



## Abstract

Calcification in many invertebrate species is predicted to decline due to ocean acidification. The potential effects of elevated  $p\text{CO}_2$  and reduced carbonate saturation state on other species, such as fish, are less well understood. Fish otoliths (earbones) are composed of aragonite, and thus, might be susceptible to either the reduced availability of carbonate ions in seawater at low pH, or to changes in extracellular concentrations of bicarbonate and carbonate ions caused by acid-base regulation in fish exposed to high  $p\text{CO}_2$ . We reared larvae of the clownfish *Amphiprion percula* from hatching to settlement at three  $\text{pH}_{\text{NBS}}$  and  $p\text{CO}_2$  levels (control: pH 8.15 and 404  $\mu\text{atm CO}_2$ ; intermediate: pH 7.8 and 1050  $\mu\text{atm CO}_2$ ; extreme: pH 7.6 and 1721  $\mu\text{atm CO}_2$ ) to test the possible effects of ocean acidification on otolith development. There was no effect of the intermediate treatment (pH 7.8 and 1050  $\mu\text{atm CO}_2$ ) on otolith size, shape, symmetry between left and right otoliths, or otolith elemental chemistry, compared with controls. However, in the more extreme treatment (pH 7.6 and 1721  $\mu\text{atm CO}_2$ ) otolith area and maximum length were larger than controls, although no other traits were affected. Our results support the hypothesis that pH regulation in the otolith endolymph of fish exposed to elevated  $p\text{CO}_2$  can lead to increased precipitation of  $\text{CaCO}_3$  in otoliths of larval fish, as proposed by an earlier study, however, our results also show that sensitivity varies considerably among species. Importantly, our results suggest that otolith development in clownfishes is robust to even the more pessimistic changes in ocean chemistry predicted to occur by 2100.

## 1 Introduction

Absorption of additional carbon dioxide ( $\text{CO}_2$ ) from the atmosphere is causing ocean pH to decline and is reducing the availability of carbonate ions required by many marine species to form calcium carbonate ( $\text{CaCO}_3$ ) shells and skeletons (Feely et al., 2004; Orr et al., 2005). As the saturation states of aragonite and calcite decline, the

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calcification rate of corals, molluscs, echinoderms and other invertebrates that secrete these forms of  $\text{CaCO}_3$  is predicted to decline (Gattuso et al., 1998; Riebesell et al., 2000; Gazeau et al., 2007; Kleypas and Yates, 2009). If the current trajectory of global  $\text{CO}_2$  emissions is maintained, atmospheric  $\text{CO}_2$  concentrations could reach between 730–1020 ppm by the end of the century (Meehl et al., 2007; Raupach et al., 2007). This would cause ocean pH to decline 0.3–0.4 units compared to current-day levels (Caldeira and Wickett, 2005) and reduce the concentration of carbonate ions in the shallow ocean by nearly 50% compared to the pre-industrial era. The consequences of such a reduction in carbonate ion concentration is likely to be serious for many calcifying species (Hoegh-Guldberg et al., 2007; Fabry et al., 2008; Doney et al., 2009; Hofmann et al., 2010). Possible impacts of elevated  $\text{CO}_2$  and ocean acidification on non-calcifying species, such as fishes, is still poorly known, but could potentially include effects on a range of physiological (Pörtner and Farrell, 2008), developmental (Pankhurst and Munday, 2011) and behavioural processes (Munday et al., 2009, 2010). Early life history stages are likely to be most vulnerable because physiological homeostasis might not be fully developed and their small body size makes them more sensitive to environmental variation (Ishimatsu et al., 2008; Melzner et al., 2009).

In general, marine fish appear to be relatively tolerant to mild increases in ambient  $\text{CO}_2$ , probably because their well-developed mechanisms for acid-base regulation allow them to compensate for cellular acidosis caused by exposure to elevated  $p\text{CO}_2$  (Pörtner et al., 2005; Ishimatsu et al., 2008; Melzner et al., 2009). A concern, however, is that fish otoliths (earbones) are composed of aragonite, and therefore, could be susceptible to the declining carbonate ion concentrations associated with ocean acidification. Just as calcification rates in corals decline as the aragonite saturation state falls, it may become more difficult for fish to precipitate aragonite in their otoliths as the availability of carbonate ions in seawater declines. Alternatively, otolith growth may be affected by regulatory mechanisms used by fish to maintain their internal pH as ambient  $p\text{CO}_2$  increases. Changes in extracellular concentrations of carbonate and bicarbonate caused by acid-base regulation in a high  $\text{CO}_2$  environment could affect

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the precipitation of  $\text{CaCO}_3$  in the otolith (Payan et al., 1997). Indeed, one recent study reported that otoliths were larger in larval fishes exposed to elevated  $\text{CO}_2$ , possibly because pH regulation caused carbonate concentrations to increase within the endolymph (Checkley et al., 2009). Maintenance of a steady pH within the endolymph, despite an influx of  $\text{CO}_2$ , would increase the abundance of bicarbonate and carbonate ions in the endolymph, both of which could potentially be used in aragonite precipitation. Finally, physiological stress can also affect otolith size, shape, and the symmetry between left and right otoliths (Gagliano and McCormick, 2004; Payan et al., 2004). Thus, even if acid-base regulation or declining aragonite saturation state do not directly affect otolith development, increased stress caused by elevated  $p\text{CO}_2$  could potentially influence otolith shape and symmetry.

Changes to ocean chemistry associated with declining pH, or changes in extracellular dissolved inorganic carbon (DIC) concentration due to acid-base regulation, could also affect otolith chemistry. The chemical composition of otoliths responds to environmental variation, including concentrations of elements in ambient water, temperature, and salinity (Campana and Thorrold, 2001). In many instances ambient water chemistry is the primary control on the incorporation of elements in otoliths, as evidenced by high correlations between the ratio of these elements to Ca at the site of otolith deposition and their concentration in the external environment (e.g. Bath et al., 2000; Martin and Thorrold, 2005). Bicarbonate ion concentration and pH has been shown to influence Sr, but not Mg, incorporation in calcitic foraminifera (Dissard et al., 2010). However, the effect of changes in aqueous carbonate chemistry on fish otolith composition remains unknown.

Fish ears detect sound, body orientation and acceleration from the position of the otoliths in the inner ear and movement of the otoliths over sensory hair cells (Helfman et al., 1997). Any substantial change to the size, shape, or symmetry of otoliths could have serious implications for individual performance and survival (Gagliano et al., 2008). To date, two studies have examined the potential effects of ocean acidification on otolith growth and development. Checkley et al. (2009) found that otolith size

increased in larval seabass exposed to  $\sim 1000 \mu\text{atm CO}_2$  for eight days. In contrast, Munday et al. (2011) detected no effects of  $\sim 850 \mu\text{atm CO}_2$  on size, shape or symmetry of otoliths on juvenile spiny damselfish, a species without a larval phase. No studies have investigated the possible effects of ocean acidification on otolith chemistry. We reared larvae of a model species, the clownfish *Amphiprion percula*, through their entire larval phase at two ocean acidification scenarios to test if exposure to elevated  $p\text{CO}_2$  and reduced carbonate ion concentrations affects otolith size, shape, symmetry (between left and right otoliths) and otolith chemistry compared with current-day controls. The experimental conditions are consistent with more extreme scenarios for the years 2100 (pH 7.8 and  $1050 \mu\text{atm } p\text{CO}_2$ ) and 2200–2300 (pH 7.6,  $1721 \mu\text{atm } p\text{CO}_2$ ) based on a business-as-usual trajectory of  $\text{CO}_2$  emissions (Caldeira and Wickett, 2005; Meehl et al., 2007). This combination of treatments allowed us to test the susceptibility of otolith development in clownfish to changes in seawater chemistry and to investigate potential mechanisms involved.

## 2 Materials and methods

### 2.1 Larval rearing

Clownfish were reared in a 70 000 l recirculating seawater system at James Cook University's experimental marine aquarium facility. Adult breeding pairs were kept in separate 70 l aquariums supplied with a continuous flow of filtered seawater at  $18 \text{ l h}^{-1}$ . Breeding pairs laid eggs on the underside of a terracotta pot placed in their aquarium. Pots were checked each morning for the presence of eggs. On discovering a new clutch of egg, the parental aquarium was assigned to one of three  $\text{pH}_{\text{NBS}}$  levels (8.15 (control), 7.8, 7.6) and pH adjusted as described below. On the night of hatching (6–8 days) the pot was removed from the parental aquarium and transferred to a 100 l larval rearing aquarium set to the same pH as the parental aquarium. Larvae were reared in a semi-closed system, where each aerated aquarium had no water flow during the

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day and was then slowly flushed with filtered seawater each night. This daily cycle ensured that larvae could feed ad-libitum throughout daylight hours and that any un-consumed food was removed each night. Larvae were fed rotifers (*Brachionus* sp.) at 5 individuals ml<sup>-1</sup> each morning for the first three days. *Artemia naupli* were added at 1 individual ml<sup>-1</sup> each morning from day three. The ratio of *Artemia naupli* to rotifers was increased each day until larvae were only fed 5 *Artemia naupli* m<sup>-1</sup> from 8 days post-hatching. A summer light cycle of 13 h light/11 h dark was simulated with fluorescent lights. Larvae were reared to the end of their larval phase (11-d post hatching), at which time behaviour consistent with competency to settle (attraction to the sides of the rearing aquarium) and the appearance of benthic colouration was observed.

## 2.2 Seawater manipulation

Seawater pH<sub>NBS</sub> in the parental aquariums and larval rearing tanks that had been allocated to the 7.8 or 7.6 treatments was adjusted by CO<sub>2</sub> injection. A separate pH-controller (Tunze Aquarientechnik, Germany) was attached to each aquarium to maintain pH at the desired level. The pH controller was connected to a laboratory-grade glass pH probe in the aquarium and to an electronic solenoid connected to a cylinder of CO<sub>2</sub>. The solenoid injected a slow stream of CO<sub>2</sub> into a diffuser (Red Sea Reactor 500) at the bottom of the aquarium whenever the pH of the aquarium seawater rose above the set point. A precision needle valve inserted before the solenoid was adjusted to ensure a slow, steady, delivery of CO<sub>2</sub> into the diffuser. Using this method is was possible to constantly maintain pH within ±0.05 units of the desired level and there was no detectable gradient in seawater pH within the aquarium. A diffuser without CO<sub>2</sub> injection was placed in each aquarium and rearing tank assigned to control conditions. The pH<sub>NBS</sub> of each aquarium was independently checked each day using a WP80 pH meter (TPS, Australia) calibrated with fresh pH buffers (Merk, Germany). CO<sub>2</sub> was only injected into aquariums to adjust pH when eggs or larvae were present. All water returned to a 60 000 l sump where it was degassed by stirring and using a 1000 l algal bio-remediation tank. Water temperature was maintained at 30°C ± 0.6

(SD) using electric heaters. Oxygen saturation was checked regularly with an Oxi 340i oxygen probe (WTW, Germany) and was always above 90%.

Total alkalinity ( $A_T$ ) of seawater at each pH level was measured weekly by titration. Average  $p\text{CO}_2$ , bicarbonate and carbonate ion concentrations were then estimated from pH and  $A_T$  in the program CO2SYS, using the constants of Millero et al. (2006). All seawater chemistry parameters are shown in Table 1. Average  $p\text{CO}_2$  for the 8.15 (control), 7.8 and 7.6 pH treatments was estimated to be 404, 1050 and 1721  $\mu\text{atm}$ , respectively.

### 2.3 Otolith morphometrics and Fourier Shape analysis

Settlement stage larvae (11-d post hatching) were sacrificed with a lethal dose of anaesthetic and stored in 75% ethanol. When required, fish were removed from the preservative, blotted dry, weighed (nearest mg) and photographed in a lateral position under a stereomicroscope. Standard length (SL) to nearest 0.01 mm was estimated for each fish from the digital photograph using image analysis (Optimas 6.5, Media Cybernetics). The sagittal otoliths were then removed and stored dry in well-plates. The left and right otolith of each individual was photographed to produce a calibrated, grey-scale image. Morphometric measurements (otolith area,  $\mu\text{m}^2$ ; maximum length,  $\mu\text{m}$ ; maximum breadth,  $\mu\text{m}$ ; rectangularity; circularity) and Fast Fourier descriptor were obtained from the images using Optimas. An automatic pixel gradient traced anti-clockwise around the silhouette of the otolith, starting from a common landmark (distal edge of the rostrum). Fourier analysis reproduces the outline of a shape by considering it to be an aggregate of simple wave forms, describable by a series of sine and cosine curves (Younker and Ehrlich 1977). Optimas provided a complex ( $a + b_i$ ) Cartesian fast Fourier transformation of the x-y coordinates of 128 equidistant points around the otolith outline. The complex Fourier descriptors were converted ( $\sqrt{a^2 + \sqrt{b^2}}$ ) to an absolute value for each harmonic (Lestrel, 1997). The images of the right otoliths were flipped horizontally so that the 128 sampling points were measuring the same part of the otolith for left and right otoliths.

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(2% HNO<sub>3</sub>) and a otolith certified reference material (Sturgeon et al. 2005), dissolved in 2% HNO<sub>3</sub> and diluted to a final Ca concentration of 8 µg g<sup>-1</sup>, were run every eight samples and used to correct for blank values and instrument mass bias, respectively, following FitzGerald et al. (2004). Detection limits were calculated as 3 standard deviations of the blanks that were run periodically throughout the analyses (*n* = 3). These limits were 5% of the average sample intensity for <sup>7</sup>Li, 3% of <sup>25</sup>Mg, 0.01% for <sup>43</sup>Ca, 8% for <sup>55</sup>Mn, 0.004% for <sup>88</sup>Sr, and 7% for <sup>138</sup>Ba. We estimated external precision (relative standard deviation, RSD) of the analyses by running a powdered otolith lab standard, dissolved in 2% HNO<sub>3</sub> and diluted to a Ca concentration of 8 µg g<sup>-1</sup>. Estimates of RSD (*n* = 3) were 2.2% for Li:Ca, 1.7% for Mg:Ca, 14% for Mn:Ca, 0.5% for Sr:Ca and 0.6% for Ba:Ca.

## 2.5 Statistical analyses

### 2.5.1 Otolith size and shape

Fish size could potentially influence otolith size and shape, therefore, we first examined the relationship between SL and each of the otolith morphometric traits. A relationship between SL and otolith maximum breadth, rectangularity and circularity was detected for either right or left otoliths, therefore ANCOVA was used to determine if these morphometric traits differed among pH treatments, using fish standard length as the covariate. There was no relationship between SL and otolith area or maximum length. Furthermore, the variance distributions of these two traits were not homogeneous (Levene's test: *P* < 0.05) and were not improved by transformations. Therefore, non-parametric Kruskal-Wallis median tests were used to determine if these morphometric traits differed among pH treatments.

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## 2.5.2 Size difference within otolith pairs

To investigate directional asymmetry of the otoliths (i.e. is the right or left otolith usually larger), signed differences in otolith morphometrics were obtained by subtracting the value for the left otolith from that of the right otolith (R–L) for otolith area, maximum length, and maximum breadth. This was not conducted for rectangularity and circularity because it is the absolute values of these parameters that have meaning. The frequency of positive versus negative scores among the pH treatments was then compared with a chi-square test of independence for each measure of otolith size.

To determine if pH treatment affected the magnitude of otolith asymmetry with respect to otolith area, maximum length, and maximum breadth, we used ANOVA to compare unsigned differences between left and right otoliths for these traits. ANCOVA was not required as there was no relationship between SL and unsigned differences for any of these traits. Unsigned differences in area and maximum length were log transformed to meet assumptions of homogeneity of variances.

## 2.5.3 Fourier analysis of otolith asymmetry

To investigate differences in the degree of asymmetry among treatments, unsigned differences between right and left otoliths in the standardised harmonic amplitude were obtained for each harmonic number. The data were analysed in two ways. First, a Kruskal-Wallis test was performed on the unsigned differences in standardised harmonic amplitudes for each harmonic number (shape descriptors). Parametric tests were not used because transformation of data did not produce normality. Second, a one-way between-groups multivariate analysis of variance (MANOVA) was performed using the unsigned differences between right and left otoliths in harmonic numbers  $H_2$  to  $H_{20}$  (shape descriptors) as dependent variables. The assumption of multivariate normality was checked by confirming normality for each of the dependent variables. The multivariate data were visualised using non-metric multidimensional scaling (MDS),

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which is presented with a stress coefficient that reflects how well the data have been reduced to two dimensions.

## 2.5.4 Otolith chemistry

A one-way MANOVA was used to compare multivariate elemental signatures among treatments. Assumptions of multivariate normality and homogeneity of covariance matrices were confirmed as above.

## 3 Results

### 3.1 Otolith Morphometrics

#### 3.1.1 Otolith size and shape

There was a significant difference among pH treatments in mean otolith area (left otoliths only) and otolith maximum length (left and right otoliths) (Table 3; Fig. 1). One-tailed t-tests (assuming unequal variance) revealed that mean area of otoliths in the 7.6 treatment was larger than controls for left otoliths ( $P = 0.02$ ), but not right otoliths ( $P = 0.11$ ) and that maximum length of otoliths in the 7.6 treatment was larger than controls for both left ( $P = 0.01$ ) and right otoliths ( $P = 0.03$ ). On average otoliths in the 7.6 treatment had a 15% greater area and were 10% longer than otoliths from control fish. There was also an apparent trend for otolith breadth to be larger in the 7.6 treatment (Fig. 1), however, this was not statistically significant (Table 3). In contrast to the 7.6 treatment, there were no differences in average size of otoliths in the 7.8 treatment compared with controls (Fig. 1;  $P > 0.1$  for all statistical comparisons).

There was a statistically significant effect of pH treatment on otolith rectangularity for right otoliths only (Table 3), with a lower rectangularity value in the pH 7.8 treatment compared with controls ( $P = 0.003$ ). However, the difference in the two values (0.76 and 0.73 for control and 7.8 respectively) represents a negligible difference in otolith

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shape, and there was no difference in shape between the pH 7.6 treatment and the controls. Therefore, this significant result does not appear to be causally related to the pH treatment. There was no significant effect of pH treatment on otolith circularity (Table 3), although there was a trend for decreased circularity for both left and right otoliths in the 7.6 treatment (Fig. 1).

Variation around the mean was lower in the pH 7.6 group for otolith area, maximum length and circularity (with one outlying value excluded) for both left and right otoliths (Fig. 1). This suggests that otoliths in this treatment were a more consistent size and shape than otoliths in the control and 7.8 groups.

Mean SL (mm) of larvae was not significantly different among treatments (ANOVA:  $F_{2,45} = 2.667$ ,  $P = 0.08$ ), however there was some variation in SL within treatments (Table 2). There was a highly significant effect of SL on otolith circularity for both left and right otoliths (Table 3). Regression analysis revealed a positive relationship between otolith circularity and fish standard length (left otoliths:  $y = 1.457x + 6.507$ ;  $r^2 = 0.517$ ; right otoliths:  $y = 1.060x + 8.880$ ;  $r^2 = 0.522$ ), indicating a shift from a square-shaped boundary in smaller fish towards a more triangular shape in larger fish.

### 3.1.2 Size difference within otolith pairs

There was no evidence of directional asymmetry, with either the left or the right otolith being the largest of a pair, and no difference in the distribution of positive and negative asymmetry among treatments for any of the otolith measurements (Chi-square  $P > 0.15$  in all cases). Similarly, there was no significant difference among pH treatments in the magnitude of otolith asymmetry with respect to otolith area ( $P = 0.24$ ), maximum length ( $P = 0.79$ ), or maximum breadth ( $P = 0.79$ ), although there was a tendency for the magnitude of difference between left and right otoliths be less in the 7.6 treatment compared to the controls and 7.8 treatment (Fig. 2). As observed above in the morphometric analysis, variation around the mean value was lower in the pH 7.6 group compared to the controls and pH 7.8 group.

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### 3.1.3 Fourier analyses of otolith asymmetry

There were no significant differences among pH treatments in the Fourier descriptors of otolith shape, either when harmonic numbers were analysed individually (Kruskal-Wallis test  $P > 0.05$  in all cases, except  $H_{13}$ ) or in the multivariate analysis of all 19 harmonic numbers (MANOVA  $F_{38,72} = 1.10$ ,  $P = 0.357$ , Wilks' Lambda = 0.4). The similarity among treatments, based on the combination of all harmonic numbers, was clearly evident in the MDS plot (Fig. 3). For  $H_{13}$  there was a significant difference in median harmonic amplitude between the control and pH 7.8 treatment (Mann-Whitney U test  $P = 0.02$ ), but no difference in any other pairwise comparison.

### 3.2 Otolith chemistry

Minor and trace element chemistry of larval clownfish otoliths appeared unaffected by the pH of ambient water in which they were reared. A one-way MANOVA found no significant differences in elemental signatures of otoliths among treatments (MANOVA  $F_{10,22} = 1.07$ ,  $P = 0.42$ , Wilks' Lambda = 0.45). Similarly, there were no consistent patterns in any of the individual elemental ratios as a function of pH (Fig. 4).

## 4 Discussion

We found that the size, shape, symmetry and elemental chemistry of otoliths in larval clownfish was unaffected by exposure to simulated levels of ocean acidification that could occur at the end of this century under a business-as-usual scenario of  $\text{CO}_2$  emissions (pH 7.8 and  $1050 \mu\text{atm } p\text{CO}_2$ ). However, in a more extreme treatment (pH 7.6 and  $1721 \mu\text{atm } p\text{CO}_2$ ) otolith area and maximum length were larger than control otoliths. Two other recent studies have examined potential effects of ocean acidification on fish otoliths. Checkley et al. (2009) detected increased otolith size in larval seabass, *Atractoscion nobilis*, exposed to elevated  $\text{CO}_2$  during the egg stage and up to 8 days post-hatching. Otoliths area was 7–9% and 15–17% larger than controls for

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fish reared at 993 and 2558  $\mu\text{atm CO}_2$ , respectively. In contrast, Munday et al. (2011) did not detect any effect of elevated  $\text{CO}_2$  on otolith size of juvenile spiny damselfish, *Acanthochromis polyacanthus*, reared for 3 weeks in treatments up to 841  $\mu\text{atm CO}_2$ . The different results of these two studies could have been due to: (1) the higher  $p\text{CO}_2$  levels used by Checkley et al. (2009), (2) the absence of a larval phase in the spiny damselfish, which could make them less susceptible to elevated  $\text{CO}_2$ , or (3) different durations of the two experiments (Munday et al., 2011). Our results presented here suggest that otolith size can indeed be affected by reduced pH and elevated  $p\text{CO}_2$ , as proposed by Checkley et al. (2009), however, sensitivity varies among species. Clownfish otoliths were not affected at  $\sim 1000 \mu\text{atm CO}_2$ , whereas seabass otoliths were significantly larger at this concentration, but otoliths of both species were larger at higher  $p\text{CO}_2$  concentrations. Thus, elevated  $p\text{CO}_2$  appears to affect otolith development in both species, but larval clownfishes are more tolerant of elevated  $\text{CO}_2$  than larval seabass. There is increasing evidence that ocean acidification affects different calcifying species in very different ways (Langer et al., 2009; Ries et al., 2009; Dupont et al., 2010; Kroeker et al., 2010) and these results indicate that the same types of variation might be observed among species without carbonate skeletons.

The variable responses observed among the three species studied to date may be related to their life histories. The spiny damselfish, *Acanthochromis polyacanthus*, studied by Munday et al. (2010) has direct developing juveniles that remain on the reef after hatching. Juveniles shelter with their parents in small caves, where  $\text{CO}_2$  levels are likely to become elevated due to respiration. Consequently, newly hatched *A. polyacanthus* may be adapted to periods of high ambient  $\text{CO}_2$ . The clownfish, *Amphiprion percula*, has pelagic larvae, but is a demersal spawner. Benthic eggs are likely to experience significant fluxes in ambient  $\text{CO}_2$  due to consumption of  $\text{CO}_2$  by photosynthesis during the day and release of  $\text{CO}_2$  by respiration of reef organisms at night (Munday et al., 2008). Hatching from benthic eggs may precondition larval clownfishes to moderate increases in ambient  $\text{CO}_2$ . In contrast, the seabass *Atractoscion nobilis* is a pelagic spawner, so both eggs and larvae are likely adapted to a low  $\text{CO}_2$  environment, and

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may be more susceptible to elevated  $\text{CO}_2$ . Further studies on a range of benthic and pelagic spawners are required to test this hypothesis.

If reduced seawater carbonate ion ( $\text{CO}_3^{2-}$ ) concentrations affected the rate of otolith precipitation we would expect to see a decline in otolith size in acidified seawater. Instead there was a trend for otolith size to increase in these conditions. Fish actively regulate their acid-base balance through bicarbonate ( $\text{HCO}_3^-$ ) accumulation and ion exchange across the gills (Claiborne et al., 2002; Evans et al., 2005; Brauner and Baker, 2009) and, thus, are able to compensate for intra- and extra-cellular acidosis caused by increased  $p\text{CO}_2$ . Changes to extracellular  $\text{HCO}_3^-$  and  $\text{CO}_3^{2-}$  concentrations caused by acid-base regulation at high  $p\text{CO}_2$  could potentially influence the precipitation of  $\text{CaCO}_3$  in structures such as otoliths (Payan et al., 1997). Checkley et al. (2009) proposed that larval fish controlled the concentration of  $\text{H}^+$  and  $\text{Ca}^{2+}$  ions in the blood plasma and endolymph (the fluid surrounding an otolith), but not the neutral molecule  $\text{CO}_2$ . Consequently, maintenance of a constant pH in the endolymph despite elevated extracellular  $p\text{CO}_2$  would lead to increased  $\text{CO}_3^{2-}$  concentrations in the endolymph due to diffusion of  $\text{CO}_2$  from blood plasma across the endolymphatic membrane. Increased  $\text{CO}_3^{2-}$  concentrations in the endolymphatic fluid may, in turn, increase the rate of aragonite precipitation at the otolith surface. However,  $\text{CO}_2$  concentrations are generally higher in endolymphic fluid than in blood plasma (Takagi, 2002), perhaps related to the removal of  $\text{H}^+$  ions produced during disassociation of  $\text{HCO}_3^-$  to  $\text{CO}_3^{2-}$  at the time of aragonite precipitation (Payan et al., 1999). Under this scenario there would be net diffusion of  $\text{CO}_2$  out of, not into, the endolymph. Alternatively,  $\text{HCO}_3^-$  accumulation as a result of acid-base compensation, either in the plasma or endolymph, may promote otolith accretion if  $\text{HCO}_3^-$  is the preferred dissolved inorganic carbon species for calcification (Allemand et al., 2007; Herfort et al., 2008). Consistent with this hypothesis, increased calcification of cuttlebone is also associated with increased extracellular  $\text{HCO}_3^-$  in cephalopods exposed to elevated  $p\text{CO}_2$  (Gutowska et al., 2010). The precise mechanisms involved in acid-base regulation in larval marine fishes are not fully understood (Brauner, 2009), nor are the cellular mechanisms involved in calcification

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of many marine species (Hofmann et al., 2010). Nonetheless, acid-base regulation and biological control of endolymph pH are likely to be central to the changes in otolith growth observed in these two species of fish when exposed to high CO<sub>2</sub> (Romanek and Gauldie, 1996; Allemand et al., 2007; Payan et al., 1997).

5 Although we detected an increase in otolith area and maximum length in the more extreme treatment (pH 7.6, pCO<sub>2</sub> 1721 µatm), we did not observe any effects on other otolith characteristics, such as otolith shape or symmetry. Although a number of studies have suggested that physiological stress can increase otolith asymmetry (Gagliano et al., 2008), we found no evidence that ocean acidification is likely to affect the magnitude  
10 of symmetry between left and right otoliths, even at relatively high CO<sub>2</sub> concentrations. Similarly we found no effects of altered seawater carbonate chemistry on the elemental chemistry of the otoliths. Elemental ratios of all five elements were within the range typically reported for aragonitic marine fish otoliths (Campana and Thorrold, 2001). We also found no visual evidence of vaterite or calcite in the any of the treatment  
15 otoliths, which if present would have resulted in relatively low Sr:Ca values (Veizer, 1983; Tomas and Geffen, 2003). Taken together, our data suggest that the larval clownfish were capable of regulating endolymphic fluid chemistry even in waters with pH values significantly lower than open ocean values.

Our results demonstrate a number of important points. First, the tendency for otolith  
20 size to increase and become less variable in the higher CO<sub>2</sub> treatment is consistent with an effect on otolith development caused by the physiological process of pH regulation in fishes. Further studies are required to pinpoint the exact processes involved. Second, our results indicate that otolith development in larval clownfishes is robust to the levels of ocean chemistry change that may occur over the next 50–100 years, even if higher  
25 levels are likely to influence otolith size. Finally, we emphasize that there is likely to be considerable variation among species in their sensitivity to elevated CO<sub>2</sub> and reduced pH. Determining the traits that make some species more susceptible than others will aid in making predictions about the longer-term and ecosystem level effects of ocean acidification.

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## References

- Allemand, D., Mayer-Gostan, N., de Pontual, H., Boeuf, G., and Payan P., Fish otolith calcification in relation to endolymph chemistry, in: Handbook of Biomineralization – Biological Aspects and Structure Formation, edited by: Bäuerlein, E., 291–308, Wiley-VCH, Weinheim, 2007.
- Bath, G. E., Thorrold, S. R., Jones, C. M., Campana, S. E., McLaren, J. W., and Lam, J. W. H.: Strontium and barium uptake in aragonitic otoliths of marine fish, *Geochim. Cosmochim. Acta*, 64, 1705–1714, 2000.
- Brauner, C. J.: Acid-base Balance, in: Fish Larval Physiology, edited by: Finn, R. N., Kapoor, B. G., 185–198, Science Publishers, Enfield, 2009
- Brauner, C. J. and Baker, D. W.: Patterns of Acid-Base Regulation During Exposure to Hypercarbia in Fishes, in: Cardio – Respiratory Control in Vertebrates, edited by: Glass, M. L. and Wood, S. C., 43–63, Springer, Berlin, 2009
- Campana, S. E. and Thorrold, S. R.: Otoliths, increments, and elements: keys to a comprehensive understanding of fish populations?, *Can. J. Fish. Aquat. Sci.*, 58, 30–38, 2001.
- Doney, S. C., Fabry, V. J., Feely, R. A., and Kleypas, J. A.: Ocean Acidification: The Other CO<sub>2</sub> Problem, *Annu. Rev. Mar. Sci.*, 1, 169–192, doi:10.1146/annurev.marine.010908.163834, 2009.
- Dupont, S., Ortega-Martinez, O., and Thorndyke, M.: Impact of near future ocean acidification on echinoderms, *Ecotoxicology*, 19, 449–462, 2010.
- Caldeira, K. and Wickett, M. E.: Ocean model predictions of chemistry changes from carbon dioxide emissions to the atmosphere and ocean, *J. Geophys. Res.*, 110, C09S04, doi:doi:10.1029/2004JC002671, 2005.
- Checkley, D. M., Dickson, A. G., Takahashi, M., Radich, J. A., Eisenkolb, N., and Asch, R.: Elevated CO<sub>2</sub> Enhances Otolith Growth in Young Fish, *Science*, 324, 1683–1683, doi:10.1126/science.1169806, 2009.
- Claiborne, J. B., Edwards, S. L., and Morrison-Shetlar, A. I.: Acid-base regulation in fishes: Cellular and molecular mechanisms, *J. Exp. Zool.*, 293, 302–319, 2002.
- Dissard, D., Nehrke, G., Reichart, G. J., and Bijma, J.: Impact of seawater pCO<sub>2</sub> on calcification

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and Mg/Ca and Sr/Ca ratios in benthic foraminifera calcite: results from culturing experiments with *Ammonia tepida*, *Biogeosciences*, 7, 81–93, 2010, <http://www.biogeosciences.net/7/81/2010/>.

Evans, D. H., Piermarini, P. M., and Choe, K. P.: The multifunctional fish gill: Dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste, *Physiol. Rev.*, 85, 97–177, doi:10.1152/physrev.00050.2003, 2005.

Fabry, V. J., Seibel, B. A., Feely, R. A., and Orr, J. C.: Impacts of ocean acidification on marine fauna and ecosystem processes, *ICES J. Mar. Sci.*, 65, 414–432, doi:10.1093/icesjms/fsn048, 2008.

Feely, R. A., Sabine, C. L., Lee, K., Berelson, W., Kleypas, J., Fabry, V. J., and Millero, F. J.: Impact of anthropogenic CO<sub>2</sub> on the CaCO<sub>3</sub> system in the oceans, *Science*, 305, 362–366, 2004.

Gagliano, M. and McCormick, M. I.: Feeding history influences otolith shape in tropical fish, *Mar. Ecol. Prog. Ser.*, 278, 291–296, 2004.

Gagliano, M., Depczynski, M., Simpson, S. D., and Moore, J. A. Y.: Dispersal without errors: symmetrical ears tune into the right frequency for survival, *Proc. R. Soc. B.*, 275, 527–534, doi:10.1098/rspb.2007.1388, 2008.

Gattuso, J.-P., Frankignoulle, M., Bourge, I., Romaine, S., and Buddemeier, R. W.: Effect of calcium carbonate saturation of seawater on coral calcification, *Global Planet. Change*, 18, 37–46, 1998.

Gazeau, F., Quiblier, C., Jansen, J. M., Gattuso, J.-P., Middelburg, J. J., and Heip, C. H. R.: Impact of elevated CO<sub>2</sub> on shellfish calcification, *Geophys. Res. Lett.*, 34, L07603, doi:10.1029/2006GL028554, 2007.

Gutowska, M. A., Melzner, F., Portner, H. O., and Meier, S.: Cuttlebone calcification increases during exposure to elevated pCO<sub>2</sub> in the cephalopod *Sepia officinalis*, *Mar. Biol.*, 157, 1653–1663, 2010.

Hofmann, G. E., Barry, J. P., Edmunds, P. J., Gates, R. D., Hutchins, D. A., Klinger, T., and Sewell, M. A.: The effects of ocean acidification on calcifying organisms in marine ecosystems: an organism-to-ecosystem perspective, *Annu. Rev. Ecol. Syst.*, 41, 127–147, 2010.

Helfman, G. S., Collette, B. B., and Facey, D. E.: *The Diversity of Fishes*. Blackwell Science, Malden, 1997.

Herfort, L., Thake, B., and Taubner, I.: Bicarbonate stimulation of calcification and photosynthesis in two hermatypic corals, *J. Phycol.*, 44, 91–98, doi:10.1111/j.1529-8817.2007.00445.x,

2008.

- Hoegh-Guldberg, O., Mumby, P. J., Hooten, A. J., Steneck, R. S., Greenfield, P., Gomez, E., Harvell, C. D., Sale, P. F., Edwards, A. J., Caldeira, K., Knowlton, N., Eakin, C. M., Iglesias-Prieto, R., Muthiga, N., Bradbury, R. H., Dubi, A., and Hatziolos, M. E.: Coral reefs under rapid climate change and ocean acidification, *Science*, 318, 1737–1742, doi:10.1126/science.1152509, |ISSN 0036-8075, 2007.
- Ishimatsu, A., Hayashi, M., and Kikkawa, T.: Fishes in high-CO<sub>2</sub>, acidified oceans, *Mar. Ecol. Prog. Ser.*, 373, 295–302, doi:10.3354/meps07823, 2008.
- Kleypas, J. A. and Yates, K. K.: Coral reefs and ocean acidification, *Oceanography*, 22, 108–117, 2009.
- Kroeker, K. J., Kordas, R. L., Crim, R. N., and Singh, G. G.: Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms, *Ecol. Lett.*, 13, 1419–1434, doi:10.1111/j.1461-0248.2010.01518.x, 2010.
- Langer, G., Nehrke, G., Probert, I., Ly, J., and Ziveri, P.: Strain-specific responses of *Emiliana huxleyi* to changing seawater carbonate chemistry, *Biogeosciences*, 6, 2637–2646, 2009, <http://www.biogeosciences.net/6/2637/2009/>.
- Lestel, P. E.: *Fourier Descriptors and their Application in Biology*, Cambridge University Press, 1997.
- Martin, G. B. and Thorrold, S. R.: Temperature and salinity effects on magnesium, manganese and barium in the otoliths of larval spot (*Leiostomus xanthurus*), *Mar. Ecol. Prog. Ser.*, 293, 223–232, 2005.
- Meehl, G. A., Stocker, T. F., Collins, W. D., Friedlingstein, P., Gaye, A. T., Gregory, J. M., Kitoh, A., Knutti, R., Murphy, J. M., Noda, A., Raper, S. C. B., Watterson, I. G., Weaver, A. J., and Zhao, Z.-C.: Global Climate Projections, in: *Climate Change 2007: The Physical Science Basis*, Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, edited by: Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K. B., Tignor, M., and Miller, H. L., 686–688, Cambridge University Press, Cambridge, UK, 2007.
- Melzner, F., Gutowska, M. A., Langenbuch, M., Dupont, S., Lucassen, M., Thorndyke, M. C., Bleich, M., and Portner, H. O.: Physiological basis for high CO<sub>2</sub> tolerance in marine ectothermic animals: pre-adaptation through lifestyle and ontogeny?, *Biogeosciences*, 6, 2313–2331, 2009, <http://www.biogeosciences.net/6/2313/2009/>.

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- Munday, P. L., Jones, G. P., Pratchett, M. S., and Williams, A. J.: Climate change and the future for coral reef fishes, *Fish Fish.*, 9, 261–285, 2008.
- Munday, P. L., Dixon, D. L., Donelson, J. M., Jones, G. P., Pratchett, M. S., Devitsina, G. V., and Doving, K. B.: Ocean acidification impairs olfactory discrimination and homing ability of a marine fish, *Proc. Natl. Acad. Sci. USA*, 106, 1848–1852, doi:10.1073/pnas.0809996106, 2009.
- Munday, P. L., Dixon, D. L., McCormick, M. I., Meekan, M., Ferrari, M. C. O., and Chivers, D. P.: Replenishment of fish populations is threatened by ocean acidification, *Proc. Natl. Acad. Sci. U.S.A.*, 107, 12930–12934, doi:10.1073/pnas.1004519107, 2010.
- Munday, P. L., Gagliano, M., Donelson, J. M., Dixon, D. L., and Thorrold, S. R.: Ocean acidification does not affect the early life history development of a tropical marine fish, *Mar. Ecol. Prog. Ser.*, in press, 2011.
- Orr, J. C., Fabry, V. J., Aumont, O., Bopp, L., Doney, S. C., Feely, R. A., Gnanadesikan, A., Gruber, N., Ishida, A., Joos, F., Key, R. M., Lindsay, K., Maier-Reimer, E., Matear, R., Monfray, P., Mouchet, A., Najjar, R. G., Plattner, G. K., Rodgers, K. B., Sabine, C. L., Sarmiento, J. L., Schlitzer, R., Slater, R. D., Totterdell, I. J., Weirig, M. F., Yamanaka, Y., and Yool, A.: Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms, *Nature*, 437, 681–686, doi:10.1038/nature04095, 2005.
- Palmer, R. A. and Strobeck, C.: Fluctuating asymmetry: measurement, analysis, patterns. *Annu. Rev. Ecol. Syst.*, 17, 391–421, 1986.
- Pankhurst, N. W. and Munday, P. L.: Effects of climate change on fish reproduction and early life history stages, *Mar. Freshwater Res.*, in press, 2011.
- Payan, P., Borelli, G., Boeuf, G., and Mayer-Gostan, N.: Relationship between otolith and somatic growth: consequence of starvation on acid-base balance in plasma and endolymph in the rainbow trout *Oncorhynchus mykiss*, *Fish Physiol. Biochem.*, 19, 35–41, 1998.
- Payan, P., De Pontual, H., Edeyer, A., Borelli, G., Boeuf, G., and Mayer-Gostan, N.: Effects of stress on plasma homeostasis, endolymph chemistry, and check formation during otolith growth in rainbow trout (*Oncorhynchus mykiss*), *Can. J. Fish. Aquat. Sci.*, 61, 1247–1255, doi:10.1139/f04-059, 2004.
- Payan, P., Kossmann, H., Watrin, A., MayerGostan, N., and Boeuf, G.: Ionic composition of endolymph in teleosts: Origin and importance of endolymph alkalinity, *J. Exp. Biol.*, 200, 1905–1912, 1997.
- Payan, P., Edeyer, A., Pontual, H., Borelli, G., and Mayer-Gostan, N.: Chemical composition of

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saccular endolymph and otolith in fish inner ear: lack of spatial uniformity, *Am. J. Physiol.*, 277, R123–R131, 1999.

Portner, H. O., Langenbuch, M., and Michaelidis, B.: Synergistic effects of temperature extremes, hypoxia, and increases in CO<sub>2</sub> on marine animals: From Earth history to global change, *J. Geophys. Res.-Oceans*, 110, C09S10, doi:10.1029/2004jc002561, 2005.

Portner, H. O. and Farrell, A. P.: Physiology and Climate Change, *Science*, 322, 690–692, doi:10.1126/science.1163156, 2008.

Raupach, M. R., Marland, G., Ciais, P., Le Quere, C., Canadell, J. G., Klepper, G., and Field, C. B.: Global and regional drivers of accelerating CO<sub>2</sub> emissions, *Proc. Natl. Acad. Sci. USA*, 104, 10288–10293, doi:10.1073/pnas.0700609104, 2007.

Riebesell, U., Zondervan, I., Rost, B., Tortell, P. D., Zeebe, R. E., and Morel, F. M. M.: Reduced calcification of marine plankton in response to increased atmospheric CO<sub>2</sub>, *Nature*, 407, 364–367, 2000.

Ries, J. B., Cohen, A. L., and McCorkle, D. C.: Marine calcifiers exhibit mixed responses to CO<sub>2</sub>-induced ocean acidification, *Geology*, 37, 1131–1134, doi:10.1130/g30210a.1, 2009.

Romanek, C. S. and Gauldie, R. W.: A predictive model of otolith growth in fish based on the chemistry of the endolymph, *Comp. Biochem. Physiol. A*, 114, 71–79, 1996.

Takagi, Y.: Otolith formation and endolymph chemistry: a strong correlation between the endolymph saturation state and pH in the endolymph of the trout otolith organ, *Mar. Ecol. Prog. Ser.*, 231, 237–245, 2002.

Tomas, J. and Geffen, A. J.: Morphology and composition of aragonite and vaterite otoliths of deformed laboratory reared juvenile herring from two populations, *J. Fish Biol.* 63, 1383–1401, 2003.

Veizer, J.: Trace elements and isotopes in sedimentary carbonates, in: *Reviews in Mineralogy volume 11, Carbonates: mineralogy and chemistry*, edited by: Reeder, R. J., 265–299, 1983.

Yunker, J. L. and Ehrlich, R.: Fourier biometrics: harmonic amplitudes as multivariate shape descriptors, *Systematic Zool.*, 26, 336–342, 1977.



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**Table 2.** Fish sample sizes and dimensions used in morphometric and Fourier analyses.

Treatment	Morphometrics			Fourier
	n	Fish standard length (mm)		n
		Mean ( $\pm$ SD)	Range	
pH 8.15	16	7.2 ( $\pm$ 1.75)	5.2–10.6	19
pH 7.8	16	8.0 ( $\pm$ 1.22)	6.0–9.7	19
pH 7.6	16	6.9 ( $\pm$ 1.22)	5.6–9.0	19

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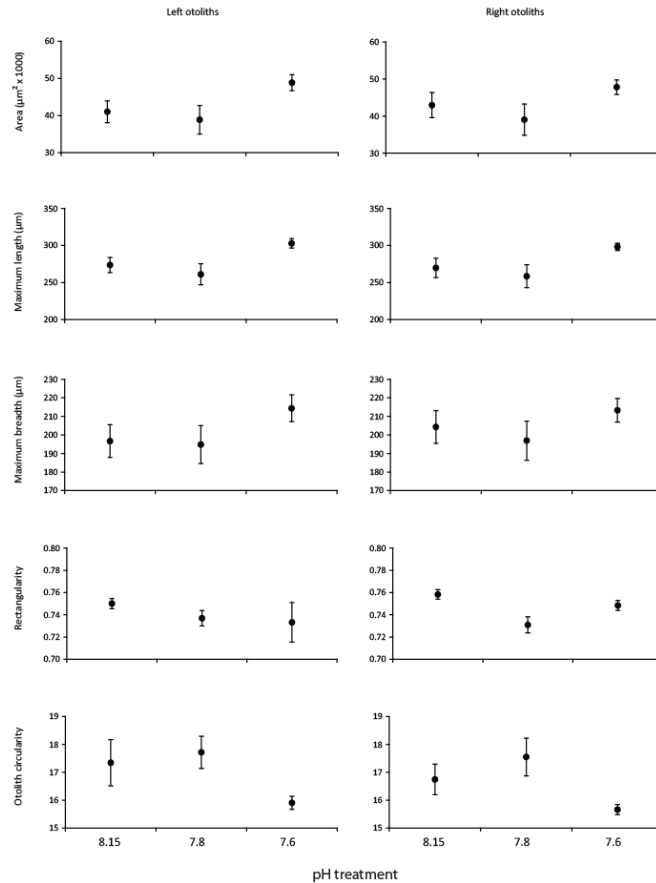
**Table 3.** Results of Kruskal-Wallis median test (area, maximum length) and ANCOVA (breadth, circularity, rectangularity) for left and right otoliths of larval clownfish reared at three pH treatments (8.15, 7.8, and 7.6).

(a) Left otoliths				
Variable	Source	d.f.	$\chi^2$ or $F$ value	$P$
Otolith area ( $\mu\text{m}^2$ )	pH treatment	2	$\chi^2 = 6.00$	0.049
Otolith maximum length ( $\mu\text{m}$ )	pH treatment	2	$\chi^2 = 9.50$	0.009
Otolith maximum breadth ( $\mu\text{m}$ )	pH treatment	2	0.583	0.562
	Fish standard length	1	3.029	0.089
Otolith circularity	pH treatment	2	1.280	0.288
	Fish standard length	1	59.70	0.000
Otolith rectangularity	pH treatment	2	0.893	0.416
	Fish standard length	1	4.739	0.030
(b) Right otoliths				
Otolith area ( $\mu\text{m}^2$ )	pH treatment	2	$\chi^2 = 0.50$	0.778
Otolith maximum length ( $\mu\text{m}$ )	pH treatment	2	$\chi^2 = 6.00$	0.049
Otolith maximum breadth ( $\mu\text{m}$ )	pH treatment	2	0.446	0.643
	Fish standard length	1	1.103	0.299
Otolith circularity	pH treatment	2	1.32	0.277
	Fish standard length	1	40.84	0.000
Otolith rectangularity	pH treatment	2	5.649	0.007
	Fish standard length	1	0.014	0.907

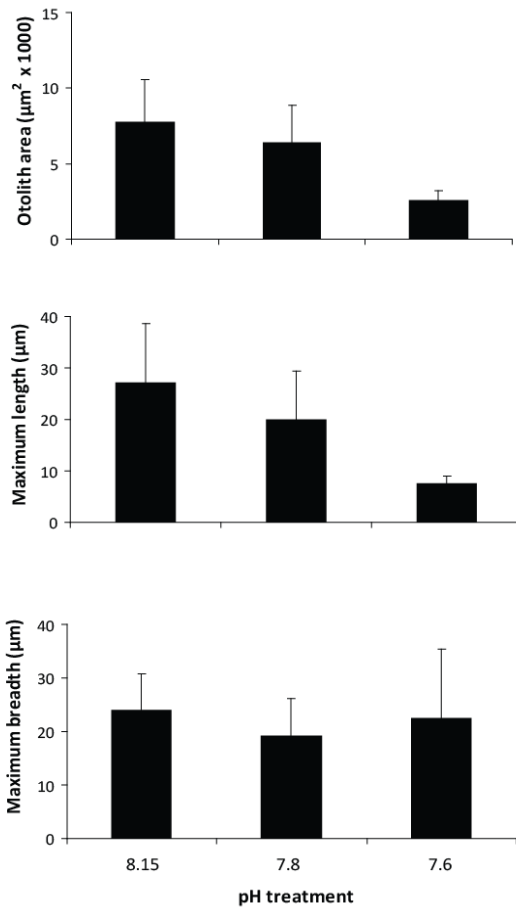


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**Fig. 1.** Mean ( $\pm$ SE) otolith area, maximum length, maximum breadth, rectangularity and circularity for left and right otoliths of larval clownfish reared at  $\text{pH}_{\text{NBS}}$  8.15, 7.8, and 7.6.



**Fig. 2.** Mean ( $\pm$ SE) unsigned differences in area, maximum length and maximum breadth between left and right otoliths of larval clownfish reared at pH<sub>NBS</sub> 8.15, 7.8, and 7.6.

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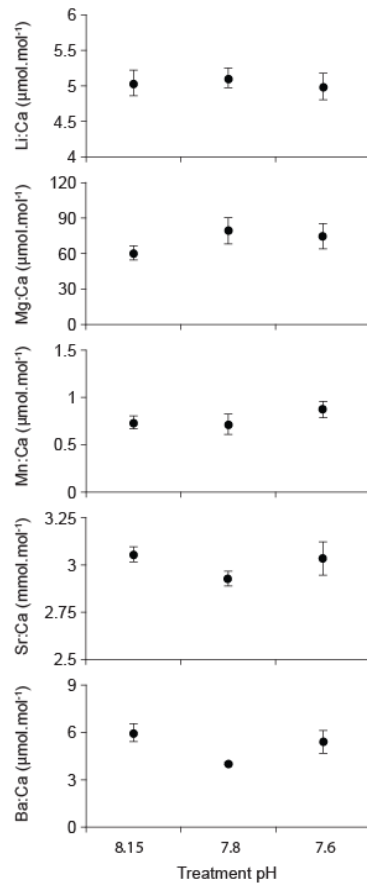
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**Fig. 3.** Mean ( $\pm$ SE) Li:Ca, Mg:Ca, Mn:Ca, Sr:Ca and Ba:Ca ratios in otoliths of larval clownfish reared at  $\text{pH}_{\text{NBS}}$  8.15 ( $n = 7$ ), 7.8 ( $n = 5$ ), and 7.6 ( $n = 6$ ).

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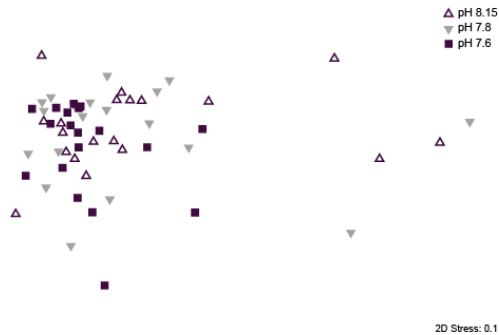
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**Fig. 4.** MDS plot of Fourier descriptors of otolith shape for each individual sampled, grouped by pH treatment.

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