

# Replenishment success linked to fluctuating asymmetry in larval fish

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**Abstract** Fluctuating asymmetry (FA), defined as random deviations from perfect symmetry, has become a popular tool with which to examine the effects of stress during the development of bilaterally symmetrical organisms. Recent studies have suggested that FA in otoliths may serve as an indicator of stress in fish larvae. We examined the relationship between otolith asymmetry and temporal patterns in the occurrence of late-stage larvae to a tropical reef (i.e. replenishment) for the Caribbean lizardfish, *Saurida suspicio* (family Synodontidae). Late-stage larvae were collected in light traps over a period of 18 consecutive lunar months in the San Blas Archipelago, Panama. Asymmetry within otolith pairs was calculated from 24 variables: area, perimeter, longest and shortest axis of the otolith and 20 shape descriptors (Fourier harmonics). Otolith asymmetry was correlated strongly with fluctuations in lunar light trap catches. Two measured variables, otolith area and one of the 20 shape descriptors, accounted for 60% of the variability in lunar replenishment of *S. suspicio*. Individuals from small replenishment pulses exhibited higher levels of asymmetry compared to larvae from large pulses. When dry and wet seasons were analysed separately, otolith asymmetry explained a surprising 70 and 97% of the variation, respectively. Although the generality of these results remain to be tested among other populations and species, otolith asymmetry may be an

important indicator, and potentially a predictor, of larval quality and replenishment success.

**Keywords** Body condition · Coral reef fish · Growth · Otolith shape · Recruitment

## Introduction

What are the larval processes that influence how many individuals join a juvenile population? This is a central question in aquatic ecology and conservation for species with complex life cycles, such as many fishes and invertebrates (Leis and McCormick 2002; Levin 2006). For these organisms, populations are maintained largely by new individuals entering the juvenile stage after a larval phase; a process known as replenishment. Despite the recent emphasis on population connectivity leading to renewed interest in larval biology (Levin 2006), we know little about what determines fluctuations in larval supply (Leis and McCormick 2002). This is due partly to the rarity and patchiness of larval assemblages coupled with the difficulty of identifying early- to late-stage larvae, particularly in the tropics (Leis and McCormick 2002).

Studies that have made use of late-stage larvae as they arrive on the reef to metamorphose and settle have yielded considerable insight into larval processes (Leis and McCormick 2002). These studies have focussed on attributes of individual larvae and used morphological, biochemical or physiological methods to suggest that patterns of survivorship are influenced by historical processes on a range of temporal scales (Leis and McCormick 2002). In recent years, the history of growth and environmental conditions incorporated within the microstructure of calcified structures, such as otoliths, has proved to be a powerful tool in early life

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history studies of marine fishes (for review see Thorrold et al. 2007). Moreover, the asymmetry within pairs of otoliths has been proposed as a useful index of body condition/health (Somarakis et al. 1997; Grønkjær and Sand 2003; Gagliano et al. 2008), and may provide an important insight into the history of larval condition that is independent of other otolith-derived characteristics, such as growth (Gagliano and McCormick 2004).

Fluctuating asymmetry (FA), defined as random, independent deviations from perfect bilateral symmetry, has become a popular measure of developmental instability, which reflects the inability of an organism to produce a regular phenotype due to genetic and environmental perturbations during ontogeny (Palmer 1996; Palmer and Strobeck 2003). Many studies have examined FA in relation to a wide variety of ecological and evolutionary research questions (Møller and Swaddle 1997). As increased environmental stress may lead to elevated values of asymmetry, FA has been used as a bio-indicator of health and quality in a diverse range of taxa, including insects (e.g. Campero et al. 2008), fish (e.g. Somarakis et al. 1997; Eriksen et al. 2008), amphibians (e.g. Parris and Cornelius 2004), reptiles (e.g. Qualls and Andrews 1999), birds (e.g. Anciaes and Marini 2000) and mammals (e.g. Badyaev et al. 2000).

While a number of morphological traits have been used for FA analyses in fishes, only a few studies have examined otoliths in relation to FA and environmental stress (e.g. El Niño conditions, Alados et al. 1993; parasitism, Escos et al. 1995; nutrition, Somarakis et al. 1997, Grønkjær and Sand 2003; salinity, Panfili et al. 2005). Somarakis et al. (1997) detected a negative relationship between morphologically derived condition indices and FA between left and right otolith in anchovy (*Engraulis encrasicolus*) larvae, suggesting that FA can be used as an indicator of larval condition. Grønkjær and Sand (2003) concluded that otolith asymmetry could be used to distinguish between cod (*Gadus morhua*) larvae of considerably different body condition. Gagliano and McCormick (2004) found that otolith shape, independent of otolith size and fish size, was sensitive to food availability in two species of tropical reef damselfish, *Amphiprion akindynos* and *Pomacentrus amboinensis*. These and other studies (Gagliano et al. 2008; M. Gagliano and M. I. McCormick, manuscript submitted) suggest that otolith microstructure contains a history of stress in larval fish. The asymmetry between left and right otolith may thus reveal information about processes important to replenishment of fish populations.

The present study explores the relationship between otolith asymmetry and the magnitude of lunar replenishment pulses in a tropical reef-associated fish, the Caribbean lizardfish *Saurida suspicio* (family Synodontidae). Previous studies have suggested that larvae within large pulses may

exhibit attributes that favour replenishment success (Wilson and Meekan 2002; Bergenius et al. 2002). According to this theory, larvae from smaller pulses are predicted to display higher levels of asymmetry, indicative of poorer conditions for larval development, growth and survival. Conversely, individuals from large pulses are predicted to exhibit lower levels of asymmetry due to more favourable conditions during the larval stage. The current study suggests that otolith asymmetry may be linked (directly or vicariously) to the processes that drive cohort strength.

## Materials and methods

### Study species

Lizardfish are a common component of light trap catches in the Caribbean (e.g. Valles et al. 2001; Wilson 2001). They provide a good model for replenishment studies due to their bipartite lifecycle with a long pelagic larval stage (pelagic larval duration: ~40 days, T. Lemberget unpublished data) followed by a bottom-dwelling adult life, as well as their frequent occurrence in light traps throughout the year in the San Blas region, Panama (Wilson 2001).

### Study site and sampling

*Saurida suspicio* larvae were collected in light traps over a period of 18 consecutive lunar months, from December 1996 to June 1998, encompassing two dry seasons and one wet season (Wilson 2001). The study was conducted in the San Blas Archipelago, which extends along the Caribbean coast of Panama (9°34'N, 78°58'W). Due to the strong correlation between light trap catches and magnitude of settlement of species in other reef systems (Milicich et al. 1992; Sponaugle and Cowen 1996), the number of larvae caught in light traps per lunar month was used as a relative measure of lunar replenishment. A detailed description of the study site and sampling protocol is outlined in Wilson (2001). Briefly, three replicate light traps were moored just below the surface, approximately 100 m apart and at a distance of 50–100 m away from the reef edge in three reef habitats: back-reef, lagoon and exposed. Most reef fish larvae arrive on the reef to settle in lunar pulses (Robertson 1992). The light traps were therefore sampled for 19 successive nights centred on the new moon in each lunar month. Larvae were preserved in 75% ethanol immediately upon light trap retrieval.

### Otolith preparation

Data were pooled across the three sampling locations due to low numbers of fish in individual light traps. Within

each lunar month, catches were subdivided into 1 mm size classes of standard length. A sub-sample of *Saurida suspicio* larvae based on the proportion of the abundance in each size class was then used for otolith asymmetry analysis. Sample sizes were low in some months because only fully intact otoliths could be used for shape analysis. A total of 222 otolith pairs were used for the asymmetry analysis. Left and right sagitta were removed from each larva and cleaned of adhering tissue. A high contrast image of each otolith, sulcus side up, was captured with a DP12 Olympus digital camera connected to a compound microscope at 4× magnification.

### Shape analysis

Image analysis software (OPTIMAS, version 6.5) was used to extract shape information. Each otolith outline was automatically traced anti-clockwise starting from the tip of the rostrum. Prior to outline detection, the image of the right otolith was reflected about the centroid, such that the right otolith would mirror the shape of the left otolith if the pair was symmetric. Four morphometric otolith measurements (longest axis, shortest axis, area and perimeter) and 20 Fourier harmonics were extracted using the centroid as the otolith centre.

Fourier Shape analysis has been thoroughly described elsewhere (Bird et al. 1986; Campana and Casselman 1993). In brief, it expresses the outline of a shape as a periodic function using additive cosine waves (i.e. harmonics), derived from equally spaced coordinates along the outline. Each harmonic has an amplitude and a phase angle component. The low order harmonics (generally the first eight harmonics) express gross shape, such as mean radius (first harmonic), elongation (second harmonic), triangularity (third harmonic) and so forth, while high order harmonics provide information about the finer details of the outline (e.g. Bird et al. 1986). The outline can be completely reconstructed by adding a sufficient number of successive harmonics (e.g. Campana and Casselman 1993).

A Fourier series for each otolith outline was extracted from 128 polar coordinates according to Christopher and Waters (1974). Due to the difficulty of normalising, and consequently interpreting, phase angles, only the amplitudes were included in the asymmetry analysis (e.g. Campana and Casselman 1993). All harmonics were standardised for variation in size and rotation by setting the “0th” harmonic to zero and dividing all harmonics by the first harmonic (e.g. Begg and Brown 2000). The reconstructive error was calculated for 30 randomly selected otolith pairs according to Jarvis et al. (1978). Twenty harmonics described 99.9% of the reconstructive error of the otolith shape. Some studies that have used Fourier harmonics to discriminate between different fish stocks

have included only the first ten harmonics in the analysis, as these tend to describe at least 95% of the shape variability (e.g. Campana and Casselman 1993). In asymmetry studies, however, where the difference in a bilateral trait is likely to be small (Palmer 1996), this approach may fail to detect subtle deviations. The first 20 harmonics, excluding the 0th and the first harmonic, were consequently used for further asymmetry analysis. These were numbered from 2 to 21, accordingly.

### Asymmetry analysis

Asymmetry was first calculated as the difference of right minus left ( $R - L$ ) side of the extracted shape variables. The absolute value ( $|R - L|$ ) was then used as an index of FA (Palmer and Strobeck 2003). Suspected outliers were tested statistically using Dixon’s test for outliers (Rorabacher 1991; Sokal and Rohlf 1995). Three outliers were detected and rejected on statistical grounds and excluded from all further analysis.

### Measurement error

It is important to establish that the observed difference between left and right otolith is caused by asymmetry and not by measurement error (Palmer and Strobeck 2003). To quantify all levels of potential error, measurement error was calculated from five otolith pairs of randomly selected larvae; three images were captured of each otolith and each otolith outline was traced three times. A two-way nested analysis of variance (ANOVA) was used to examine the magnitude of measurement error in relation to differences in the shape of otoliths between individual fish [factors: Individual, Image (Individual)]. The error term indicates measurement error due to tracing. Measurement error was examined for left and right sagitta separately.

### Assumptions of FA analysis

#### *Departure from normality*

Based on the distribution and mean of the signed values ( $R - L$ ), bilateral asymmetry can be classified into three types: FA, directional asymmetry and antisymmetry (Palmer 1994; Palmer and Strobeck 2003). FA variables should exhibit a normal distribution with a zero mean as a result of random, independent variations between the sides. Directional asymmetry, which arises when one of the sides is consistently larger than the other (Palmer 1994; Palmer and Strobeck 2003), has a normal distribution with a mean different to zero. Antisymmetry occurs when one side is randomly larger than the other, usually causing a bimodal distribution about the mean (Palmer 1994; Palmer and

Strobeck 2003). The shape variables were tested for ideal FA by examining the distribution, mean, skew and kurtosis of the signed values ( $R - L$ ) for each lunar month. Critical values for skew were obtained from Zar (1999), while kurtosis was examined according to the table presented in Palmer and Strobeck (2003). Contrary to standard kurtosis tables (e.g. Zar 1999), this table takes into account the fact that kurtosis distributions are highly skewed for sample sizes  $<200$ . It is therefore likely to provide a more correct measure of kurtosis (Palmer and Strobeck 2003). The mean ( $R - L$ ) was compared to zero by one-sample  $t$  tests (Palmer and Strobeck 2003). To alleviate the problem of compounded errors from multiple non-independent tests, a Bonferroni correction for multiple tests ( $n = 18$ ;  $\alpha = 0.003$ ) was applied for the mean and the skew (Palmer 1994). Due to the skewed distribution of kurtosis discussed above, no Bonferroni correction was applied for kurtosis values. Instead, a higher significant level ( $\alpha = 0.01$ ) was adopted.

#### Size dependency

The presence of size dependency complicates interpretations of FA because the observed trend may be due to size differences rather than FA (Palmer and Strobeck 2003). We corrected for size by using a residual-based FA index, rather than the commonly used index that divides the absolute value of the trait difference by the trait mean [ $|R - L|/((R + L)/2)$ ] (Palmer and Strobeck 2003). The latter has often been criticised for not removing the size effect due to the lack of independence of the numerator and denominator (Palmer and Strobeck 2003). Hence, a residual-based index is likely to be a more robust way of correcting for size. Pearson's correlation coefficient was used to examine the relationship between the unsigned value ( $|R - L|$ ) of the FA trait and the trait size [ $(R + L)/2$ ] (Palmer and Strobeck 2003). If a significant positive correlation was present, the standardised residuals of the regression model ( $|R - L|$ ) on [ $(R + L)/2$ ] were used as an index of asymmetry rather than the absolute value of ( $R - L$ ). Assumptions of normality and homoscedasticity were examined using residual analysis.

#### Body condition index

The standardised residuals from the regression analysis of larval length versus weight were used as an index of body condition (e.g. Meka and McCormick 2005). Length was measured as standard length in centimetres and wet weight was measured in grams. Weights and lengths were not corrected for shrinkage in the 75% ethanol preservative. However, since individual fish were in the preservative for similar lengths of time, the length/weight relationship

should still give a useful relative index of body condition. The relationship between mean body condition and lunar replenishment ( $n = 18$  months) and between body condition and FA ( $n = 222$  individuals) were examined.

#### Growth

Daily-formed otolith increments retain a permanent record of larval growth, which can be indicative of relative feeding success among larvae. Otolith asymmetry coupled with growth analysis may therefore provide clues about periods during which larvae are more susceptible to developmental instability. The relationship between FA and otolith growth was examined for 42 larvae from the same cohort (lunar month 10). Lunar month 10, which represents the largest replenishment pulse, was chosen to get sufficient number of larvae that had been exposed to the same pelagic environment. Although any observed relationship between FA and growth may be weak, since all larvae were from the same lunar month, growth increments are more likely to reflect relative feeding success than other environmental differences.

Otolith growth sections were prepared according to Wilson and McCormick (1997) by grinding the otoliths with lapping film (0.3–12  $\mu\text{m}$ ) to produce transverse sections through the nucleus. The sections were view and photographed under a compound microscope at 40 $\times$  magnification. The distance between growth increments was measured along the longest otolith axis using the OPTIMAS software program. Assuming that the first increment is formed at the time of hatching (e.g. Wellington and Victor 1989), average otolith growth was calculated in three different ways for FA comparisons: (1) from time of hatching to time of capture, (2) over short time intervals during the larval phase (1–5, 6–10, 11–15, 16–20, 21–25, 26–30, 31–35 and 36–38 days, where the 38th day signifies the age of the youngest larvae and was used as a cut-off point), and (3) average growth during the 5 days prior to capture.

#### Data analysis

Otolith asymmetry was investigated using 24 shape variables (4 morphometric and 20 Fourier harmonics) that were first calculated for each individual larva and then averaged for each lunar month. This cohort-based approach enabled us to compare otolith asymmetry with the magnitude of larval abundance in the 18 lunar months of sampling. A stepwise multiple regression analysis was used to examine the relationship between FA variables and the magnitude of lunar replenishment, where number of larvae per lunar month was  $\log_{10}$  transformed. Regression analysis is not used here to attain an equation for predicting

cohort strength, but rather as a means of exploring the dataset because it allows us to examine each variable's contribution to the observed lunar replenishment pattern (Philippi 1993). Multiple regression is well suited to detect leading indicators for any time series (e.g. growth) from other time series without lagged effects (Davies 2002). To investigate the robustness of the relationships found, the multiple regression was rerun, firstly excluding the lunar month with fewest larvae (lunar month 15) and secondly, excluding the lunar months where otolith asymmetry was based on very few samples (otolith pairs <10: lunar month 2, 4, 7, 15). In addition, wet and dry seasons were analysed separately. Analyses were repeated using forward and backward model selection to confirm the composition of these best fit models (Post 2005).

The relationship between body condition and the magnitude of replenishment was examined by Pearson's correlation coefficient. A stepwise regression analysis was used to investigate the relationship between the 24 FA variables and body condition and between the FA variables and the different estimates of growth. SPSS (Version 11.0.4 for Macintosh) was used for all statistical analysis.

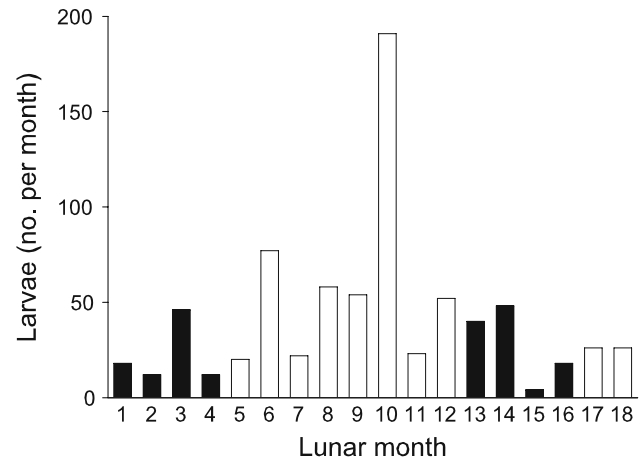
## Results

### Lunar replenishment pattern

In total, 747 *S. suspicio* larvae were collected in light traps during the 18 lunar months of sampling. Larvae were caught throughout the sampled period, but the lunar pulses of replenishment varied in magnitude. Overall, more larvae were caught in the wet season compared to the dry season. The highest and lowest pulses occurred in lunar month 10 (wet season) and lunar month 15 (dry season) with 191 and 4 larvae, respectively (Fig. 1).

### Measurement error

A breakdown of the variance for the left and right otoliths showed that, in 23 of the 24 variables, less than 0.8% of the variability in otolith shape could be attributed to measurement error (i.e. differences among images within an otolith and differences among tracings for an individual image). In all but one case, more than 99.2% of the variability in each of the shape measures was accounted for by differences among individual fish. In one variable, harmonic 20, 12.5% of the variability was due to measurement error while 87.5% of the variability was due to differences among individuals. Thus, the likelihood of measurement error confounding asymmetry results is very small, and even harmonic 20 may contain a useful amount of data with which to define differences among individuals.



**Fig. 1** Total numbers of Caribbean lizardfish (*Saurida suspicio*) larvae caught in light traps per lunar month during dry (black bars) and wet (white bars) seasons in the San Blas Archipelago, Panama, from December 1996 to June 1998

### Assumptions of FA

#### Departure from normality

One-sample *t* tests suggested that no directional asymmetry was present ( $P > 0.003$ , Bonferroni correction). Examination of skew revealed that harmonic 19 in lunar month 11 and harmonic 12 in lunar month 10 exhibited significant negative skew ( $P < 0.003$ ). Significant kurtosis was detected in 10 out of 432 cases (24 variables  $\times$  18 months). Five showed significant positive kurtosis (harmonic 19 in lunar month 3, harmonic 12 in lunar month 10, harmonic 9 and harmonic 19 in lunar month 11 and harmonic 5 in lunar month 12;  $P < 0.01$ ), and five exhibited significant negative kurtosis (harmonic 14 in lunar month 1, harmonic 19 in lunar month 5, harmonic 19 in lunar month 7, harmonic 18 in lunar month 11 and harmonic 7 in lunar month 12;  $P < 0.01$ ). These values are conservative because the Bonferroni correction was not applied (see “Materials and methods”).

#### Size dependency

There was no correlation between trait size  $[(R + L)/2]$  and unsigned asymmetry  $(R - L)$  when the lunar months were analysed separately. After pooling all months, however, the unsigned asymmetry in otolith area, perimeter and shortest axis exhibited a significant positive correlation with their respective trait sizes, while longest axis showed a non-significant positive correlation (area: Pearson's  $r = 0.318$ ,  $P < 0.001$ ; perimeter: Pearson's  $r = 0.295$ ,  $P < 0.001$ ; shortest axis: Pearson's  $r = 0.136$ ,  $P = 0.043$ ; longest axis: Pearson's  $r = 0.118$ ,  $P = 0.079$ ). The standard residuals generated from each regression model were

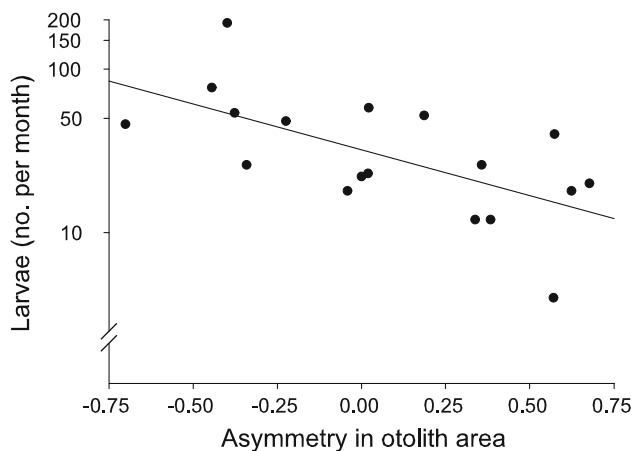


then used as asymmetry indices. Although the longest axis did not exhibit a significant correlation, a residual-based asymmetry index was still applied to make it comparable with the other morphometric indices. No size dependency was detected in the already standardised Fourier harmonics.

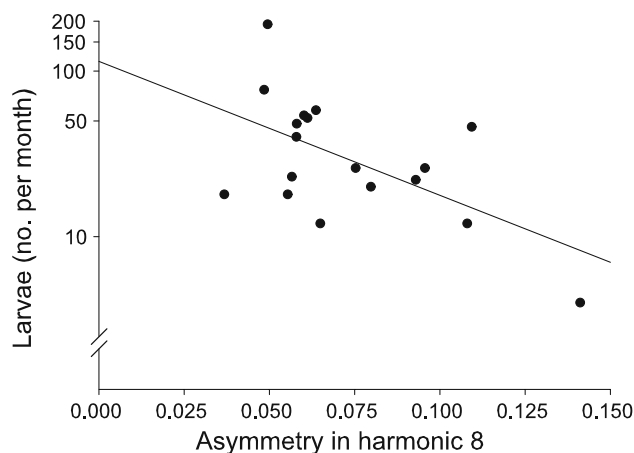
Otolith FA and relationship with replenishment

Larvae from small replenishment pulses exhibited higher asymmetry values compared to larvae from large pulses. Multiple regression between number of larvae per lunar month ( $\log_{10}$  transformed) and 24 FA variables showed that FA in otolith area ( $R_p = -0.558$ ) and harmonic 8 ( $R_p = -0.503$ ) was significantly negatively correlated with magnitude of lunar replenishment (Figs. 2, 3; Table 1a). Combined, they accounted for 59.7% of the observed variability in lunar replenishment of *S. suspicio*. When the smallest replenishment pulse (lunar month 15) was excluded from the analysis to explore the robustness of this correlation, only FA in otolith area ( $R_p = -0.588$ ) was significantly correlated with lunar replenishment, accounting for 30.2% of the variability in replenishment (Table 1b). Furthermore, when the four months with lowest sample sizes (<10 fish each) were excluded from the analysis, FA in otolith area ( $R_p = -0.547$ ) still accounted for 24.1% of the variability in lunar light trap catches (Table 1c), suggesting a robust relationship.

When the dry and wet season were analysed separately, the relationship between FA in otolith shape measures and replenishment was much stronger than for the whole



**Fig. 2** Relationship between number of *S. suspicio* larvae caught in light traps per lunar month and the asymmetry between left and right otolith area, calculated from a residual-based asymmetry index (residuals from the regression model  $(|R - L|)$  on  $[|R - L| / ((R + L) / 2)]$ . The zero value on the x-axis therefore does not depict perfect symmetry, but reflects the asymmetry value derived from standardised residuals. Data are from 18 lunar months of larval collections (see Fig. 1) in  $\log_{10}$  scale



**Fig. 3** Relationship between number of *S. suspicio* larvae caught in light traps per lunar month ( $\log_{10}$  transformed) and asymmetry between left and right harmonic eight, calculated as the absolute value of  $(R - L)$  from the standardised harmonics. Data are from 18 lunar months of larval collections (see Fig. 1) in  $\log_{10}$  scale

**Table 1** Relationship between fluctuating asymmetry (FA) variables and lunar replenishment pattern of *Saurida suspicio*. The analysis was performed using a stepwise multiple regression. The partial correlation coefficients ( $R_p$ ), adjusted regression coefficients for the model ( $r^2_{Adj}$ ) (for all variables listed) and  $P$  values are given. The dependent variable was the number of larvae per lunar month ( $\log_{10}$  transformed), while the independent variables available for model selection were harmonic 2 to harmonic 21, otolith area, perimeter, longest and shortest axis

Variable	$R_p$	$r^2_{Adj}$	$P$ value
All months included <sup>a</sup>			
Otolith area	-0.558	0.597	<0.0001
Harmonic 8	-0.503		
One lunar month excluded <sup>b</sup>			
Otolith area	-0.588	0.302	0.013
Four lunar months excluded <sup>c</sup>			
Otolith area	-0.547	0.241	0.043
Dry season <sup>d</sup>			
Harmonic 10	-0.542		
Harmonic 16	0.512	0.967	<0.0001
Perimeter	-0.414		
Wet season <sup>e</sup>			
Harmonic 8	-0.747	0.695	0.006
Harmonic 13	0.481		

<sup>a</sup> For 18 successive lunar months ( $n = 222$ )  
<sup>b</sup> When the lunar month with lowest number of larvae was excluded (lunar months = 17,  $n = 219$ )  
<sup>c</sup> When lunar months with less than ten fish were excluded (lunar months = 14,  $n = 200$ )  
<sup>d</sup> During dry season (lunar months = 8,  $n = 90$ )  
<sup>e</sup> During wet season (lunar months = 10,  $n = 132$ )

dataset. FA in harmonic 10, harmonic 16 and perimeter were correlated with the replenishment during the dry season, accounting for 96.7% of the variability (Table 1d), while FA in harmonic 8 and harmonic 13 accounted for 69.5% of the variability in number of larvae caught during the wet season (Table 1e). Surprisingly, FA in harmonic 16 in the dry season and FA in harmonic 13 in the wet season were positively correlated with lunar light trap catches. These are both high order harmonics that describe the finer details of the otolith outline.

#### Body condition

There was no significant relationship between body condition and lunar replenishment. A stepwise regression analysis showed a very weak significant relationship between body condition and the FA in one shape descriptor (harmonic 15:  $n = 222$ ,  $R_{Adj} = 0.019$ ,  $r_p = 0.154$ ,  $P < 0.05$ ).

#### Otolith growth

To explore the relationship between growth and otolith FA, multiple regressions were undertaken on otolith growth estimates (i.e. ten different measures, undertaken in separate analyses) and 24 FA attributes for 42 larvae from the same cohort (lunar month 10). Regression analysis identified two time intervals during which FA in one single shape descriptor was significantly, although weakly, correlated with growth. FA in harmonic 14 was negatively correlated with otolith growth from 6 to 10 days after hatching ( $n = 42$ ,  $R_{Adj} = 0.18$ ,  $P < 0.05$ ), while FA in harmonic 4 was positively correlated with otolith growth from 16 to 20 days after hatching ( $n = 42$ ,  $R_{Adj} = 0.084$ ,  $P < 0.05$ ). There was no significant relationship between other estimates of growth and FA ( $n = 42$ ,  $P > 0.05$ ).

### Discussion

The asymmetry of morphological features has been used as a proxy for the stress or fitness of individuals and populations in a broad range of taxa (Lens et al. 2002). While some studies have found FA to be of little value (e.g. Bjorksten et al. 2000) many studies have documented higher levels of asymmetry in individuals from environments that lead to some form of stress, regardless of whether the source of stress is nutritional (e.g. starvation: Campero et al. 2008), social (e.g. dominance status: Witter and Swaddle 1994), chemical (e.g. acidification: Øxnevad et al. 2002) or genetic (Leary and Allendorf 1989). The strength of the association between FA and stress varies considerably between taxa, traits, and types of stress (Lens et al. 2002). However, because stress elevates energy

expenditure, which compromises developmental stability (Møller and Swaddle 1997), FA is often believed to be a more sensitive indicator of health than other measures of fitness (Clarke 1995). The strong correlation between FA and magnitude of replenishment found in the present study suggests that FA either has a direct impact on survival or co-varies with processes that influence cohort strength. Whatever the mechanism underlying the relationship between FA and replenishment, if this relationship proves consistent then it may be useful to include FA in models that predict future population dynamics. Previous studies have concluded that it is the potential of FA to predict future, stress-associated changes in fitness that makes FA a powerful tool in ecology and conservation (Leary and Allendorf 1989; Clarke 1995; Lens et al. 2002).

Few studies have explored the influences of asymmetry on dispersal and recruitment processes. Indeed, this study is the first to explore the relationship between FA and temporal replenishment pattern in an organism with a complex life cycle. Here, otolith asymmetry correlated strongly with fluctuations in the intensity of replenishment of *S. suspicio* in the San Blas Archipelago, and explained a surprising 70–97% of the variation, depending upon season. Larvae from small replenishment pulses exhibited elevated values of otolith asymmetry compared to larvae from larger pulses, in keeping with the hypothesised link between asymmetry and sub-optimal conditions for development during the larval phase. Other studies have found poor relationships between their measure of FA and dispersal success. Asymmetry of the wing, tail and tarsus did not affect orientation during migration in three European birds (*Sylvia atricapilla*, *Phoenicurus phoenicurus*, *Erithacus rubecula*) (Matessi 1997). Likewise, the FA of wing shape did not differentiate between dispersal rates in a butterfly (*Melitaea cinxia*) from the north coast of Estonia (Breuker et al. 2007). Our results suggest that the information incorporated into the otolith shape is in some way related (either directly or indirectly through other variables) to the strength of replenishment pulses. This provides us with a valuable tool to investigate the processes that generate fluctuations in replenishment of demersal fishes.

When data from wet and dry seasons were combined, there was a strong negative correlation between otolith asymmetry and the replenishment of *S. suspicio* that accounted for 60% of the variation. However, the exclusion of a single lunar month, which represents the smallest pulse (with only three usable larvae), reduced the power of otolith asymmetry to explain fluctuations in replenishment to 30%. This data point may be of biological importance because it is the smallest replenishment pulse, and is therefore expected to have the highest level of asymmetry. The positive link between otolith growth and magnitude of replenishment detected in two Caribbean reef fishes

(Bergenius et al. 2002; Wilson and Meekan 2002) suggests that individuals within the smallest pulse are of biological interest. These individuals may represent the lower levels of development, growth or condition able to survive in the system at that point in time. Thus, the data point may actually be one of the most important in the dataset. Alternatively, it may be a spurious data point, which artificially inflates the correlation due to low sample size. Ideally, it would have been best to sample fish larvae that died prior to settlement, since this would have allowed us to directly test the prediction that survivors (as measured here) would display lower levels of asymmetry compared to those that died earlier in the larval phase. However, the problems of capturing an unbiased sample of tropical fish larvae and then identifying them to species, makes attempting to hindcast conditions in the pelagic phase from settlement-stage larvae the best option currently available.

So what is the mechanism underlying the correlation between otolith FA and magnitude of replenishment? Asymmetry may simply co-vary with other historical events or processes that affect replenishment, or it may affect performance and survival directly (Møller and Swaddle 1997). The exact mechanisms are unclear, probably not mutually exclusive, and all are speculative to a greater or lesser extent.

There may be temporal patterns in reproductive output that influence the availability of settlement stage larvae and their asymmetry. Seasonality in larval abundance is the norm and is generally linked to gross seasonality in the reproductive output of populations. If the environmental cues that promote fish breeding also promote larval symmetry, then a negative correlation between asymmetry and replenishment may result. This might be the case in cold water and temperate environments where spawning of stocks is often timed with environmental conditions that promote larval growth (e.g. Cushing 1990). However, in low latitude locations close to the equator, such as the present study site, fish reproduction and settlement often occur throughout the year (e.g. Wilson 2001; Srinivasan and Jones 2006), making this mechanism less likely to be important. Robertson et al. (1993) concluded that the poor correlation they detected between production and settlement of fishes in the San Blas Archipelago was likely to be due to variable processes during the planktonic larval phase.

Otolith FA may also co-vary with temporal trends in parental stress as local populations move in and out of breeding state. Cycles in activity, the intensity of behavioural interactions and stress within social systems are often closely linked to temporal changes in breeding biology (e.g. Pankhurst et al. 1999; Leary et al. 2008). Typically, higher courtship and breeding intensity is associated with higher corticosteroids (e.g. Fichtel et al.

2007; Ostner et al. 2008). Maternal cortisol has been shown to directly influence larval size in tropical damselfish and salmonids (McCormick 1998; Eriksen et al. 2006), and has also been recently shown to promote otolith asymmetry (Eriksen et al. 2008; M. Gagliano and M. I. McCormick, manuscript submitted). Parents in more stressful situations produce larvae that are smaller (McCormick 1999; Eriksen et al. 2006) and more asymmetrical (Eriksen et al. 2008; M. Gagliano and M. I. McCormick, manuscript submitted). In contrast to the previous mechanism, current research thus predicts that stress should positively correlate with breeding activity and larval production, leading to a positive association between the availability of early-stage larvae and the levels of FA. If larval asymmetry is associated with survival, then we would expect this relationship to result in a decoupling of the spawning and replenishment cycles—a characteristic that is evident for many fish stocks (e.g. Robertson et al. 1993). To explore this hypothesis it will be necessary to quantify the levels of parental stress and larval FA (at hatching, prior to any selective loss) and how it changes throughout the year.

Alternatively, otolith asymmetry may directly affect performance because otoliths are central to balance and hearing, and asymmetric otolith pairs are likely to exhibit a different centre of mass compared to symmetrical pairs (Popper et al. 2005). Experimental evidence shows that fish with asymmetrical otoliths are more susceptible to motion sickness when exposed to altered gravitational environments (e.g. Hilbig et al. 2002). Although this demonstrates that otolith asymmetry can affect motion dynamics in fishes, the thresholds at which this may occur in natural environments are unknown. Furthermore, Gagliano et al. (2008) found that otolith FA influenced whether a late-larval stage damselfish (*Pomacentrus amboinensis*) was attracted to high or low frequency sounds. Sound is thought to be important in the detection of reefs (e.g. Simpson et al. 2005), and also in successful habitat selection once settled (Higgs 2005). Otolith FA may have a crucial impact on replenishment through a direct effect on hearing. Clearly, experiments are required to investigate the relationship between otolith FA, hearing and the capacity for behavioural compensation.

Otolith asymmetry may also influence survival through subtle effects on performance in the early larval phase. These effects may be amplified through development due to the covariance of traits that influence survival success, such as growth, swimming speed, burst speed, prey capture success and success in avoiding prey (e.g. Fisher et al. 2000). A longitudinal study that compared the frequency of otolith FA in newly hatched versus newly metamorphosed damselfish from the same cohort (Gagliano et al. 2008) suggested that mortality might be selective for larvae that



display otolith asymmetry. A recent review of FA in fish otoliths also suggested that FA is established in early development, and that the embryonic FA signature is maintained during larval development (Lychakov et al. 2006). However, Gagliano and McCormick (2004) experimentally manipulated the food availability to juveniles of two species of damselfish and found that it affected otolith shape, indicating that some degree of compensation of initial FA may be possible. The extent to which fishes can compensate for embryonic FA and the factors (and intensities) that influence FA at particular developmental stages require further study.

FA has frequently been used as a measure of individual quality, but the relationship between FA and other measures of fitness are often unclear, ranging from not evident (e.g. Dufour and Weatherhead 1998) to poor and highly complex (Leung et al. 2000). In the present study, we found poor relationships between otolith FA and body condition and between otolith FA and larval growth history. Otolith asymmetry may provide a record of larval stress that is not reflected in measures of body condition at settlement, possibly due to compensatory processes during the larval stage. Alternatively, these findings may be the result of our index of condition (residuals of a length vs weight relationship) being a poor measure of fish health among lunar pulses (Fey and Hare 2008). Otolith growth analysis identified two time intervals during the larval phase (6–10 and 16–20 days after hatching) during which FA and growth were significantly correlated. Although these correlations were very weak and inconclusive, possibly because all larvae were from the same lunar month, they suggest that processes during the early part of the larval stage may be important to growth and asymmetry. As noted, recent experimental evidence suggests that otolith asymmetry may even be derived from events prior to hatching (Eriksen et al. 2008; M. Gagliano and M. I. McCormick, manuscript submitted). Because otoliths are metabolically inert and conservative in their response to nutritional changes (Molony and Choat 1990), FA in late-stage larvae is likely to reflect a cumulative effect of environmental stress factors (Parsons 1990). Consequently, it may be difficult to identify the onset and the sources of asymmetry. Experiments are needed to test the sensitivity of otolith asymmetry to different types and magnitudes of environmental stressors to better understand causes of FA and how it is linked to cohort strength in fish populations.

In conclusion, replenishment of *S. suspicio* was strongly correlated with otolith asymmetry, suggesting that processes promoting or co-varying with otolith asymmetry may be major determinants of replenishment success in this species. Further work is needed to determine the temporal consistency of this correlation. As otolith asymmetry is likely to retain a record of early larval stress, it may

become a useful variable to include in models for predicting cohort strength. These findings encourage further research into (1) the mechanisms that drive otolith asymmetry; and (2) the influences of otolith asymmetry on larval performance, quality/health and ultimately survival. The current study examined only otolith asymmetry among lunar cohorts. It is necessary for future research to shift the focus to larvae at an individual level and use experiments to explore the causes and effects of otolith asymmetry in greater detail.

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