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Interacting effects of elevated temperature and ocean acidification on the aerobic performance of coral reef fishes

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ABSTRACT: Concerns about the impacts of ocean acidification on marine life have mostly focused on how reduced carbonate saturation affects calcifying organisms. Here, we show that levels of CO_2-induced acidification that may be attained by 2100 could also have significant effects on marine organisms by reducing their aerobic capacity. The effects of temperature and acidification on oxygen consumption were tested in 2 species of coral reef fishes, *Ostorhinchus doederleini* and *O. cyanosoma*, from the Great Barrier Reef, Australia. The capacity for aerobic activity (aerobic scope) declined at temperatures above the summer average (29°C) and in CO_2-acidified water (pH 7.8 and ~1000 ppm CO_2) compared to control water (pH 8.15). Aerobic scope declined by 36 and 32% for *O. doederleini* and *O. cyanosoma* at temperatures between 29 to 32°C, whereas it declined by 33 and 47% for *O. doederleini* and *O. cyanosoma* in acidified water compared to control water. Thus, the declines in aerobic scope in acidified water were similar to those caused by a 3°C increase in water temperature. Minimum aerobic scope values of ~200 mg O_2 kg^{-1} h^{-1} were attained for both species in acidified water at 32°C, compared with over 600 mg O_2 kg^{-1} h^{-1} in control water at 29°C. Mortality rate increased sharply at 33°C, indicating that this temperature is close to the lethal thermal limit for both species. Acidification further increased the mortality rate of *O. doederleini*, but not of *O. cyanosoma*. These results show that coral reef fishes are sensitive to both higher temperatures and increased levels of dissolved CO_2, and that the aerobic performance of some reef fishes could be significantly reduced if climate change continues unabated.

KEY WORDS: Global warming · Ocean acidification · Hypercapnia · Marine fish · Metabolism · Aerobic scope

INTRODUCTION

Predicting the effects of rapid climate change on ecological communities depends on understanding the sensitivity of individuals and populations to environmental change (Harley et al. 2006, Tewksbury et al. 2008). For marine animals, a decreased capacity to perform aerobically at higher temperatures is hypothesized to be the key physiological mechanism that will determine the response of many species to climate change (Pörtner & Knust 2007, Pörtner & Farrell 2008). The limited capacity of the circulatory and ventilatory systems of aquatic species to keep pace with increased O_2 demands at higher temperatures causes a reduction in aerobic scope and sets the boundaries of whole-organism thermal tolerance (Pörtner & Knust 2007). Simultaneously, extra CO_2 dissolved in the ocean due to increasing atmospheric CO_2 concentrations is expected to compound the effects of higher temperatures on aerobic capacity (Pörtner & Farrell 2008). Thus, predicting the impacts of climate change on marine organisms depends on understanding how tempera-

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ture and elevated CO_2 levels interact to affect the performance of individuals (Rosa & Seibel 2008), and ultimately how this interaction affects their capacity to sustain viable populations.

Atmospheric CO_2 concentrations have increased from an estimated 280 ppm around 1700 AD to over 380 ppm today, causing earth average surface temperature to increase by ~0.7°C (Brohan et al. 2006). Depending on future CO_2 emission scenarios, the average surface temperature is projected to increase by 1.1–6.4°C by 2100, with best estimates placing the range between 2.0 and 4.5°C (Meehl et al. 2007). Average sea surface temperature increases at a slower rate than average air temperature, but is still predicted to increase by up to 3°C in tropical and temperate seas within the next 100 yr (Poloczanska et al. 2007, Lough 2008, Munday et al. 2009). Although increases in average temperature may be greater in temperate regions than in the tropics, it is thought that tropical species might respond more strongly to climate change because they have evolved in a more thermally stable environment than species at higher latitudes; thus, they might be expected to have narrower thermal tolerances and to live closer to their thermal optima (Hoegh-Guldberg et al. 2007, Tewksbury et al. 2008).

Recent research has demonstrated that some coral reef fishes are sensitive to relatively small increases in maximum summer temperatures. Nilsson et al. (2009) demonstrated that the aerobic scope of 2 cardinalfish species (family Apogonidae) declined sharply with a 2°C increase in average summer water temperature, and Munday et al. (2008a) found that the growth of juvenile and adult spiny damselfish (family Pomacentridae) was compromised with a 3°C increase in average summer water temperature. Moreover, observed shifts in the geographical distributions of other marine fishes towards higher latitudes in conjunction with increases in sea temperatures (Holbrook et al. 1997, Perry et al. 2005) suggest that populations of many marine species are sensitive to small increases in ambient temperature.

As atmospheric CO_2 concentrations increase, the amount of CO_2 dissolved in the ocean also increases, which in turn causes ocean pH to decline (a process known as ocean acidification). On a 'business-as-usual' A2-SRES (Special Report on Emissions Scenarios, scenario A2) emission trajectory, atmospheric CO_2 concentrations are predicted to range between 730 and 1020 ppm by 2100 (Meehl et al. 2007). This would cause ocean pH to drop by 0.3 to 0.4 points compared to current-day levels, making the ocean more acidic than at any time in the past 400 000 yr (Feely et al. 2004). Although the combined effects of increasing water temperature and ocean acidification have been tested for a few calcifying organisms, such as corals and coralline algae (Reynaud et al. 2003, Anthony et al. 2008), their effects on the vast majority of other marine organisms, including fishes, are largely unknown (Harley et al. 2006, Fabry et al. 2008, Ishimatsu et al. 2008, Munday et al. 2008b).

In this study, we investigated the combined effects of temperature and CO_2-induced ocean acidification on the aerobic scope of 2 cardinalfish species, *Ostorhinchus doederleini* and *O. cyanosoma.* These species were chosen because previous research has shown that they are sensitive to increased water temperature (Nilsson et al. 2009), but the effects of CO_2-induced acidification on O_2 uptake and aerobic scope are unknown. Specifically, we used respirometry to determine how increased temperature and CO_2-induced ocean acidification affected the aerobic capacity of the 2 species at Lizard Island on the northern Great Barrier Reef, Australia. For each species, we measured resting O_2 consumption and the maximum rate of O_2 consumption while swimming at current-day average summer sea-surface temperatures experienced at Lizard Island (~29°C, Lough 1999) and at temperatures likely to be experienced with increasing frequency at this location over the next century (31, 32 and 33°C). For each temperature, we tested the additional effect of ocean acidification by measuring resting and maximum O_2 consumption while swimming in seawater at current-day pH values (~8.15) and in seawater that had been acidified by the addition of CO_2 to simulate a 0.35 unit decline in ocean pH by 2100 (pH 7.80 and ~1000 ppm CO_2).

MATERIALS AND METHODS

Experimental design. This study was conducted in January 2008 (austral summer) at Lizard Island on the northern Great Barrier Reef (14° 40′ S, 145° 28′ E), Australia. Adult *Ostorhinchus doederleini* (1.25 to 4.01 g) and *O. cyanosoma* (1.70 to 3.66 g) were collected from the reef using a hand net after lightly anaesthetizing them with clove oil (Munday & Wilson 1997). The fish were immediately transferred to temperature controlled indoor aquaria supplied with a continuous flow of fresh seawater pumped from the ocean. Control individuals were kept at the ambient ocean temperature, which during the experimental period was 28.5 to 29.5°C. Treatment fish were kept in identical aquaria where water temperature was increased over a period of 24 h to 31, 32 or 33°C (±0.5°C) with aquarium heaters. A 12 h light:12 h dark light cycle was maintained with fluorescent lighting.

For each fish species, 2 replicate aquaria were kept at each temperature. One aquarium was supplied with seawater at the ambient ocean pH (average 8.15, range 8.02 to 8.21). The other aquarium at each tem-

perature was supplied with seawater adjusted to pH 7.8 (range 7.75 to 7.85) to simulate future ocean acidification. pH was adjusted using the standard method of bubbling additional CO_2 into a reservoir tank (Leclercq et al. 2002, Michaelidis et al. 2005, Anthony et al. 2008), which then supplied equilibrated seawater to each of the test aquaria. pH in the 60 l reservoir tank was regulated with an automated pH controller (Tunze Aquarientechnik) connected to an electronic solenoid valve. A laboratory-grade glass pH probe continuously monitored pH in the reservoir. The solenoid injected bubbles of CO_2 into a diffuser (Red Sea Reactor 500) at the bottom of the reservoir tank whenever the pH rose above 7.8. The diffuser rapidly dissolved CO_2 into the seawater and also served as a vigorous stirrer. The equivalent atmospheric concentration of CO_2 for the pH treatment was estimated by sealing replicate tanks in which the pH of the water had been adjusted and then measuring the increase in pCO_2 in a narrow headspace above the water surface with an infrared CO_2 probe (Vaisala). The estimated concentration of CO_2 in the pH treatment was between 1000 and 1050 ppm. This value closely matches that of other studies that have found a 0.3 to 0.4 unit decline in seawater pH corresponding to ~1000 ppm CO_2 (Havenhand et al. 2008, Rosa & Seibel 2008). Water O_2 level varied between 95 and 100% of air saturation.

Seven to 13 individuals of each species were acclimated to each combination of temperature and CO_2 acidification for 1 wk prior to measurements. One week was considered sufficient because fishes rapidly became accustomed to captivity, and acclimation periods up to 3 wk did not affect rates of O_2 consumption (Nilsson unpubl. data). Furthermore, previous studies have found that compensation of blood acid–base balance of fish exposed to hypercapnia is usually stabilized within 1 to 5 d (Michaelidis et al. 2007). Fishes were fed twice daily to satiation with frozen blood worms and commercial fish food (INVE Aquaculture Nutrition pellets), but starved for 24 h before respirometry. Aquaria were checked hourly during daylight hours and any mortality recorded.

Respirometry. The methods for measuring resting O_2 consumption (MO_{2rest} as described by Nilsson & Östlund-Nilsson 2004) and maximal O_2 uptake using swim respirometry (MO_{2max} as described by Nilsson et al. 2007) followed the protocols described by Nilsson et al. (2009). Briefly, the respirometry chamber for measuring resting O_2 consumption consisted of a plexiglass cylinder (internal diameter: 80 mm) that could be sealed at both ends. An O_2 electrode (OXI 340i, WTW) continuously recorded O_2 levels within the chamber. The chamber was placed in an aquarium containing seawater at the test temperature and acidification treatment. The sides of the aquarium were covered to limit visual disturbance to fish in the chamber. At the start of a trial, a single fish was placed in the respirometer and allowed to acclimate for 2 h with water flowing through the chamber. Fish settled and became calm within minutes of being introduced to the chamber. Longer acclimation periods (24 h) in the chamber did not further reduce the rate of O_2 uptake in these fishes (Nilsson et al. 2009). After the acclimation period, the chamber was closed and O_2 levels continuously recorded for 30 to 40 min. All recordings were taken at O_2 levels of 70 to 100% of air saturation.

Maximum O_2 consumption of each individual was then tested in a respirometer where the fish was forced to swim against a water current. The swim-respirometry chamber consisted of a plexiglass cylinder with an internal diameter of 80 mm and a total water volume of 500 ml. The chamber could be opened at the bottom, where a petri dish was tightly fitted. The tip of an O_2 electrode (same as above) was inserted 10 mm above the bottom of the chamber. A removable wire mesh (5 mm mesh width) was positioned horizontally in the middle of the chamber. Above the mesh, a centrally placed cylinder created a circular swim chamber, and the water was set in motion by a 6 cm long magnetic stirring bar in the compartment below the mesh. The respirometer was placed at the bottom of the temperature-controlled aquarium, below which a magnetic plate was placed to drive the stirring bar in the respirometer. Water speed was regulated with the magnetic stirrer. As soon as the water was set in motion, the fish started swimming against the current. Water speed was set to a point where each fish swam at or just above the aerobic maximum speed. This was achieved by increasing the water speed to a point where the fish was barely able to maintain a steady position in the chamber. Water O_2 was recorded for 10 min, during which time a linear fall in O_2 was observed. Recordings were taken at water O_2 concentrations between 90 to 100% of air saturation.

Resting and swimming O_2 consumption was tested for 6 to 8 fish at each combination of temperature (29, 31 and 32°C) and CO_2 acidification (control seawater & pH 7.8). Respirometry was not conducted at 33°C because of the high mortality in the CO_2-acidification treatment (Table 1). All experiments were carried out between 08:00 to 18:00 h.

Data analysis. Factorial ANOVA was used to test the effects of temperature and CO_2 acidification on resting O_2 consumption, maximum O_2 uptake while swimming, and aerobic scope for each species. Aerobic scope was calculated as the difference between maximum swimming and resting O_2 consumption. Where ANOVA revealed significant effects, Newman-Keuls multiple comparison tests were used to compare means between temperatures and between CO_2-

acidified water and control water. Resting O_2 values for *Ostorhinchus cyanosoma* were square root transformed prior to analysis to improve the distribution of residuals.

Logistic regression was used to test if CO_2 acidification affected the probability of survival at each temperature. Analysis was conducted using Statistica version 8.

RESULTS

Aerobic capacity

Temperature and CO_2 acidification affected the O_2 consumption and aerobic scope of both species of fish. Resting O_2 consumption increased with increasing temperature and with CO_2 acidification (Table 1, Fig. 1). For both species, resting O_2 consumption in acidified water exhibited an asymptotic relationship with increasing temperature, rising significantly between 29 and 31°C, but with no further increase from 31 to 32°C (Fig. 1). For *Ostorhinchus doederleini*, resting O_2 consumption in acidified water was significantly higher than in control water at 31°C but not at either of the other 2 temperatures (Fig. 1a). For *O. cyanosoma*, resting O_2 consumption in acidified water was significantly higher than in control water at 29°C, but not at 31 or 32°C (Fig. 1b), resulting in a significant interaction between temperature and acidification (Table 1).

Maximum O_2 consumption did not change significantly with temperature for either species (Table 1, Fig. 2). For *Ostorhinchus doederleini*, maximum O_2 consumption did not differ between CO_2-acidified water and control water (Fig. 2a, Table 1). In contrast, maximum O_2 consumption was lower for *O. cyanosoma* in CO_2-acidified water than in control water (Fig. 2b, Table 1), although the mean values were only significantly different at 32°C.

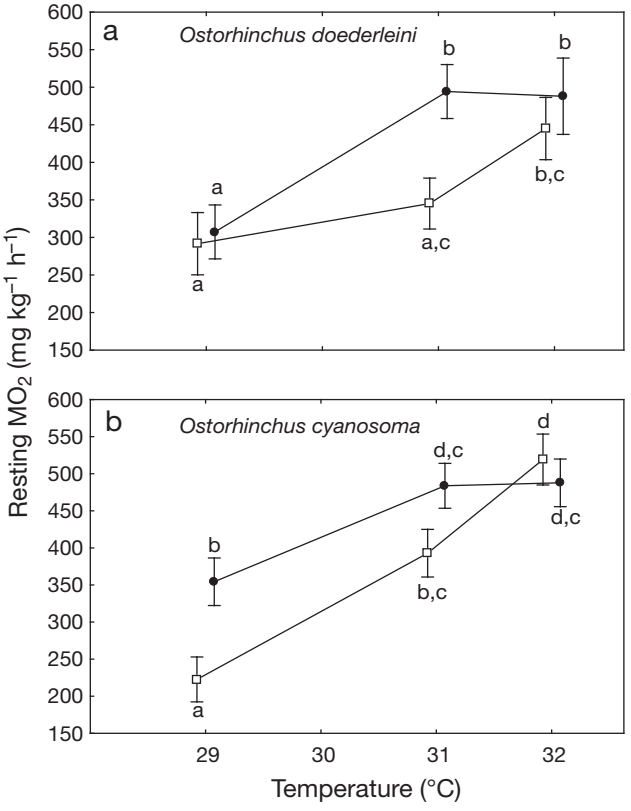

Fig. 1. (a) *Ostorhinchus doederleini* and (b) *O. cyanosoma*. Mean resting MO_2 (±SE) at 6 combinations of water temperature (29, 31, 32°C) and seawater pH (control, 7.8). (□) Control seawater, (●) CO_2-acidified seawater

Table 1. *Ostorhinchus doederleini* and *O. cyanosoma*. ANOVA for resting oxygen consumption (resting MO_2), maximum swimming oxygen consumption (swimming MO_2), and aerobic scope (swimming – resting oxygen consumption) of individuals from Lizard Island at 8 combinations of water temperature (29, 31, 32, 33°C) and pH (8.15, 7.8)

	O. doederleini				*O. cyanosoma*			
Source	df	MS	*F*	p	df	MS	*F*	p
Resting MO_2								
Temp	2	91 689	8.89	<0.001	2	135.30	28.67	<0.001
pH	1	45 742	4.43	0.042	1	35.41	7.50	0.008
Temp × pH	2	18 838	1.83	NS	2	19.26	4.08	0.02
Error	35	10 317			43	4.72		
Swimming MO_2								
Temp	2	35 634	1.08	NS	2	4786	0.13	NS
pH	1	74 527	2.26	NS	1	750 201	20.86	<0.001
Temp × pH	2	104 297	3.16	NS	2	89 444	2.49	NS
Error	33	32 968			43	35 970		
Aerobic scope								
Temp	2	82 801	3.35	0.047	2	160 580	4.39	0.018
pH	1	239 081	9.68	0.003	1	1 184 650	32.46	<0.001
Temp × pH	2	36 355	1.47	NS	2	46 822	1.28	NS
Error	33	24 707			43	36 501		

Aerobic scope decreased with both increasing temperature and CO_2 acidification for both species (Table 1). There was no interaction between temperature and acidification. When temperature was considered alone, the overall mean aerobic scope of *Ostorhinchus doederleini* declined by 36% from 483 (±42) mg O_2 kg^{-1} h^{-1} at 29°C to 307 (±56) mg O_2 kg^{-1} h^{-1} at 32°C. For *O. cyanosoma*, the mean aerobic scope declined by 32% from 609 (±46) mg O_2 kg^{-1} h^{-1} at 29°C to 410 (±49) mg O_2 kg^{-1} h^{-1} at 32°C. The underlying reason for the decreasing aerobic scope with increasing temperature was the increase in resting O_2 consumption but not in maximum O_2 consumption, with

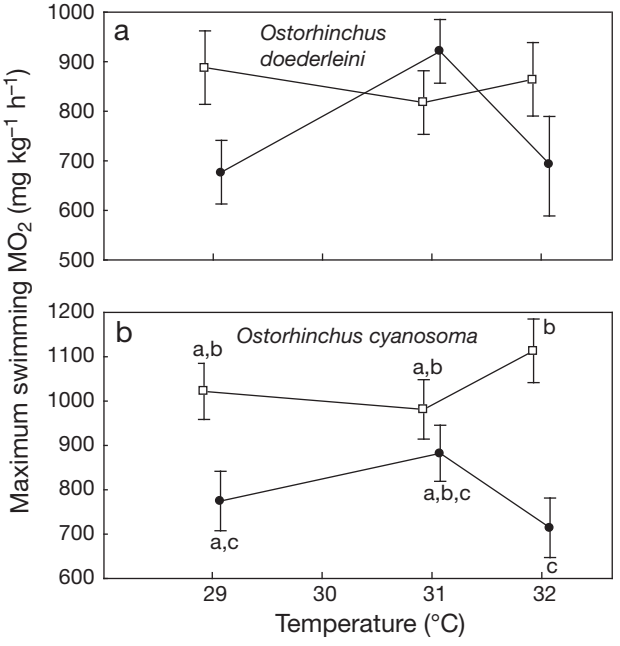

Fig. 2. (a) *Ostorhinchus doederleini* and (b) *O. cyanosoma*. Mean swimming MO_2 (±SE) at 6 combinations of water temperature (29, 31, 32°C) and seawater pH (control, 7.8). (□) Control seawater, (●) CO_2-acidified seawater

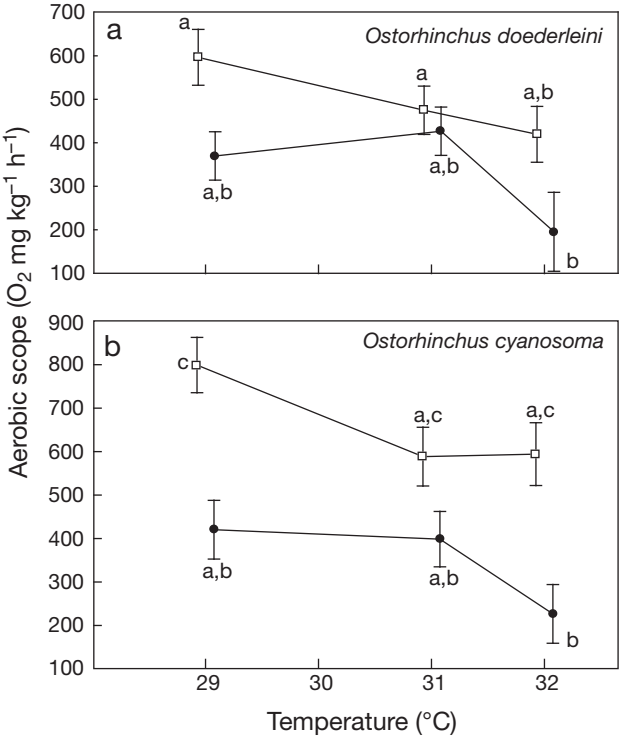

Fig. 3. (a) *Ostorhinchus doederleini* and (b) *O. cyanosoma*. Mean aerobic scope (±SE) at 6 combinations of water temperature (29, 31, 32°C) and seawater pH (control, 7.8). (□) Control seawater, (●) CO_2-acidified seawater

temperature. The aerobic scope was also lower for fish kept in CO_2-acidified water than for fish in control water (Table 1). When acidification was considered alone, the overall mean aerobic scope of *O. doederleini* declined by 33% from 497 (±35) mg O_2 kg^{-1} h^{-1} in control water to 330 (±40) mg O_2 kg^{-1} h^{-1} in acidified water. For *O. cyanosoma*, the mean aerobic scope declined by 47% from 661 (±39) mg O_2 kg^{-1} h^{-1} in control water to 348 (±38) mg O_2 kg^{-1} h^{-1} in acidified water. Therefore, the percent decline in aerobic scope in acidified water (33 to 47%) was similar to that caused by increasing temperature from 29 to 32°C (32 to 36%).

Despite the significant main effects, the only significant differences in aerobic scope for *Ostorhinchus doederleini* that were detected by post-hoc tests when all means were considered together were between fish in control water at 29°C and those in acidified water at 32°C (Fig. 3a). There were no significant differences in aerobic scope between fish in control water and those in acidified water within temperature treatments. In contrast, the aerobic scope for *O. cyanosoma* was significantly different between fish in control and those in acidified water at 29 and 32°C (Fig. 3b). The mean aerobic scope in acidified water was similar for the 2 species at each of the 4 test temperatures (Fig. 3), with minimum values of 195 (±91) mg O_2 kg^{-1} h^{-1} for *O. doederleini* at 32°C (Fig. 3a) and 226 (±68) mg O_2 kg^{-1} h^{-1} for *O. cyanosoma* at 32°C (Fig. 3b).

Mortality

There was no mortality in either species at 29, 31 or 32°C in control water and only a small number of fish died at these temperatures in acidified water (Table 2; 4 of 34 *Ostorhinchus doederleini* and 2 of 27 *O. cyanosoma*). In contrast, over ⅓ of individuals from both species died within a week in control water at 33°C (3 of 8 *O. doederleini* and 4 of 9 *O. cyanosoma*). This indicates that 33°C is close to the lethal thermal limit of both species at Lizard Island. CO_2 acidification significantly increased mortality of *O. doederleini* (Wald = 8.41, p = 0.004), but did not affect the mortality rate of *O. cyanosoma* (Wald = 0.87, p = 0.35). Four of 12 *O. doederleini* died in CO_2-acidified water at 32°C and all of them died in acidified water at 33°C (Table 2).

DISCUSSION

Aerobic function affects all aspects of individual performance and, thus ultimately, population sustainability. We found that both increased temperature and CO_2-induced acidification reduced the aerobic scope of 2 common coral reef fish species. Aerobic scope

Table 2. *Ostorhinchus doederleini* and *O. cyanosoma*. Number and percent (in parenthesis) of individuals from Lizard Island that died when kept at 8 combinations of water temperature (29, 31, 32, 33°C) and pH (8.15, 7.8) for 1 wk

Temperature (°C)	Species and seawater pH			
	O. doederleini		*O. cyanosoma*	
	8.15	7.80	8.15	7.80
29	0	0	0	1 (11.1)
31	0	0	0	0
32	0	4 (33.3)	0	1 (11.1)
33	3 (37.5)	13 (100)	4 (44.4)	3 (33.3)

decreased with increasing temperature, as has been previously described (Nilsson et al. 2009). Importantly, acidification had an additional effect that further reduced aerobic scope, and the percent declines in aerobic scope in acidified water (equivalent to ~1000 ppm atmospheric CO_2) were of a similar magnitude to the declines caused by a 3°C increase in water temperature above summer averages. Increasing levels of atmospheric CO_2 over the coming century will act to both increase average global temperatures and acidify the ocean (Meehl et al. 2007). A fall in aerobic scope with increasing temperature is thought to be the key physiological mechanism determining how most marine species will be affected by climate change, setting the limits for species distributions and causing range shifts (Pörtner & Knust 2007). Our results suggest that the aerobic capacity of some species will be further compromised by the effects of additional CO_2 dissolved in seawater as atmospheric CO_2 continues to rise. Consequently, levels of atmospheric CO_2 that could be attained by 2100 could have significant impacts on the success of some marine fishes by reducing their capacity for aerobic activity.

The effects on aerobic scope observed here could have been due to the direct effects of an elevated CO_2 level (~1000 ppm), the reduced seawater pH (0.35 units), or a combination of both. It is likely that hypercapnia played a significant role because CO_2 readily diffuses across fish gills where it acts to acidify the blood and other tissues (Ishimatsu et al. 2005, 2008, Pörtner et al. 2005). Reduced seawater pH will also acidify blood and tissue, but is expected to act more slowly. Bicarbonate accumulation and active ion transport are used by fish to compensate for increasing acidosis and to regulate their acid–base balance (Claiborne et al. 2002). However, these mechanisms are likely to have some physiological cost. Furthermore, elevated tissue pCO_2 may hinder the effective transport of O_2, especially in species or life stages with high metabolic demands (Pörtner et al. 2005). Whatever specific mechanisms were responsible for the reduction in aerobic scope we observed, our experiments show that continued CO_2-induced acidification of the ocean could affect the aerobic capacity of some marine fishes, and thus potentially influence the sustainability of local populations.

The underlying reason for the reduced aerobic scope in CO_2-acidifed water for the 2 species tested here was that resting O_2 consumption either increased or remained stable in acidified water compared to control water (Fig. 1), whereas maximum O_2 consumption either decreased or remained stable in acidified water compared to control water (Fig. 2). This effect was more apparent in *Ostorhinchus cyanosoma*, which exhibited a larger percent decline in aerobic scope than *O. doederleini*. Although increased CO_2 often causes metabolic depression in marine invertebrates (Pörtner et al. 2004, Michaelidis et al. 2005, Rosa & Seibel 2008), similar effects are not usually observed in fish. Instead, resting O_2 consumption of fish is usually unaffected by hypercapnia (Pörtner et al. 2004, Ishimatsu et al. 2005, 2008). This indicates that fish are generally able to meet O_2 demands when exposed to elevated CO_2 levels provided that no exercise is necessary (Ishimatsu et al. 2005). We observed small increases in resting O_2 consumption for both species of coral reef fishes studied here, even after a week of acclimation; this suggests some energetic cost involved in acid–base compensation for these species, even under the relatively low levels of hypercapnia used in our experiments.

While resting O_2 consumption tended to increase slightly in CO_2-acidifed water, maximum O_2 consumption during swimming was unaffected in *Ostorhinchus doederleini* and reduced in *O. cyanosoma*. Decreased O_2 consumption during swimming under hypercapnic conditions has been observed in other fishes (Ishimatsu et al. 2005), but only at levels of ambient CO_2 that are much greater than used in our experiments to simulate ocean acidification. The significant decline in maximum swimming O_2 consumption for *O. cyanosoma* exposed to water acidified with ~1000 ppm CO_2 demonstrates that this species is especially sensitive to even small increases in ambient CO_2. This is reflected in the greater percent decrease in the aerobic scope of *O. cyanosoma* (47%) compared with *O. doederleini* (33%) in acidified water.

The sensitivity to CO_2 acidification displayed by the 2 species of coral reef cardinalfish has to our knowledge not been documented in other physiological studies on fish. Fishes are usually regarded as being relatively tolerant to a wide range of dissolved CO_2 levels and water pH (Ishimatsu et al. 2005, Pörtner et al. 2004, 2005). This is probably because most previous studies on pH and CO_2 effects on fish had been done on temperate species or tropical freshwater species that are adapted to habitats with large natural variations in CO_2 and acidity levels (Freda & McDonald 1988, Mor-

ris et al. 1989, Ishimatsu et al. 2005). For example, the rainbow trout *Oncorhynchus mykiss* not only lives in both freshwater and seawater, but also tolerates pH values from 6 to 9 without any measurable negative effects on physical performance (Randall & Brauner 1991, Morgan et al. 2001). In contrast, coral reef fishes do not experience such substantial changes in CO_2 and pH levels, which may make them more sensitive to changes in these environmental parameters. In a previous study, Nilsson et al. (2009) found that the aerobic performance of the 2 cardinalfish species studied here was more strongly affected by changes in environmental conditions (temperature) than a number of other coral reef fishes. Therefore, although coral reef fishes might be expected to be more sensitive to changes in CO_2 and pH than other fishes, we expect that the responses of *Ostorhinchus doederleini* and *O. cyanosoma* to acidification would be towards the extremes exhibited by coral reef fishes. Further studies are now needed to determine whether the effect of ocean acidification on the aerobic performance of the 2 cardinalfishes observed here is representative of other coral reef species or not.

In one of the few other studies testing the effects of moderately low levels of hypercapnia on marine fish, Michaelidis et al. (2007) observed changes in enzyme activity consistent with a shift from aerobic to anaerobic activity in gilthead bream (*Sparus aurata*) exposed to seawater where pH had been decreased by 0.75 of a unit (8.05 to 7.3) through the addition of CO_2. Such shifts in metabolic pathways are expected to have energetic costs and could affect individual performance. Although the reduction in pH used by Michaelidis et al. (2007) was twice that used in our experiments, the equivalent CO_2 levels (4.5 × ambient) were still much less than those used in most previous studies on hypercapnia in marine fishes (Ishimatsu et al. 2005, 2008) and illustrate that low to moderate levels of hypercapnia can have physiological consequences for some marine fishes. Similar effects on metabolic pathways might have been responsible for the reduced aerobic scope we observed in *Ostorhinchus doederleini* and *O. cyanosoma.*

Natural seawater pH at Lizard Island varied from a minimum of 8.08 to a maximum of 8.21 over the course of our experiments, with minimum values usually attained during the early morning. pH may decline in shallow reef habitats overnight due to the effect of accumulating CO_2 from respiration of reef organisms and values below 8.00 have been recorded at some locations (Ohde & van Woesik 1999, Kuffner et al. 2008). Therefore, reef fishes might already be exposed to elevated CO_2 and pH values approaching 7.8, especially if they shelter in the reef matrix overnight. However, regular tidal flushing of shallow reef water and photosynthesis by reef algae and corals during daylight hours would mean that elevated CO_2 and low pH values are temporary. The intermittent nature of low pH episodes in coral reefs may explain why the cardinalfishes we tested were sensitive to continued exposure to CO_2 acidification for a week even though pH values of 7.8 may already occur in some reef habitats.

Mortality of both cardinalfish species increased markedly at 33°C. Although 29°C is the average summer sea temperature at Lizard Island, maximum temperatures already exceed 30°C and extremes of up to 32.7°C have been recorded at this location (Lough 1999). Sea surface temperatures are predicted to increase by 1 to 3°C over the next 50 to 100 yr as a result of global warming (Lough 2008, Munday et al. 2009). Therefore, temperatures near 33°C will be experienced with greater frequency over the coming century. However, acute mortality at extreme temperatures is unlikely to be the primary threat to these species, or other coral reef fishes. The reduced capacity for aerobic function associated with moderate increases in temperature is likely to affect key aspects of individual performance, including feeding, growth and reproduction, and therefore threatens population sustainability before conditions become lethal to short-term individual survival.

Mortality of *Ostorhinchus doederleini* at elevated temperatures was exacerbated by high CO_2 and low pH, with all individuals dying within a week at 33°C in acidified water. This suggests that the physiological condition of individuals was already severely compromised at 33°C and that the additional stress of hypercapnia was sufficient to cause increased mortality. *O. doederleini* has an anti-tropical distribution (Randall et al. 1991), which suggests that it is cannot tolerate temperatures above 30°C that already occur in equatorial regions. Our results indicate that ocean acidification will further limit the low latitude locations where this species can persist in the future.

To date, studies on the effects of additional CO_2 in tropical seas has mostly focused on the effects of reduced carbonate saturation states on calcifying organisms (e.g. Kleypas et al. 2006, Anthony et al. 2008). This study shows that levels of atmospheric CO_2 that could be attained by 2100 could also have significant impacts on the success of some tropical marine fishes by reducing their capacity for aerobic activity. This supports recent suggestion that ocean acidification poses a significant physiological challenge to some sea animals, particularly when coupled with rising water temperature (Pörtner et al. 2005, Pörtner & Farrell 2008, Rosa & Seibel 2008). The capacity for aerobic activity underpins the sustainability of animal populations, and thus determines the locations where species can persist. Whether populations of fishes or other reef organisms can adapt to permanent acidification is unknown, but should be a priority area for further research.

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