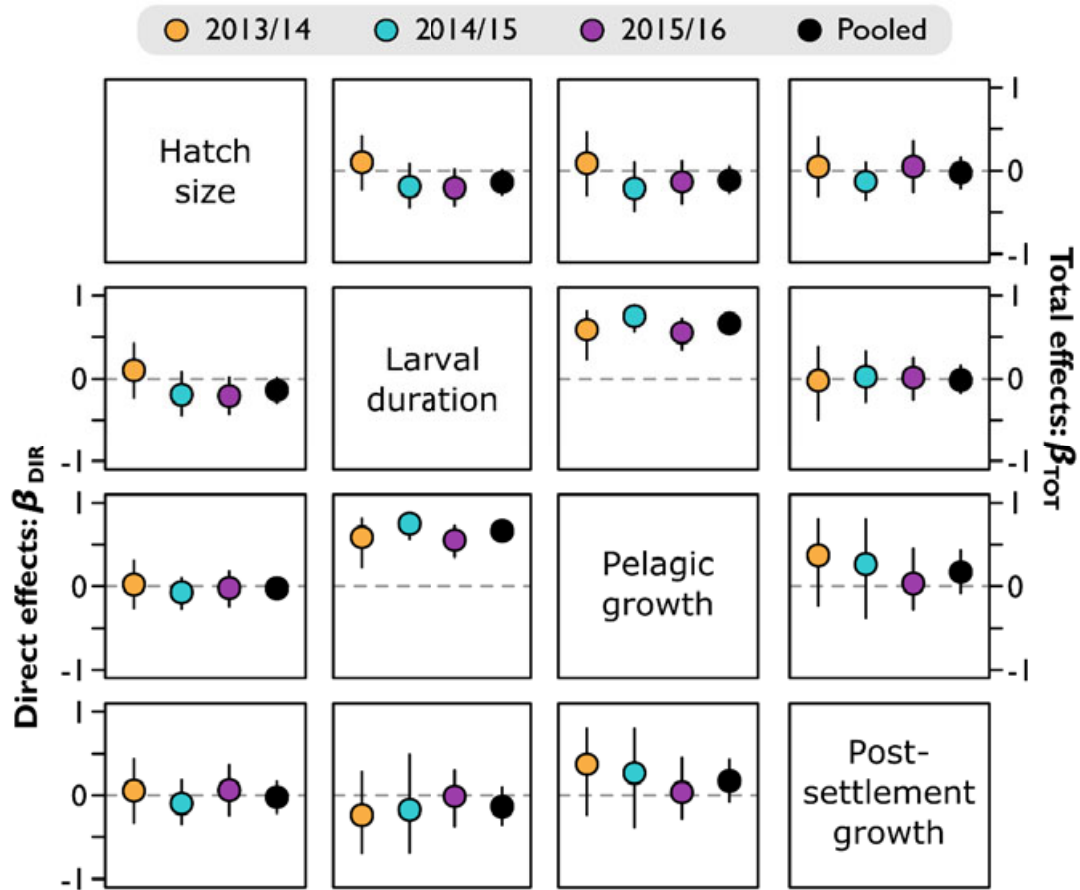


Fig. 2. Estimates of standardized direct effects ( $\beta_{DIR}$ : below the diagonal) and total effects ( $\beta_{TOT}$ : above the diagonal) quantifying the effect of an earlier expressed trait on a later expressed trait in *Bathygobius cocosensis*, as estimated from path models in different breeding seasons (see key): 2013/14 ( $n = 26$ ); 2014/15 ( $n = 48$ ); 2015/16 ( $n = 50$ ), and pooled ( $n = 124$ ). Traits are arranged in chronological order from left to right and the plot should be interpreted as earlier expressed traits affecting later expressed traits. Error bars are bootstrap 95% confidence intervals. Dashed lines indicate an effect size of zero.



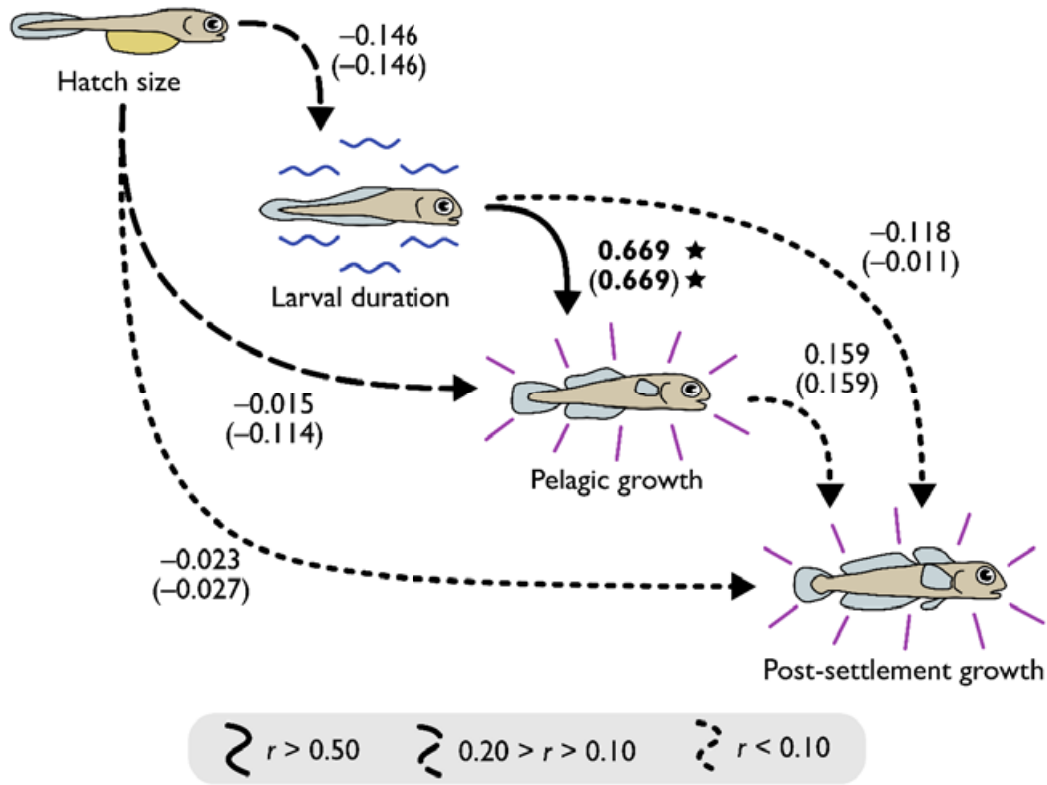


Fig. 3. Relationships among early life-history traits in *Bathygobius cocosensis*, as determined from path analyses, in our pooled dataset (all breeding seasons,  $n = 124$ ). Traits are arranged in chronological order from left to right. Arrows indicate direction of effect and the line type (dotted, dashed, or solid) for each arrow represents the strength of correlation ( $r$ , see legend) between the two traits. Numbers are the standardized effect sizes for direct effect (total effect), that is,  $\beta_{DIR}$  ( $\beta_{TOT}$ ), where the TOTAL EFFECT = DIRECT EFFECT + INDIRECT EFFECT. Bold values marked with a “★” are statistically significant (where the bootstrap 95% confidence interval does not contain zero).

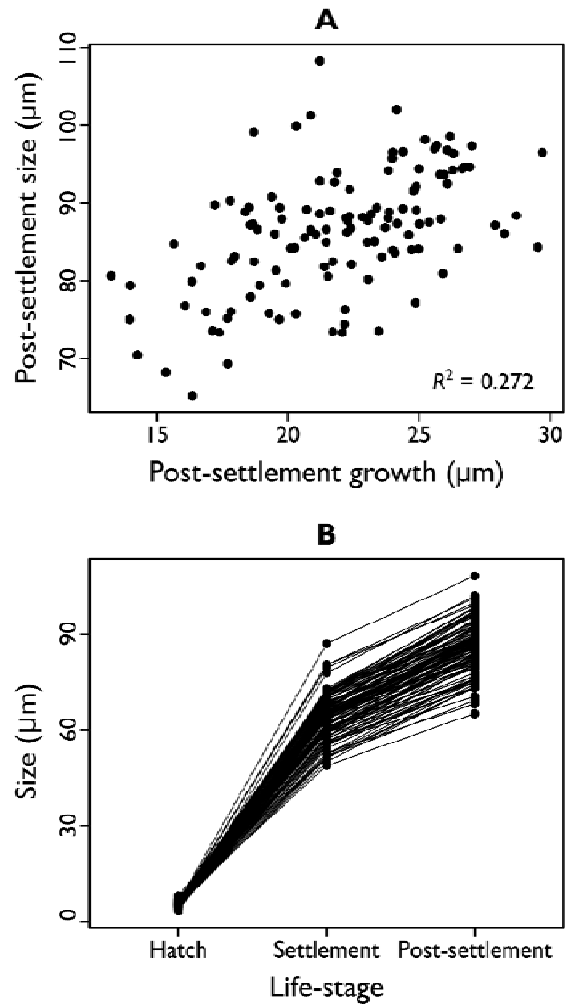


Fig. 4. Size and growth of *Bathygobius cocosensis* juveniles pooled across seasons ( $n = 124$ ). (A) The correlation between post-settlement growth with post-settlement size and the associated  $R^2$ . (B) The lapilli otolith size for each individual plotted against life-stage based on the distance from the nucleus to a specific increment: “Hatch” = the hatch check; “Settlement” = the settlement check; and “Post-settlement” = the fourth increment after the settlement check (see Fig. S2).

Table 1. Early life-history traits examined in *Bathygobius cocosensis*. The coefficient of variation (CV) for each trait was calculated for the entire sample (across seasons), with a sample size of  $n = 124$ . See Fig. S2 for illustration of trait definitions.

Trait	Units	Description	CV
Hatch size	$\mu\text{m}$	The distance between the otolith nucleus and the hatch check; a proxy for somatic size-at-hatching and maternal investment.	0.145
Larval duration	days	The number of days larvae spent as plankton; this was calculated as the number of daily increments from, and including, the hatch check to settlement check.	0.126
Pelagic growth	$\mu\text{m}$	The distance between the hatch check and the settlement check; the amount of growth attained during the pelagic stage.	0.114
Settlement size	$\mu\text{m}$	The distance between the nucleus and the settlement check; a proxy for somatic size-at-settlement.	0.104
Post-settlement growth	$\mu\text{m}$	The distance between the settlement check and the fourth-day-post-settlement increment; the amount of growth attained in the four days after settlement.	0.161
Post-settlement size	$\mu\text{m}$	The distance between the nucleus and the fourth-day-post-settlement increment; a proxy for somatic size four days after settlement.	0.090

Table 2. ANCOVA table for early life-history trait variation in juvenile *Bathygobius cocosensis* as a function of breeding season (SEASON: broad-scale temporal variation) and hatch dates (HATCH DATE: fine-scale temporal variation). Significant  $p$ -values ( $\alpha = 0.05$ ) are indicated with a “★”.

	SEASON		HATCH DATE	
	DF = 2, 120		DF = 1, 120	
Trait	$F$	$p$	$F$	$p$
Hatch size	0.119	0.889	0.713	0.401
Larval duration	7.156	0.001★	17.831	< 0.001★
Pelagic growth	2.035	0.135	3.038	0.084
Post-settlement growth	2.826	0.063	0.477	0.491
Settlement size	1.998	0.140	2.724	0.101
Post-settlement size	0.470	0.626	2.912	0.090

Table 3. Predictability of early life-history trait variation in juvenile *Bathygobius cocosensis* based on variation in preceding traits. The total explained variation ( $R^2$ -values, range from 0–1) in a trait based on path analyses (combination of indirect and direct effects) is presented for each breeding season and the pooled dataset.

<b>Trait</b>	<b>2013/14</b>	<b>2014/15</b>	<b>2015/16</b>	<b>Pooled</b>
Larval duration	0.008	0.036	0.046	0.020
Pelagic growth	0.358	0.600	0.322	0.445
Post-settlement growth	0.100	0.051	0.004	0.016