

Ocean acidification does not affect the early life history development of a tropical marine fish

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ABSTRACT: Determining which marine species are sensitive to elevated CO₂ and reduced pH, and which species tolerate these changes, is critical for predicting the impacts of ocean acidification on marine biodiversity and ecosystem function. Although adult fish are thought to be relatively tolerant to higher levels of environmental CO₂, very little is known about the sensitivity of juvenile stages, which are usually much more vulnerable to environmental change. We tested the effects of elevated environmental CO₂ on the growth, survival, skeletal development and otolith (ear bone) calcification of a common coral reef fish, the spiny damselfish *Acanthochromis polyacanthus*. Newly hatched juveniles were reared for 3 wk at 4 different levels of $PCO_{2(seawater)}$ spanning concentrations already experienced in near-reef waters (450 $\mu\text{atm CO}_2$) to those predicted to occur over the next 50 to 100 yr in the IPCC A2 emission scenario (600, 725, 850 $\mu\text{atm CO}_2$). Elevated PCO_2 had no effect on juvenile growth or survival. Similarly, there was no consistent variation in the size of 29 different skeletal elements that could be attributed to CO₂ treatments. Finally, otolith size, shape and symmetry (between left and right side of the body) were not affected by exposure to elevated PCO_2 , despite the fact that otoliths are composed of aragonite. This is the first comprehensive assessment of the likely effects of ocean acidification on the early life history development of a marine fish. Our results suggest that juvenile *A. polyacanthus* are tolerant of moderate increases in environmental CO₂ and that further acidification of the ocean will not, in isolation, have a significant effect on the early life history development of this species, and perhaps other tropical reef fishes

KEY WORDS: Carbon dioxide · Hypercapnia · Development · Growth rate · Survival · Otolith · Skeleton · Calcification · Coral reef fish

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INTRODUCTION

Carbon dioxide (CO₂) concentrations at the ocean surface are in approximate equilibrium with the atmosphere. Consequently, as CO₂ concentration increases in the atmosphere, more CO₂ is dissolved in the shallow ocean. Through a process known as ocean acidification, increased uptake of CO₂ at the ocean surface causes ocean pH to decline and changes the availability of dissolved carbonate and bicarbonate ions

(Sabine et al. 2004, Royal Society 2005, Denman et al. 2007). Atmospheric CO₂ concentrations are predicted to exceed 500 ppm mid-century and could reach 850 ppm by 2100 if current CO₂ emissions trajectories are maintained (Meehl et al. 2007, Raupach et al. 2007). This would cause ocean pH to decline 0.3 to 0.4 units compared to current-day values (Caldeira & Wickett 2005, Royal Society 2005, Meehl et al. 2007) and make the ocean more acidic than at any time in the past 850 000 yr (Feely et al. 2004, Kump et al. 2009).

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A pH decline of this magnitude will likely have serious consequences for marine organisms that require carbonate ions to build shells and skeletons, especially corals and other invertebrates that precipitate aragonite skeletons (Feely et al. 2004, Orr et al. 2005, Kleypas et al. 2006, Hoegh-Guldberg et al. 2007, Moy et al. 2009). Increased levels of dissolved CO_2 and reduced pH could also affect non-calcifying species, including squid (Rosa & Seibel 2008) and fish (Munday et al. 2009a,b), although the potential impacts on these taxa are poorly understood (Fabry et al. 2008, Munday et al. 2008a).

Exposure to elevated environmental CO_2 (hypercapnia) can lead to extra- or intracellular acidosis in some marine animals, with effects on growth, development and survival (Pörtner et al. 2004, Kurihara 2008, Widdicombe & Spicer 2008). Adult fishes, however, appear to be relatively tolerant of changes in ambient CO_2 (Ishimatsu et al. 2005, 2008, Melzner et al. 2009). Fish actively regulate their acid–base balance through bicarbonate accumulation and ion exchange across the gills (Claiborne et al. 2002, Evans et al. 2005, Melzner et al. 2009), and small changes in tissue pH associated with elevated CO_2 can usually be compensated by these mechanisms. Although a great deal is known about how adult fish control their acid–base balance (Brauner & Baker 2009), less is known about the susceptibility of early life stages to elevated CO_2 (Brauner 2009). Furthermore, most of the past research on this topic has been conducted at CO_2 concentrations far higher than those predicted to occur as a result of global climate change (Ishimatsu et al. 2008), and has often focused on species known to tolerate considerable environmental variation. For other species, especially those with high metabolic demands (Pörtner & Farrell 2008), or from relatively stable marine environments such as coral reefs (Munday et al. 2009b), exposure to elevated CO_2 could potentially have significant physiological costs. Such costs would be expected to be most pronounced during the early life history when regulatory processes may be less well developed and when individuals are more susceptible to environmental variation due to their relatively small body size.

Another concern for marine fishes is that ocean acidification could affect otolith (ear bone) or skeletal development (Munday et al. 2008a). 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 changes to the size, shape, or density of otoliths could have serious implications for ecological performance and individual fitness. Otoliths are composed of aragonite, which will become less abundant as the ocean becomes more acidic (Sabine et al. 2004), and

this may affect otolith growth. Alternatively, otolith growth may be affected by the mechanisms used to compensate extracellular pH. Indeed, a recent study reported that otoliths were larger in larval fishes exposed to elevated CO_2 , possibly because pH regulation caused carbonate ion concentrations to increase within the otolith endolymph (Checkley et al. 2009). Finally, physiological stress can increase otolith size, shape and symmetry (Gagliano & McCormick 2004, Payan et al. 2004), leading to reduced performance and higher mortality (Gagliano et al. 2008). Therefore, even if ocean acidification does not directly affect otolith development, increased stress caused by elevated CO_2 could influence otolith shape and symmetry.

Direct effects of ocean acidification on skeletal development are not expected, because the skeleton is composed primarily of calcium phosphate and cartilaginous material, rather than calcium carbonate. Nonetheless, it is possible that the additional buffering of tissue pH with bicarbonate and non-bicarbonate ions under hypercapnic conditions could interfere with normal skeletal growth. No studies have investigated the potential effects of ocean acidification on fish skeletal growth.

We tested the effects of CO_2 -induced ocean acidification on early life history development of a tropical marine fish, the spiny damselfish *Acanthochromis polyacanthus*. We focused on the juvenile stage immediately after hatching because: (1) larval and juvenile fishes are often more sensitive to environmental conditions than adults (Rombough 1997, Pörtner & Farrell 2008) and (2) rapid somatic, otolith and skeletal growth during this stage make it more likely to detect any effects of ocean acidification over relatively short experimental periods. Juveniles were reared in seawater diffused with air (present-day control) or with CO_2 -enriched air to produce concentrations of $PCO_{2(\text{seawater})}$ of ~450, 600, 725 and 850 μatm . The control value (450 μatm) is consistent with observations of $PCO_{2(\text{seawater})}$ between 400 and 500 μatm near coral reefs (Fagan & Mackenzie 2007) due to the release of CO_2 during calcification and respiration. The highest treatment level (850 μatm PCO_2) is consistent with the IPCC A2-SRES emission trajectory, where CO_2 concentrations in the atmosphere and shallow ocean are predicted to reach approximately 850 ppm (estimated range 730 to 1020 ppm) in the year 2100 (Meehl et al. 2007). Somatic growth (length and weight) of juveniles was compared after 3 wk of exposure to the different treatments. Otoliths were then extracted for comparisons of otolith size, shape, asymmetry (between left and right otoliths) and chemical composition. Finally, specimens were cleared and stained, and the dimensions of major skeletal elements were compared among treatments.

MATERIALS AND METHODS

Study species. *Acanthochromis polyacanthus* is a moderate-sized (total length 140 mm) planktivorous damselfish that is common on coral reefs in the western Pacific. Adults form breeding pairs and lay eggs in small caves in the reef matrix. *A. polyacanthus* does not have a pelagic larval phase. Instead, newly-hatched juveniles remain with the adults for up to 45 d before dispersing from their natal site (Kavanagh 2000). As a result of this life history, *A. polyacanthus* can be reared in captivity with high success and is routinely used as a model species in reef fish ecology and climate change research (e.g. Donelson et al. 2010).

Breeding and juvenile rearing. Juvenile and broodstock damselfish were reared at James Cook University's experimental marine aquarium facility. Broodstock were 3 yr old breeding pairs of *Acanthochromis polyacanthus* that were offspring of 10 wild-caught pairs from Pelorus Island on the Great Barrier Reef, Australia. Breeding pairs were kept in separate 60 l aquaria supplied with a continuous flow of filtered seawater. Water temperature was adjusted to approximate the long-term average water temperature at Pelorus Island throughout the year. Water temperature was maintained between 28 and 29°C during the summer breeding period. Adults were fed twice daily to satiation with INVE Aquaculture Nutrition pellets. Half of a terracotta pot on the bottom of each aquarium served as a shelter and breeding site. Aquaria were checked each morning for the presence of newly hatched juveniles.

Five clutches of newly-hatched juveniles, each from a different parental pair, were used in the experiment. Clutches were tested sequentially, as they became available. On the day of hatching, 80 fish from each clutch were randomly selected and separated into 4 groups of 20 fish. Rearing in small groups replicated the natural social environment of *Acanthochromis polyacanthus* juveniles. Each group of 20 fish was transferred into 1 of 4 rearing tanks (60 l) that was continuously diffused with a air or CO₂-enriched air to produce dissolved CO₂ concentrations of ~450, 600, 725 and 850 µatm CO₂ (see below). The 600 µatm treatment was not available for 2 of the clutches; therefore, these clutches were only reared in the 450, 725 and 850 µatm CO₂ treatments. Two different rows of rearing tanks were used (i.e. a total of 8 rearing tanks). Three of the 5 clutches were reared in 1 set of 4 experimental tanks, and 2 of the 5 clutches were reared in the other set of 4 experimental tanks. Newly hatched juveniles were reared for 21 d.

Aerated rearing tanks had no water flow during the day, but were flushed for 40 min each night with clean, filtered, seawater that had been aerated all day with the same concentration of CO₂-enriched air. This cycle ensured the fish could feed ad libitum throughout daylight hours and that any unconsumed food was removed each night while maintaining the appropriate CO₂ environment in each tank. Juveniles were fed *Artemia* nauplii at 2 ind. ml⁻¹ each morning from the day of hatching until Day 7 and then 100 mg (5 mg fish⁻¹) of 2/4 Aquaculture Nutrition NRD (protein 55%, fat 9%, fibre 1.9%) until the end of the experiment.

Seawater manipulations. Rearing tanks were continuously diffused with 1 of 4 concentrations of CO₂ in air: average 397 (unmanipulated air), 551, 741 or 1036 ppm CO₂. Control or CO₂-enriched air was bubbled into each tank at 1.5 l min⁻¹ through a fine-pore airstone. The concentration of CO₂-enriched air for each treatment was controlled by a scientific-grade pressure regulator and precision needle valve and measured continuously with an in-line infrared CO₂ probe (see Munday et al. 2009c for details). Rearing tanks were sealed on top with a clear acrylic lid to limit CO₂ exchange with the atmosphere. The pH of rearing tanks was measured each day between 07:00 and 08:00 h, approximately 12 h after tanks had been flushed with clean treatment seawater the previous evening. pH was measured on the National Bureau of Standards (NBS) scale with a portable meter (Hach HQ11D) calibrated with fresh buffers (Merck). Total alkalinity in seawater was estimated using the program CO2SYS. Average PCO_{2(seawater)} was estimated to be 455, 598, 723 and 841 µatm; therefore, we report values of ~450, 600, 725 and 850 µatm. All seawater carbonate parameters are shown in Table 1. Oxygen saturation in the tanks was checked regularly with an oxygen electrode (OXI 340i; WTW) and was always above 90% saturation. A summer light cycle of 13 h light:11 h dark was simulated with fluorescent lights.

Seawater for the experiment came from a 70 000 l recirculating seawater system at James Cook Univer-

Table 1. Summary of seawater parameters in control and acidification treatments. PCO₂ in air, pH and total alkalinity were measured. Other values were estimated using the program CO2SYS. Values are mean (±SD). NBS: National Bureau of Standards

PCO ₂ (air) (ppm)	pH _{NBS}	Total alkalinity (µmol kg ⁻¹)	PCO ₂ (seawater) (µatm)	HCO ₃ ⁻ (µmol kg ⁻¹)	CO ₃ ²⁻ (µmol kg ⁻¹)
397 ± 27	8.07 ± 0.07	1858 ± 120	455	1491	144
551 ± 56	7.95 ± 0.1	1766 ± 70	598	1486	109
741 ± 43	7.88 ± 0.13	1774 ± 118	723	1529	95
1036 ± 61	7.83 ± 0.1	1810 ± 111	841	1585	88

sity. Biological activity in the recirculating system and occasional water exchanges caused fluctuations in pH and total alkalinity of seawater during the experiment (Table 1). The pH of individual tanks also varied within treatments because the concentration of CO₂-enriched air rather than the pH of the treatment water per se was controlled. Average pH of treatments varied approximately 0.2 units over the 21 d duration of the experiment and up to 0.12 units between days (Fig. 1). This range is consistent with variation in pH and PCO₂ recorded on reefs due to fluctuations in respiration, photosynthesis, calcification and water exchange (Ohde & van Woesik 1999, Kuffner et al. 2008, Gagliano et al. 2010) and therefore provided a realistic environment for rearing juvenile *Acanthochromis polyacanthus*.

Survival and growth. All fish remaining in each tank were sacrificed at 21 d post hatching and fixed in 4% phosphate-buffered formalin. Fish were removed from the fixative within 48 h of preserving, blotted dry, weighed (nearest mg) and then photographed in a lateral position under a stereomicroscope. Once photographed, the fish were stored in 75% ethanol. Standard length (SL) to the nearest 0.1 mm was estimated for each fish from the digital photograph using image analysis (Optimas 6.5, Media Cybernetics).

The number of fish surviving in each tank was compared among CO₂ treatments using 1-way analysis of variance (ANOVA). The length and weight of fish at the end of the experiment was compared among treatments and clutches using a mixed-model nested ANOVA, with clutch (1 to 5) as a random factor nested within CO₂ treatment. Clutch was nested within CO₂ treatment because not all clutches were tested at all 4 treatment levels. Fish were considered as individual replicates because the rearing conditions mimicked

their natural social environment. Furthermore, using a mean value of SL or weight of fish from each tank yielded the same (non-significant) result for the main effect of CO₂ treatment as the nested ANOVA.

Otolith development. For each clutch, left and right sagitta were removed from 5 fish from each CO₂ treatment. Each sagittal otolith was photographed, and dimensions and shape descriptors were obtained from a calibrated grey-scale image. The grey-scale images were used to measure 5 dimensions, viz. maximum otolith length (mm), perimeter (mm), area (mm²), rectangularity and circularity, 2 indices which calculate how well an otolith fits into a rectangular and a circular shape, respectively. The 2-dimensional shape of each otolith was then obtained from the outline of the otolith image over 128 equidistant sampling points and quantified using Fast Fourier analysis (see Gagliano & McCormick 2004 for details).

For statistical analyses, all 5 dimensions and the first 20 standardised shape Fast Fourier descriptors were used. Additionally, for all otolith traits of each individual fish, the signed asymmetry value (R–L) of the otolith pair was estimated to establish whether otoliths exhibited fluctuating asymmetry (FA). To ensure that otolith pairs exhibited true FA, the distribution of these values in each otolith pair character was examined (i.e. distribution with a mean equal to 0 and normal variation), and signs of different forms of asymmetry, such as directional asymmetry (i.e. skewed distributions with kurtosis values smaller than 0) and antisymmetry (i.e. bimodality) were tested for as described by Palmer & Strobeck (1986).

After initial otolith measurements were completed, all otolith characters on both right and left sides were then randomly re-measured with no knowledge of sample identity, and the possibility of inflated asymmetry estimates (i.e. non-directional asymmetry) due to measurement error (ME) was tested for. To do this, mixed-model ANOVAs with otolith side as a fixed factor and individual as a random factor were used. The side effect tested for directional asymmetry, while the side-by-individual interaction tested for non-directional asymmetry. None of the characters showed directional asymmetry (all $p > 0.05$). The interaction variance was highly significant for all characters under consideration ($p < 0.001$), indicating that ME variance was significantly smaller than FA variance, hence providing justification for FA significance tests among treatments (Palmer 1994). Since the direction of asymmetry was not relevant,

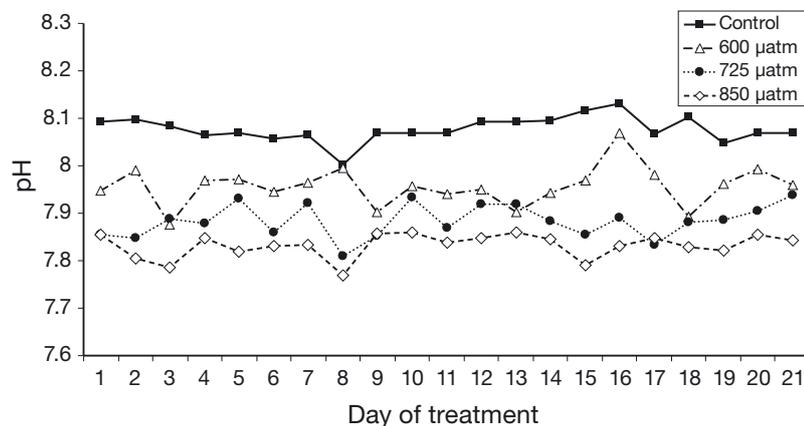


Fig. 1. Mean pH_{NBS} (NBS: National Bureau of Standards) of seawater in controls and 3 elevated PCO₂ treatments (600, 725, 850 µatm) over the 21 d duration of the rearing experiment

absolute asymmetry values were then used to compare relative levels of FA between treatments. Specifically, mixed-model nested ANOVAs with clutch ID as the hierarchically nested random factor were used to test whether individuals reared under CO₂-enriched conditions exhibited a significantly higher mean FA at the end of the experimental period than their control counterparts.

Skeletal development. For each clutch, 5 fish from each CO₂ treatment were cleared and stained to reveal skeletal elements. Methods for clearing and staining followed Taylor & van Dyke (1985) with slight modification. The following procedure was used: (1) degreasing in xylene for 24 h, (2) cartilage staining in alcian blue solution for 24 h, (3) neutralisation in saturated borax for 48 h, (4) bleaching in 10% H₂O₂ in 0.5% KOH solution for 24 h, (5) clearing for up to 78 h (depending on fish size) in trypsin solution, (6) bone staining in alizarine red solution for 24 h, (7) cleaning in trypsin solution for 12 to 24 h and (8) glycerine storage using a 3-step process from 40 to 100% glycerine every 12 h.

Cleared specimens were photographed under a stereomicroscope. SL and measurements of up to 29 skeletal elements (Fig. 2, Table 2) were then estimated for each fish from the digital photograph using image analysis (Optimas 6.5, Media Cybernetics). Skeletal elements were chosen that: (1) were clearly visible in the majority of specimens, (2) had distinct reference points for measurements, and (3) provided a thorough representation of the skeleton. Not all skeletal ele-

ments could be visualised in all individuals; therefore, the number of individuals for which a specific element could be measured differed among elements (Table 2). Because the size of skeletal elements is associated with body size, the size of each element was regressed against SL prior to analysis. The residuals of this regression were then compared among CO₂ treatments for each skeletal element using 1-way ANOVA.

RESULTS

Survival and growth

An average of 16 fish (mean = 16.44 ± 2.85 SD) survived per tank. Survivorship did not differ among CO₂ treatments ($F_{3,14} = 0.78$, $p = 0.53$). Juveniles grew substantially during the experimental period. Newly hatched juveniles averaged 5.22 mm SL and weighed 3.5 mg. By the end of the experiment, they averaged 13.1 mm SL and weighed 66 mg. SL at the end of the experiment differed among clutches ($F_{3,14} = 26.85$, $p < 0.001$, Fig. 3a), but not among CO₂ treatments ($F_{3,14} = 0.45$, $p = 0.7$). Similarly, weight at the end of the experiment differed among clutches ($F_{3,14} = 36.33$, $p < 0.001$, Fig. 3b), but not among CO₂ treatments ($F_{3,14} = 0.04$, $p = 0.98$). Clutch identity explained 57% of the variance in SL and 65% of the variance in weight. In contrast, CO₂ treatment explained only 9% of the variance in SL and <1% of the variance in weight.

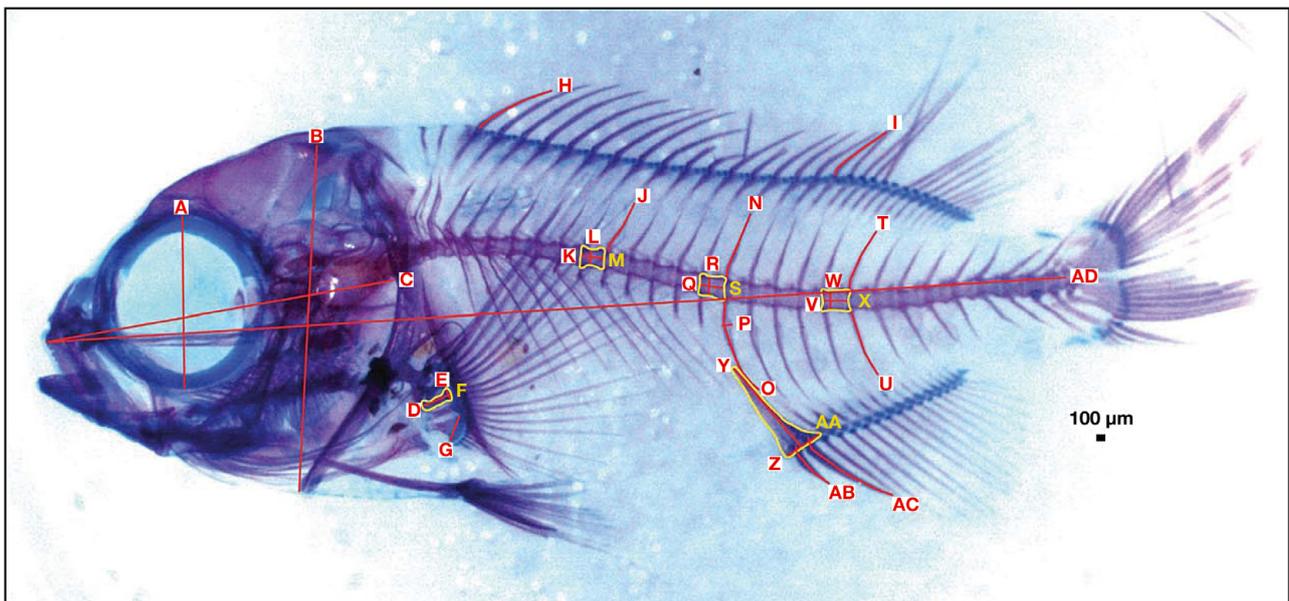


Fig. 2. *Acanthochromis polyacanthus*. Photograph of cleared and stained juvenile showing the 29 skeletal measurements compared among treatments. The name of each element is shown in Table 2

Table 2. *Acanthochromis polyacanthus*. Skeletal elements compared among CO₂ treatments (in µatm) and symbols used to illustrate the elements in Fig. 2. The number of individuals for which each element was measured, and ANOVA summary statistics are shown. ns: not significant; na: not applicable

Character	Symbol	Individuals measured				ANOVA	
		450 µatm	600 µatm	725 µatm	850 µatm	F	p
Eye diameter	A	21	15	23	24	3.33	0.023
Head depth	B	25	15	25	24	0.66	ns
Head length	C	20	13	24	23	0.37	ns
Pectoral fin, second radial length	D	19	9	19	21	0.27	ns
Pectoral fin, second radial width	E	19	10	18	18	0.04	ns
Pectoral fin, second radial area	F	14	7	15	16	0.13	ns
Pectoral fin, fourth radial width	G	14	10	15	15	0.59	ns
Dorsal fin, first spine length	H	19	14	18	20	0.45	ns
Dorsal fin, last spine length	I	16	10	19	21	0.08	ns
Central precaudal neural spine length	J	21	15	23	25	1.37	ns
Central precaudal vertebra length	K	8	7	12	14	1.40	ns
Central precaudal vertebra diameter	L	19	14	23	24	2.59	ns
Central precaudal vertebra area	M	7	4	10	13	0.25	ns
First caudal neural spine length	N	22	14	25	25	2.09	ns
First caudal haemal spine length	O	21	12	21	24	2.38	ns
First caudal haemal spine width	P	22	15	24	25	0.95	ns
Last precaudal vertebra length	Q	17	10	15	19	0.06	ns
Last precaudal vertebra diameter	R	22	15	24	24	4.32	0.007
Last precaudal vertebra area	S	15	10	14	18	1.77	ns
Central caudal neural spine length	T	22	14	25	25	1.38	ns
Central caudal haemal spine length	U	22	14	25	24	2.31	ns
Central caudal vertebra length	V	15	11	17	20	0.11	ns
Central caudal vertebra diameter	W	20	15	24	25	2.68	ns
Central caudal vertebra area	X	15	10	17	20	0.85	ns
First & second interhaemal spine length	Y	19	9	18	22	2.25	ns
First & second interhaemal spine width	Z	24	15	25	25	0.37	ns
First & second interhaemal spine area	AA	18	9	18	22	0.31	ns
First anal spine length	AB	23	13	20	25	1.72	ns
Second anal spine length	AC	18	15	23	24	4.41	0.006
Standard length	AD	25	15	25	25	na	na

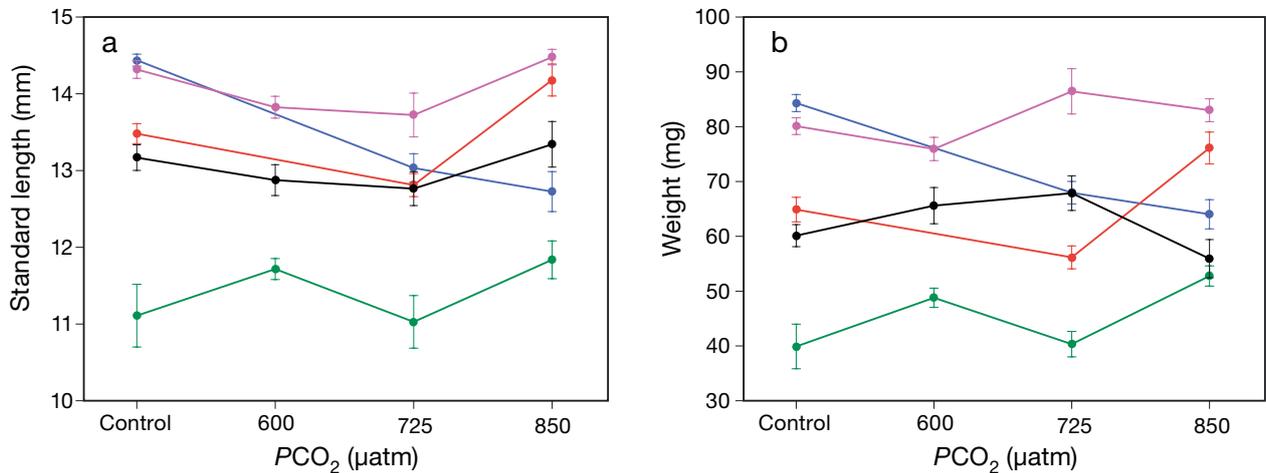


Fig. 3. *Acanthochromis polyacanthus*. Mean (\pm SE) (a) standard length and (b) weight of juveniles reared for 3 wk at 4 levels of PCO_{2(seawater)}. Each line represents a clutch from a different parental pair

Otolith development

There was no effect of CO₂ treatment on otolith area, maximum length, perimeter, rectangularity or circularity ($p > 0.5$ for all comparisons; Fig. 4). However, all of these measures differed strongly among clutches ($p < 0.001$ for all comparisons), and this parental effect accounted for up to 87% of the total variance observed. Similarly, there was no effect of CO₂ treatment on the overall otolith shape among treatments ($p > 0.05$ for all Fast Fourier descriptors), but the shape of otoliths differed significantly among individuals depending on their clutch of origin. Clutch effects accounted for over 40% of the total variance in otolith shape. Lastly, FA of otoliths from fish reared under CO₂-enriched conditions did not differ from control individuals ($p > 0.05$ for all comparisons). Nonetheless, otolith asymmetry

differed significantly among clutches, and this component accounted for more than 25% of the total variance.

Skeletal development

Dimensions of only 3 of 29 skeletal elements differed among CO₂ treatments (Table 2): eye diameter ($F_{3,14} = 3.34$, $p = 0.02$), diameter of the last precaudal vertebra ($F_{3,14} = 4.32$, $p = 0.007$) and length of the second anal spine ($F_{3,14} = 4.41$, $p = 0.007$). None of these values was significant when alpha was Bonferroni corrected ($p = 0.002$) to account for the large number of consecutive tests. More importantly, there was no consistent pattern of variation in mean values in relation to the CO₂ treatments, either when comparing among CO₂ treat-

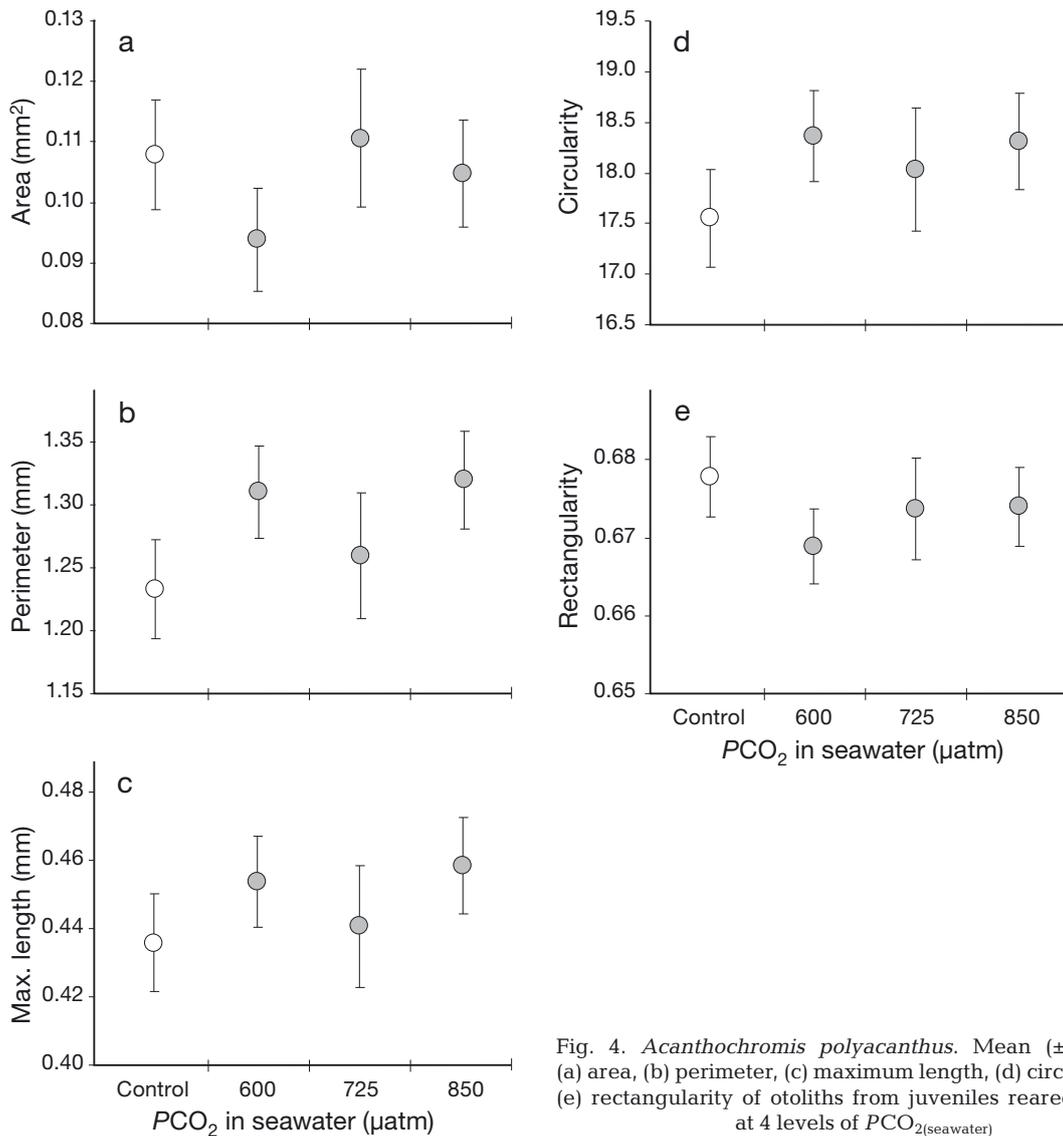


Fig. 4. *Acanthochromis polyacanthus*. Mean (\pm 95% CI) (a) area, (b) perimeter, (c) maximum length, (d) circularity and (e) rectangularity of otoliths from juveniles reared for 3 wk at 4 levels of PCO_{2(seawater)}

ments for each element, or among elements within CO₂ treatments. Therefore, these differences among treatments appeared to be random, not causally related to the experimental treatments.

DISCUSSION

Determining which marine species are sensitive to elevated CO₂ and reduced pH, and which species can tolerate these changes, is critical for assessing the impacts of ocean acidification on marine biodiversity and ecosystem function (Fabry et al. 2008, Melzner et al. 2009, Hofman & Todgham 2010). There is now ample evidence that CO₂-induced acidification can affect the early life history development of some invertebrates, especially calcifying species of echinoderms, molluscs and corals (Kurihara 2008, Widdicombe & Spicer 2008). In contrast, we found no evidence for negative effects of elevated CO₂ on growth, survival, skeletal development and otolith calcification during the early life history of a tropical marine fish. This is the first comprehensive analysis of how CO₂ concentration relevant to climate change over the next 50 to 100 yr might impact life history traits and calcification in a marine fish. Our results are consistent with studies on some temperate and cold-water fishes (Kikkawa et al. 2003, Ishimatsu et al. 2004), which indicate that they can tolerate CO₂ levels well above those predicted to occur in the shallow ocean over the coming century due to increasing anthropogenic CO₂ emissions. Together, these results suggest that early life history development of marine fishes will be more tolerant to ocean acidification than that of many invertebrates, probably because fish generally have a superior capacity for acid–base regulation (Pörtner et al. 2004, Brauner 2009, Melzner et al. 2009).

Although fish are generally thought to be tolerant of mild hypercapnia, much of the previous research on this topic has been conducted on adult fish, often on species that are tolerant to environmental variation (e.g. rainbow trout), and at CO₂ levels that have little relevance to anthropogenic climate change (Ishimatsu et al. 2008). Early life history stages are usually considered to be more sensitive than adults to environmental change, either because their physiological compensatory mechanisms are not fully developed, or because their small size increases the cost of homeostasis and makes them more susceptible to environmental fluctuations (Brauner 2009, Melzner et al. 2009). The absence of any detectable effects on growth, survival and development of *Acanthochromis polyacanthus* in the first few weeks after hatching suggests that juveniles of this species have well-developed mechanisms for acid–base regulation that are active soon after

hatching. The early ontogenetic development of gills in fish is associated with the capacity for ion regulation during early life (Rombough 2007, Fu et al. 2010), and likely explains why we observed no effect of hypercapnia on the early life history development of *A. polyacanthus*.

Another concern for marine fishes is that elevated PCO₂ could affect calcification of the internal skeleton or otoliths (Munday et al. 2008a), either because of the changed saturation states of carbonate ions in seawater, or due to changes in extra-cellular concentrations of bicarbonate and non-bicarbonate ions caused by acid–base regulation under hypercapnia. Otoliths are expected to be the most susceptible calcified structure in fishes, because they are composed of aragonite. Nevertheless, we found no significant effect of elevated CO₂ on the size, shape or asymmetry of otoliths in juvenile *Acanthochromis polyacanthus*. After 3 wk of exposure to elevated CO₂ there were no significant differences in any of the otolith parameters of treatment fish compared with current-day controls. Although there was a tendency for otoliths of CO₂-treated fish to show greater circularity, and thus reduced rectangularity and a smaller perimeter, than control fish, the differences were not statistically significant. In contrast, very clear differences in all otolith traits were detected among clutches from different parents, indicating that such differences could easily be distinguished when present. The similarity in otolith size and shape among treatments suggests that the carbonate chemistry of the otolith endolymph does not change markedly when individuals are exposed to elevated environmental CO₂, despite any changes to extracellular ion concentrations resulting from acid–base regulation, or the reduced environmental availability of aragonite.

The absence of any effect on otolith size and shape in this study contrasts with a recent study by Checkley et al. (2009) that detected increased otolith size in larval sea bass exposed to elevated CO₂ during the egg stage and up to 8 d post hatching (DPH). The different results for the 2 studies could be due to: (1) differences in sensitivity of the species tested, (2) differences in the ontogenetic stages and duration of exposure to elevated CO₂, (eggs and larvae for 8 d versus post-hatching juveniles for 21 d) or (3) the slightly higher CO₂ levels used by Checkley et al. (2009) (~1000 and 2500 µatm) compared to the current study (up to ~850 µatm). To date, these are the only 2 studies to investigate otolith growth and development under near-future scenarios of ocean acidification. Importantly, they point to the possibility that the effects of elevated CO₂ on otolith development may differ between species. There is increasing evidence that ocean acidification affects different calcifying species in very

different ways (Ries et al. 2009, Dupont et al. 2010). Consequently, there is good reason to expect interspecific variation in the response of otolith calcification to ocean acidification.

Alternatively, the different ontogenetic stages and durations of exposure to elevated CO_2 could explain the contrasting results for otolith development between studies. Gas exchange is cutaneous in embryonic and early larval stages until the gills develop, and this may affect the ability for acid–base regulation (Rombough 2007, Brauner 2009, Fu et al. 2010). Checkley et al. (2009) examined the effects of elevated CO_2 on otolith size in the early larval stage of sea bass (7 to 8 DPH). In contrast, we examined otolith development in juvenile *Acanthochromis polyacanthus* after 3 wk of exposure to elevated CO_2 . It is possible that otolith development in *A. polyacanthus* could have been affected by elevated CO_2 during the first few days post hatching, but subsequently mediated by improved acid–base regulation in larger juveniles. The larger volume of otolith accretion in juveniles compared to small larvae could expunge any minor differences in otolith size that might develop immediately after hatching. Clearly, much more research is required to determine whether elevated CO_2 will have a significant effect on otolith development in a broad range of larval and juvenile fishes.

This is the first study to investigate the potential effects of near-future ocean acidification on skeletal development in marine fishes. Skeletal tissue is not expected to be susceptible to reduced ocean pH, because it is primarily formed of calcium phosphate minerals (hydroxylapatite) and complex cartilaginous tissues (Witten et al. 2010), rather than calcium carbonate. However, it is still possible that changes to extracellular concentrations of bicarbonate and non-bicarbonate ions due to acid–base regulation in hypercapnia-exposed fish could affect skeletal development. As expected, we observed no consistent effects of exposure to elevated CO_2 on the size of representative skeletal elements throughout the body.

Acanthochromis polyacanthus is a common reef fish that is often used as a model species in reef fish ecology and climate change research. It is possible, however, that young *A. polyacanthus* might be more tolerant of elevated CO_2 than some other reef fishes, because they lack a planktonic larval phase. Newly hatched juveniles remain with their parents and shelter near the reef matrix, where they likely experience natural fluctuations in ambient CO_2 due to diurnal cycles of photosynthesis and respiration by reef organisms (Ohde & van Woesik 1999, Kuffner et al. 2008). Furthermore, juvenile *A. polyacanthus* shelter in small caves at night with their parents, where pH and PCO_2 could potentially reach levels similar to

those tested here (Gagliano et al. 2010). Consequently, *A. polyacanthus* juveniles might be adapted to periods of moderately elevated CO_2 because of their close association with the reef environment. Pelagic larvae are likely to experience lower and more stable CO_2 conditions in the open ocean, and thus might be more sensitive to mild increases in PCO_2 . Munday et al. (2009c) observed no negative effects of elevated CO_2 on early life history traits of *Amphiprion percula*, a reef fish that has a pelagic larval phase. However, more research on a greater range of species is clearly required before conclusions can be reached about the sensitivity of pelagic larvae to elevated PCO_2 .

The results of this study, in combination with our understanding of the physiological capacity of marine fishes (e.g. Ishimatsu et al. 2008, Melzner et al. 2009), suggest that the early life history development of at least some reef fishes might not be seriously affected by the changes in CO_2 concentrations that are predicted to occur in the tropical ocean over the next 50 to 100 yr. In contrast, the impairment of larval behaviour caused by elevated CO_2 that has recently been described (Munday et al. 2009a, Dixson et al. 2010) will probably have significant impacts on juvenile success and recruitment to adult populations. PCO_2 concentrations that could occur in the shallow ocean by the end of this century (700 to 850 ppm) cause newly-settled reef fish to exhibit bolder behaviour in their natural habitat, leading to markedly higher mortality rates from predation (Munday et al. 2010). Consequently, the effects of elevated CO_2 on the behaviour of juvenile reef fishes may be more important than possible negative effects on growth or development.

Although early life history traits of *Acanthochromis polyacanthus* appear to be tolerant to near-future levels of ocean acidification, this species is highly sensitive to small increases in average summer temperatures that could occur as a result of global warming. Spawning rate, clutch size, size at hatching, juvenile growth and juvenile survival are all significantly affected by temperature increases of 1.5 to 3°C (Munday et al. 2008b, Donelson et al. 2010). Consequently, this species appears to be at greater risk from warming oceans than it does from ocean acidification. Nevertheless, future studies should consider potential interacting effects of temperature and elevated environmental CO_2 on the early life history success of this and other reef fishes. Both temperature and environmental CO_2 act to reduce the aerobic performance of water-breathing animals (Pörtner et al. 2004, Pörtner & Farrell 2008), including adults of some reef fishes (Munday et al. 2009b), and consequently, their interacting effects could have very significant impacts on individual performance and population sustainability.

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