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Species-specific effects of near-future CO_2 on the respiratory performance of two tropical prey fish and their predator

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ABSTRACT

Ocean surface CO_2 levels are increasing in line with rising atmospheric CO_2 and could exceed 900 μatm by year 2100, with extremes above 2000 μatm in some coastal habitats. The imminent increase in ocean pCO_2 is predicted to have negative consequences for marine fishes, including reduced aerobic performance, but variability among species could be expected. Understanding interspecific responses to ocean acidification is important for predicting the consequences of ocean acidification on communities and ecosystems. In the present study, the effects of exposure to near-future seawater CO_2 (860 μatm) on resting ($\dot{M}O_{2rest}$) and maximum ($\dot{M}O_{2max}$) oxygen consumption rates were determined for three tropical coral reef fish species interlinked through predator–prey relationships: juvenile *Pomacentrus moluccensis* and *Pomacentrus amboinensis*, and one of their predators: adult *Pseudochromis fuscus*. Contrary to predictions, one of the prey species, *P. amboinensis*, displayed a 28–39% increase in $\dot{M}O_{2max}$ after both an acute and four-day exposure to near-future CO_2 seawater, while maintaining $\dot{M}O_{2rest}$. By contrast, the same treatment had no significant effects on $\dot{M}O_{2rest}$ or $\dot{M}O_{2max}$ of the other two species. However, acute exposure of *P. amboinensis* to 1400 and 2400 μatm CO_2 resulted in $\dot{M}O_{2max}$ returning to control values. Overall, the findings suggest that: (1) the metabolic costs of living in a near-future CO_2 seawater environment were insignificant for the species examined at rest; (2) the $\dot{M}O_{2max}$ response of tropical reef species to near-future CO_2 seawater can be dependent on the severity of external hypercapnia; and (3) near-future ocean pCO_2 may not be detrimental to aerobic scope of all fish species and it may even augment aerobic scope of some species. The present results also highlight that close phylogenetic relatedness and living in the same environment, does not necessarily imply similar physiological responses to near-future CO_2.

1. Introduction

The average concentration of carbon dioxide (CO_2) in the atmosphere has increased from approximately 280 ppm in pre-industrial times (Barnola et al., 1987) to >400 ppm in 2013 (Dlugokencky and Tans, 2013) and is projected to exceed 900 ppm by the year 2100 if the current emission trajectory is maintained (Meinshausen et al., 2011). Because atmospheric and ocean surface pCO_2 are in equilibrium, CO_2 in the ocean is also increasing at approximately the same rate as in the atmosphere (Doney, 2010). Moreover, due to the hydrolysis of CO_2 in seawater, ocean surface pH is 0.1 unit lower today than preindustrial values and is predicted to be a further 0.3–0.4 units lower by 2100, which translates to a 100–150% increase in $[H^+]$ (Solomon et al., 2007). Some coastal regions could experience changes to $[H^+]$ that are at least 2–3 times the global average due to CO_2 enhancement from eutrophication (Melzner et al., 2012) and amplification of natural CO_2 and pH variation (Shaw et al., 2013). Such changes in ocean chemistry are predicted to affect physiological functions of many marine organisms, with potentially far-reaching effects on marine diversity and ecosystem processes (Fabry et al., 2007; Pörtner, 2008; Gattuso and Hansson, 2011).

Ocean acidification has been hypothesized to have negative consequences for the performance of marine fishes, primarily through an effect of the capacity for oxygen supply and delivery (Pörtner et al., 2004). Aerobic scope, which represents the oxygen available for any activities beyond that required for basic maintenance (Fry, 1947, 1971; Fry and Hart, 1948), is expected to decline with increasing pCO_2 (Pörtner and Farrell, 2008). Reduced aerobic scope could affect individual fitness, since less energy can be devoted to digestion, growth and reproduction (Munday et al., 2009b; 2012). Reduced aerobic scope could also affect the outcome of key ecological interactions and ultimately the structure of ecological communities (Pörtner, 2008; Nilsson et al., 2009).

In accordance with these predictions, the aerobic capacity of two cardinalfish species from the Great Barrier Reef (GBR) was significantly reduced by exposure to CO_2-acidified water (Munday et al., 2009a). However, it may be expected that not all fish species of coral reef

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ecosystems will be similarly affected by CO_2-induced ocean acidification. Fishes are the most diverse group of vertebrates and the ontogenetic and lifestyle traits of some species could provide pre-adaptation to high ambient CO_2 (Ishimatsu et al., 2008; Melzner et al., 2009b). Indeed, the aerobic scope of some fish species appears to be unaffected by hypercapnia (McKenzie et al., 2003; Ishimatsu et al., 2008; Baker and Brauner, 2012). Interspecific differences in the response of aerobic scope to near-future seawater CO_2 could have important ecological ramifications, especially for species that interact through competitive or predator–prey relationships (Munday et al., 2012). Many studies implicate species interactions to be an important proximate cause of extinction due to climate change, particularly due to decreases in food availability (Cahill et al., 2012).

The objective of the present study was to assess species-specific effects of near-future seawater CO_2 on aerobic performance among tropical reef fish species involved in predator and prey relationships. The model predator species investigated was the brown dottyback (*Pseudochromis fuscus* Müller & Troschel 1849), a common mesopredator on the GBR. Two closely related damselfishes, the lemon damselfish (*Pomacentrus moluccensis* Bleeker 1853) and the Ambon damselfish (*Pomacentrus amboinensis* Bleeker 1868) were chosen as the model prey species. *P. fuscus* is known to be a major predator of recently settled juveniles of these two damselfishes on the GBR (Holmes and McCormick, 2010). Respirometry was utilized to measure the effects of exposure to elevated CO_2 on resting ($\dot{M}O_{2rest}$) and maximum ($\dot{M}O_{2max}$) oxygen consumption rates. In fish, physiological alterations in response to elevated ambient CO_2 can occur within minutes (*i.e.*, ventilatory responses; Gilmour and Perry, 2006), hours (*i.e.*, blood acid-base regulation; Brauner and Baker, 2009; Heisler, 1993; Esbaugh et al., 2012) to days (*i.e.*, neurological disruptions; Nilsson et al., 2012). In terms of neurological impairments, four days of near-future CO_2 exposure has been shown to disrupt a number of sensory systems and alter the behavior of reef fishes, including the three species examined in the present study (Cripps et al., 2011; Ferrari et al., 2011a; Ferrari et al., 2011b; Nilsson et al., 2012). Longer exposure to elevated CO_2 does not induce further behavioral effects (Munday et al., 2010). Therefore, in our first experiment, we measured oxygen consumption after exposing the three species to ambient or elevated CO_2 for four days, to enable direct comparisons with previous studies on coral reef fish. In a second experiment, we measured oxygen consumption of the damselfishes, following acute exposure to near-future seawater CO_2 to determine if exposure to elevated CO_2 induces an immediate effect on aerobic performance. In both experiments, the elevated CO_2 treatment (860 μatm) was selected to approximate the level predicted for the atmosphere and ocean surface in 2100 under the IPCC A2 emissions scenario (Meehl et al., 2007). Finally, a third experiment was conducted to understand the effects of more extreme fluctuations in seawater pCO_2 and consequently pH that may occur in some coastal habitats, including shallow coral reef flats (Shaw et al., 2013). Four groups of *P. amboinensis* were exposed to one of four pH levels spanning from the present-day control pH of 8.1 down to pH 7.5 at 0.2 unit increments. The desired pH level was obtained by adding increasing volumes of 100 mM hydrochloric acid to the seawater. The addition of a strong acid to a closed system, such as a closed respirometer, has similar consequences on pCO_2 and pH as equilibrating water of an open system with CO_2 gas (Gattuso and Lavigne, 2009). Corresponding pCO_2 levels were approximately 450 μatm at pH 8.1, 860 μatm at pH 7.9, 1400 μatm at pH 7.7 and 2400 μatm at pH 7.5.

2. Materials and methods

2.1. Experimental fish

The experiments were conducted at Lizard Island Research Station (LIRS; 14°40′S, 145°28′E) between December and January (austral summer). Juvenile *P. moluccensis* (mean ± SD, 40.9 ± 5.6 mg) and *P. amboinensis* (53.9 ± 12.4 mg) were caught at night using light traps moored 2 m below the surface and approximately 100 m off the reef (Meekan et al., 2001). In this location, the fish are trapped immediately before their arrival to the reef at the end of their planktonic larval stage (Meekan et al., 1993). Every morning, juveniles were collected from the traps and transferred to the laboratory where they were exposed to ambient or elevated CO_2 for four days (see Section 2.2). Adult *P. fuscus* (4.51 ± 0.78 g) were collected from shallow reefs (<6 m) in the Lizard Island lagoon using a hand-net after lightly anesthetizing them with a mixture of clove oil, ethanol and seawater (Munday and Wilson, 1997). Captured fish were transported to the research station where they were maintained for two days prior to exposure to CO_2 treatments. Fish were maintained at ambient ocean temperatures, which ranged from 28.3 to 30.4 °C (Table 1; 29.4 ± 0.1 °C) during the experimental period. Damselfishes were fed freshly hatched *Artemia* nauplii three times daily, and *P. fuscus* were fed twice daily to satiation with INVE Aquaculture Nutrition pellets. Feeding was discontinued 18–24 h prior to resting oxygen consumption measurements (see section 2.3.1). Animal care and experimental protocols complied with regulations at James Cook University and Lizard Island Research Station, and were approved by the James Cook University Ethic Committee (Approval # A1722). Fish were collected under permit G10/33239.1 from the GBR Marine Park Authority.

2.2. CO_2 exposure

Fish were exposed to either aerated control water (pCO_2 = 451 μatm) or 860 μatm CO_2 water (termed near-future seawater CO_2 in the present study) for four days (Table 1). Near-future seawater CO_2 concentrations were maintained by CO_2-dosing to a set pH_{NBS} (National Bureau of Standards) following standard techniques for ocean acidification research (Gattuso et al., 2010). Seawater was pumped from the ocean into two 60 l header tanks, one equilibrated with air (ambient control) and the other with CO_2 to achieve the pH expected to correspond to the ocean CO_2 concentration projected for 2100 (Meehl et al., 2007). The pH level was based upon preliminary observations of total alkalinity, salinity and temperature of seawater at Lizard Island. A pH-controller (Aqua Medic GmbH, Bissendorf, Germany) was attached to the CO_2-treated header tank to maintain pH at the desired level. A solenoid injected a slow stream of CO_2 into a submersible pump at the bottom of the header tank whenever the seawater pH rose above the set point. The pump ensured rapid dissolution of CO_2 into the seawater and also served as a vigorous stirrer. The pump in the control seawater header tank was injected with a slow stream of air. Seawater from each header tank was supplied at a rate of ca 500 ml min^{-1} to four replicate 35 l aquaria for each species. pCO_2 in the aquaria was checked twice daily with a CO_2-permeable membrane connected to an infrared CO_2 probe (Vaisala GMP343, Vaisala, Helsinki, Finland) in a closed loop (Hari et al., 2008). Water samples were collected at the start, middle and end of the experiment in order to precisely determine pCO_2. Total alkalinity (A_T) of seawater was estimated by Gran titration (Gran, 1950; 1952) using certified reference material from Dr. A. G. Dickson (Scripps Institution of Oceanography), and average seawater pCO_2 was calculated with CO2SYS (http://cdiac.ornl.gov/oceans/co2rprt.html) from measured A_T and pH and using the constants of Mehrbach et al. (1973) refit by Dickson and Millero (1987).

Table 1
Mean (±SE) seawater parameters in the experimental system. pCO_2 was estimated with the program CO2SYS from measured pH, salinity and total alkalinity (A_T) of water samples.

Treatment	pH_{NBS}	Temperature (°C)	Salinity (ppt)	A_T (μmol kg^{-1} SW)	pCO_2 (μatm)
Control	8.11–8.17	29.4 ± 0.1	34.5	2272 ± 13	451 ± 15
Near-future CO_2	7.90–7.92	29.4 ± 0.1	34.5	2267 ± 2	860 ± 14

2.3. Experimental set-up and protocol

$\dot{M}O_{2rest}$ and $\dot{M}O_{2max}$ were used as proxies for resting and maximum metabolic rates and were measured by respirometry as previously utilized for assessing the effects of climate change variables on other fish (see Ishimatsu et al., 2008 for review), especially tropical reef fish species (Nilsson and Östlund-Nilsson, 2004; Nilsson et al., 2007a; Nilsson et al., 2007b; Munday et al., 2009a; Gardiner et al., 2010; Nilsson et al., 2010). Respirometry chambers were immersed in temperature-controlled (29 °C) aquaria continuously supplied with either air- or near-future CO_2-equilibrated seawater.

2.3.1. Resting oxygen consumption

Cylindrical 26.7-ml static respirometers were used for *P. moluccensis* and *P. amboinensis* juveniles. After four days of exposure to current day or near-future CO_2 seawater, one fish was transferred to each respirometry chamber. The chamber was left open and the fish left undisturbed to habituate to the chamber for 1–2 h whereupon the chamber was cautiously closed without disturbing the fish. Previous experiments have shown that habituation periods longer than 2 h in the chamber do not further reduce $\dot{M}O_2$ (Nilsson and Östlund-Nilsson, 2004; Nilsson et al., 2010). All fishes included in this study settled down rapidly and remained virtually motionless during the measuring period. Once the chamber had been sealed, water oxygen concentration was recorded continuously with an oxygen probe (CellOx 325, WTW, Germany; calibrated daily) connected to an oxygen meter (OXI 340i, WTW, Germany). The oxygen probe was fitted with a magnetic propeller (BOD stirring accessory, WTW, Germany) set in motion with a magnetic stir plate situated outside the aquarium along the glass wall. The propeller ensured gentle water mixing inside the respirometer and water renewal along the O_2 probe membrane during the habituation and recording periods. The oxygen meters were connected to a data acquisition system (PowerLab 4/20, ADInstruments, Colorado Springs, USA). $\dot{M}O_{2rest}$ was calculated from the steady rate of oxygen consumption observed between 100 and 90% of air saturation. The decrease of water oxygen concentration was recorded until it reached approximately 10% of air saturation in order to calculate the critical oxygen concentration (O_{2crit}), which is the lowest O_2 concentration where the fish is still able to maintain $\dot{M}O_{2rest}$. O_{2crit} was reached 2.5–3 h after the chamber was sealed. For each species, two parallel setups allowed for the simultaneous recording of one fish in near-future CO_2 water and another fish in control conditions. Similar to a number of other prior experiments conducted to determine the O_{2crit} of a fish using this classic protocol, the measurement of O_{2crit} required the respirometer to remain closed until almost all O_2 was depleted from the system. Consequently, CO_2 concomitantly increased in the respirometer due to the respiration of the fish and the fish simultaneously experienced hypoxia and increasing hypercapnia during the O_{2crit} measurement. However, most of the excreted CO_2 would convert rapidly to bicarbonate. Assuming a respiratory quotient of 1.0, calculations of the rise in dissolved inorganic carbon (DIC) in the respirometers caused by the respiration of the fish estimate that the maximum build-up of CO_2 for each 10% fall in O_2 was 9.7 and 12.2% for control and near-future CO_2 fish respectively.

For *P. fuscus*, $\dot{M}O_{2rest}$ was measured in 1615-ml intermittent-flow respirometers. Fish were first habituated to the chambers for 90 min. Preliminary experiments determined that 90 min was ample time for this species to ensure O_2 consumption rates had reached the lowest possible values under the experimental conditions. Beyond this time, O_2 consumption rates did not significantly vary. Submersible pumps supplied a water flow (150 l h^{-1}) from the aquaria through the chambers and after the habituation period, water flow to each chamber was stopped for 15 min every 30 min over a period of 90 min. The time the water flow was interrupted was short enough to ensure O_2 did not fall below 80% of air saturation. Water oxygen concentration (mg l^{-1}) was continuously recorded at a frequency of 1 Hz using oxygen-sensitive REDFLASH dye on contactless spots (2 mm) adhered to the inside of each chamber and connected *via* fiber-optic cable to a Firesting Optical Oxygen Meter (Pyro Science e. K., Aachen, Germany). Data were analyzed using LabChart 6.1.3 (ADInstruments, Colorado Springs, USA).

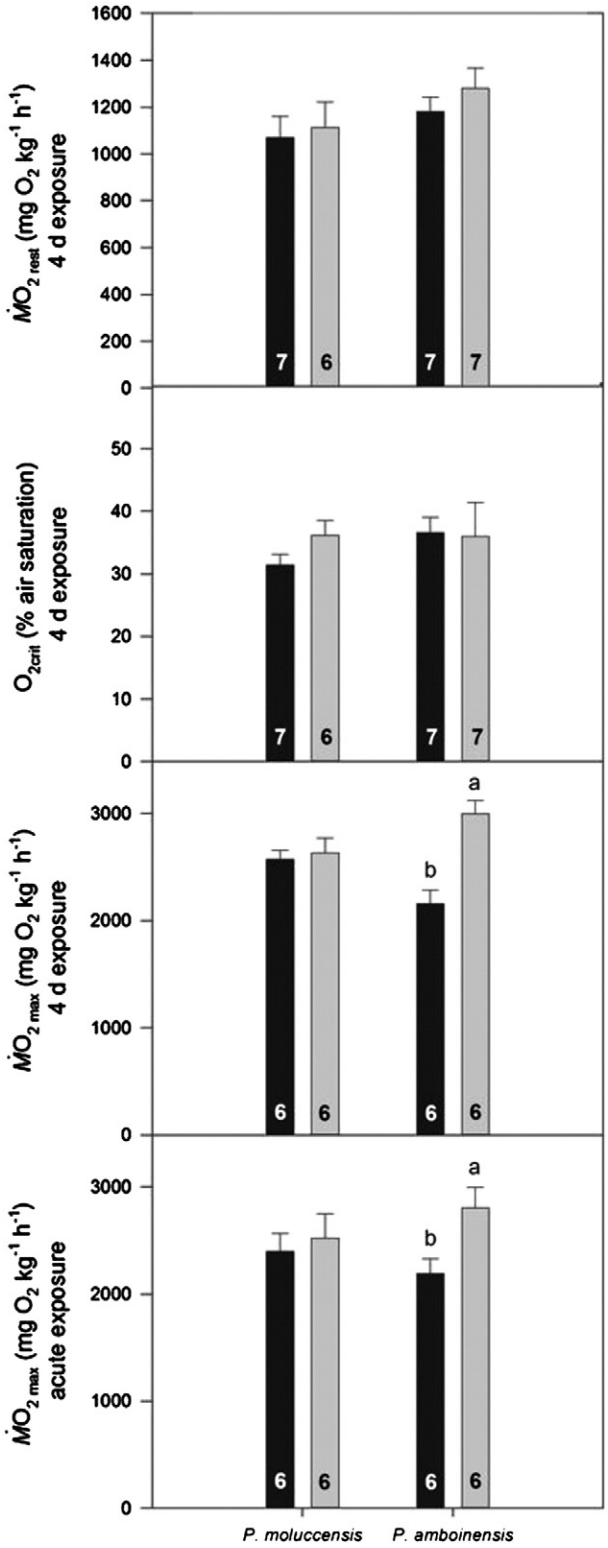

Fig. 1. Respiratory performance (mean ± SE) of *P. moluccensis* and *P. amboinensis*. A. Resting oxygen consumption rate ($\dot{M}O_{2rest}$; mgO_2 kg^{-1} h^{-1}), B. critical oxygen concentration (O_{2crit}; % of air saturation), C. maximum oxygen consumption rate ($\dot{M}O_{2max;}$ mgO_2 kg^{-1} h^{-1}) after a 4 d exposure to control seawater (451 μatm; ■) or near-future CO_2 seawater (860 μatm; ■) and D. $\dot{M}O_{2max}$ after an acute exposure to near-future CO_2 seawater. Letters that differ indicate statistically significant differences (see text for *P* values). N numbers are indicated at the bottom of each bar.

2.3.2. Maximum rate of oxygen consumption

$\dot{M}O_{2max}$ was measured in custom made cylindrical swimming chambers previously utilized by a large number of studies for small tropical coral reef fish species (see Nilsson et al., 2007b for diagram and detailed description of the set-up; Gardiner et al., 2010; Nilsson et al., 2009; Munday et al., 2009a; Nilsson et al., 2007a; Nilsson et al., 2010; Nilsson and Östlund-Nilsson, 2004). The volume of the chambers ranged between 215 and 267 ml for *P. moluccensis* and *P. amboinensis* juveniles and was 1594 ml for *P. fuscus*. The decrease in [O_2] was measured with an O_2 electrode (WTW CellOx 325, as above). It has been suggested that the aerobically fuelled muscle mass in some fish is not large enough to force them to reach the maximum rate of oxygen uptake during maximal swimming performance (Goolish, 1991). Therefore, fish were fed *ad libitum* prior to estimating $\dot{M}O_{2max}$, since it is likely that the combined oxygen needs of digestion and maximal swimming would be high enough to engage the full capacity of the respiratory system (Bennett and Hicks, 2001; Gardiner et al., 2010). Up to three *P. amboinensis* (to increase the total fish weight so that reliable recordings could be made), one *P. moluccensis* or one *P. fuscus* (same fish as for $\dot{M}O_{2rest}$ measurements) were placed in the swimming chamber. The water speed was regulated with a magnetic stirrer located beneath the chamber. As soon as the water was set in motion, the fishes started swimming against the current, apparently guided by landmarks provided by items such as the oxygen electrode and the edges of the surrounding aquarium. The speed was consistently increased to a point where it was assumed that the fishes swam at their maximum speed. The water speed corresponded to the point at which the fishes could just barely maintain a steady position in the chamber. At a slightly higher speed, the fishes were no longer able to maintain position for more than a few seconds and stopped swimming. Nilsson et al. (2007b) showed that water speeds of approximately 50 and 125 cm s^{-1} could be achieved near the inner and outer wall of the chamber, respectively. The speeds are more than sufficient for each of the species examined to reach their maximal swimming speed. Pre-settlement larvae of both *Pomacentrus* species as well as *P. fuscus*, which are more efficient swimmers than the post-settlement larvae and adults studied here (Nilsson et al., 2007b) exhibit an average maximum sustained swimming speed ranging from less than 30 cm s^{-1} to a maximum of 36 cm s^{-1} (Fisher et al., 2005). The decrease in oxygen concentration was recorded at a frequency of 1 Hz in the chamber at the maximum swimming speed for up to 6 min, during which time oxygen concentration remained above 90% of air saturation.

First, $\dot{M}O_{2max}$ was measured in the three species that had been maintained in control or near-future CO_2 seawater for four days. Then, in order to assess an acute effect of near-future CO_2, $\dot{M}O_{2max}$ was measured in *P. moluccensis* and *P. amboinensis* maintained in control conditions for four days and acutely exposed to near-future CO_2 while in the respirometer. Finally, to further understand the effects of increased CO_2 and low pH in more extreme habitats, $\dot{M}O_{2max}$ of four groups of *P. amboinensis* was measured after acute exposure of the fish to pH levels of 8.1 (control, present day level, corresponding to approximately 450 μatm CO_2), 7.9 (corresponding to approximately 860 μatm CO_2), 7.7 (corresponding to approximately 1400 μatm CO_2) and 7.5 (corresponding to approximately 2400 μatm CO_2). The desired pH level was obtained by diluting increasing volumes of 100 mM hydrochloric acid in the seawater utilized in the experimental set-up. The water was prepared immediately prior to the experiment, and within 2 min, the fish was placed in the respirometry chamber, which was sealed, thereby preventing the water from equilibrating with the atmosphere. $\dot{M}O_{2max}$ was then measured as described above. Average seawater pCO_2 was calculated with CO2SYS (http://cdiac.ornl.gov/oceans/co2rprt.html) from measured pH and assuming the same A_T as in the control conditions. The addition of a strong acid to a closed system like a closed respirometer has rather similar consequences on water chemistry as equilibrating water of an open system with CO_2 gas, thereby allowing reasonable comparison between both techniques (Gattuso and Lavigne, 2009). However, without the addition of CO_3^{2-} or HCO_3^-, the technique leads to a slightly lower A_T. For example, Gattuso et al. (2010) report that the A_T of seawater with a salinity of 35 ppt will decrease by 6% when pH is decreased from 8.1 to 7.8 *via* the addition of HCl in a closed system. This would reduce pCO_2 estimates in our system by 6%. Therefore, our estimates of pCO_2 from hydrochloric acid addition may be marginally higher than what was achieved in the respirometer prior to the start of the experiment and addition of respiratory CO_2 from the fish.

Because the measurements of $\dot{M}O_{2max}$ in the small damselfish (*P. amboinensis*) required the pooling of individuals (see above), $\dot{M}O_{2rest}$ and $\dot{M}O_{2max}$ had to be measured on different sets of fish. The procedure precluded the calculation of individual aerobic scope in damselfish. As for the measurement of $\dot{M}O_{2rest}$, two parallel setups allowed for the simultaneous measurement of $\dot{M}O_{2max}$ of fish exposed to near-future or higher CO_2, and those exposed to control conditions.

2.4. Data analyses and statistics

Oxygen consumption ($\dot{M}O_2$ in mgO_2 kg^{-1} h^{-1}) was calculated for each fish or pool of fish using the following formula:

$$\dot{M}O_2 = \Delta[O_2] \times \Delta t^{-1} \times VOL_{resp} \times M^{-1}$$

where $\Delta[O_2]$ is the decrease in water oxygen concentration (Δ mgO_2 l^{-1}), Δt the recording time (h), VOL_{resp} is the volume of the respirometer minus the volume of the fish (l), and M the mass of the fish (kg). Fish $\dot{M}O_2$ was corrected for background (microbial) respiration measured after each recording. For *P. moluccensis* and *P. amboinensis*, $\dot{M}O_{2rest}$ was measured in a closed respirometer from $\Delta[O_2]$ data above 90% of air saturation. O_{2crit} was calculated for each damselfish as the concentration of O_2 at the intersection of the regression lines from the data recorded >80% of air saturation (well above O_{2crit}) and the data recorded <20% air saturation (well below O_{2crit} as evident from the break in the curve at O_{2crit}). For *P. fuscus*, three slopes ($\Delta[O_2] \times \Delta t^{-1}$) were averaged to calculate $\dot{M}O_{2rest}$. $\dot{M}O_{2max}$ was calculated for all fish from within the first minute of recording. Unpaired *t*-tests were utilized to assess the effect of seawater pCO_2 on $\dot{M}O_{2rest}$ and $\dot{M}O_{2max}$ for each species, and the effect of seawater CO_2 concentration on O_{2crit} in *P. moluccensis* and *P. amboinensis*. In the experiment examining the effect of extreme pH on $\dot{M}O_{2max}$ in *P. amboinensis*, no significant differences were found between the three control groups ($P = 0.236$). Therefore, the control data were pooled. Then, a one-way ANOVA was used to test for the effect of pH on $\dot{M}O_{2max}$. In all instances, $P < 0.05$ was considered significant.

3. Results

$\dot{M}O_{2rest}$ of the two damselfish prey species was unaltered after exposure to near-future CO_2 for four days (Fig. 1A). Similarly, four days of exposure to near-future CO_2 had no significant effect on O_{2crit} for either of the two damselfish prey species (Fig. 1B).

$\dot{M}O_{2max}$ of *P. moluccensis* was unaffected by four days exposure as well as acute exposure to near-future CO_2 (Fig. 1C and D). In contrast, $\dot{M}O_{2max}$ of *P. amboinensis* was significantly higher after four days (+39%; $t_{[10]} = 4.665$; $P = 0.009$) as well as acute (+28%; $t_{[10]} = 2.597$; $P = 0.029$) exposure to near-future CO_2 when compared to controls (Fig. 1C and D). Furthermore, the two CO_2 treatments resulted in a similar increase in $\dot{M}O_{2max}$. $\dot{M}O_{2max}$ of *P. amboinensis* measured after four days exposure to near-future CO_2 was not significantly different than $\dot{M}O_{2max}$ measured after acute exposure to near-future CO_2 ($P = 0.417$). Similarly, $\dot{M}O_{2max}$ of the respective control groups did not differ significantly ($P = 0.858$).

Exposure to near-future CO_2 for four days had no effect on either $\dot{M}O_{2rest}$ or $\dot{M}O_{2max}$ of the predator, *P. fuscus* (Fig. 2). Consequently, there was no significant effect of near-future CO_2 on net aerobic scope or factorial aerobic scope in this species.

When acutely exposed to pH 7.9 (approximately 860 μatm pCO_2), *P. amboinensis* exhibited a $\dot{M}O_{2max}$ that was significantly higher than

that of the control fish, as well that of fish acutely exposed to pH 7.7 (approximately 1400 μatm pCO_2) or 7.5 (approximately 2400 μatm pCO_2; Fig. 3; $P < 0.0001$). The increase in $\dot{M}O_{2max}$ (+32%) was quantitatively similar to the increased $\dot{M}O_{2max}$ displayed by *P. amboinensis* after the four-day and acute exposures to near-future CO_2. However, at pH 7.7 and 7.5, $\dot{M}O_{2max}$ of *P. amboinensis* was no longer elevated and was not significantly different from the control (Fig. 3).

4. Discussion

CO_2-driven ocean acidification has been predicted to have detrimental effects on marine organisms by reducing the scope for aerobic performance (Pörtner and Farrell, 2008; Pörtner and Knust, 2007; Seibel and Walsh, 2001). However, contrary to expectations, none of the three tropical reef fish examined here, *P. moluccensis*, *P. amboinensis* or *P. fuscus*, exhibited an elevated $\dot{M}O_{2rest}$ or reduced $\dot{M}O_{2max}$ when exposed to the average pCO_2 projected to occur in the ocean surface by the year 2100. Rather, $\dot{M}O_{2rest}$ of the two juvenile damselfish prey species (*P. moluccensis* and *P. amboinensis*) and the adult predator (*P. fuscus*) was maintained after a four-day exposure to near-future seawater CO_2. Early life stages of fish and other marine organisms are believed to be more sensitive to pH changes because of their high metabolic demand (Brown and Sadler, 1989; Pörtner et al., 2005). The unchanged $\dot{M}O_{2rest}$ of juvenile *P. moluccensis* and *P. amboinensis* after four day exposure to near-future CO_2 thus suggests that the hypothesized metabolic costs of living in a high CO_2 environment, namely altered acid-base balance, ionoregulation and cardiorespiratory function (Pörtner et al., 2004), were insignificant for these species at rest. Nevertheless, the unchanged $\dot{M}O_{2rest}$ of the species examined after four days exposure to near-future CO_2 does not preclude that physiological changes occurred, including compensatory ones. For example, gill ionoregulatory machinery is rapidly altered (within 8 h to 2 d) in response to hypercapnia exposure in the eelpout (*Zoarces viviparous*), without measurable effect on resting metabolic rate over the same time period (Deigweiher et al., 2008). Similarly, long-term (4–12 months) exposure to hypercapnia leads to upregulated gill Na^+/K^+-ATPase activity and protein expression in the Atlantic cod (*Gadus morhua*), but resting and low activity metabolic rates remain unaltered (Melzner et al., 2009a). Furthermore, the gilthead bream

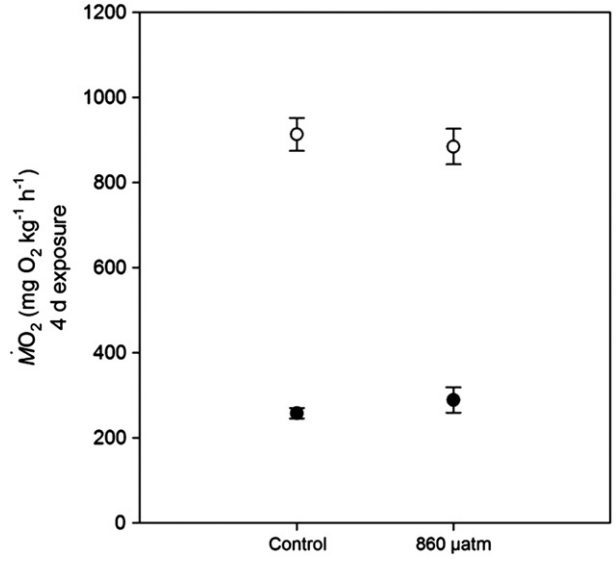

Fig. 2. Respiratory performance (mean ± SE) of *P. fuscus*. Resting oxygen consumption rate ($\dot{M}O_{2rest}$; mgO_2 kg^{-1} h^{-1}) (●) and maximum oxygen consumption rate ($\dot{M}O_{2max;}$ mgO_2 kg^{-1} h^{-1}) (○) after a 4 d exposure to control seawater (451 μatm; n = 8) or near-future CO_2 seawater (860 μatm; n = 7).

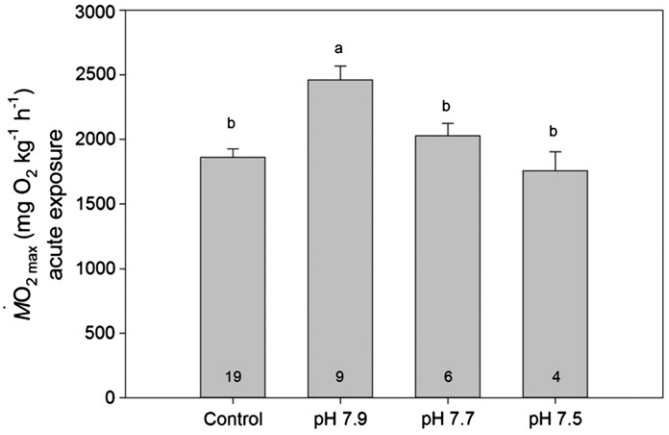

Fig. 3. Maximum oxygen consumption rate ($\dot{M}O_{2max;}$ mgO_2 kg^{-1} h^{-1}; mean ± SE) of *P. amboinensis* at different levels of seawater pH. N numbers are indicated at the bottom of each bar.

(*Sparus auratus*) exhibits a shift from aerobic to anaerobic metabolic pathways during hypercapnic exposure (Michaelidis et al., 2007). Whether similar compensatory physiological changes also occur in coral reef fish during exposure to near-future climate change CO_2 levels remains to be investigated.

Like observed for $\dot{M}O_{2rest}$, $\dot{M}O_{2max}$ of *P. moluccensis* and *P. fuscus* was also unaffected by exposure to near-future CO_2. In contrast, $\dot{M}O_{2max}$ of *P. amboinensis* was higher under near-future CO_2 conditions than under control conditions. The lack of a detrimental effect of increased CO_2 on $\dot{M}O_{2rest}$ and $\dot{M}O_{2max}$, as reported here for *P. moluccensis* and *P. fuscus*, is not unprecedented for fish (McKenzie et al., 2003; Ishimatsu et al., 2008; Melzner et al., 2009a). However, to the best of our knowledge, an augmented aerobic capacity of a juvenile marine teleost in response to elevated CO_2 has not been documented previously. The validity of the present results for *P. amboinensis* are supported by the findings that $\dot{M}O_{2max}$ was increased with elevated CO_2 in separate groups of fish that were exposed to near-future CO_2 using three different experimental techniques. The specific treatments consisted of a four-day exposure to near-future CO_2 maintained by CO_2-dosing to a set pH_{NBS}, an acute exposure to near-future CO_2 maintained by CO_2-dosing to a set pH_{NBS} and an acute exposure to near-future CO_2 obtained by the addition of strong acid into a closed system. Across the three methodologies, all 21 individuals exposed to near-future CO_2 and the resulting acidosis exhibited an increased $\dot{M}O_{2max}$. Moreover, the magnitude of the increase in $\dot{M}O_{2max}$ was consistent among the different experimental protocols (+28–39%). Finally, $\dot{M}O_{2max}$, $\dot{M}O_{2rest}$ and O_{2crit} of *P. amboinensis* in control conditions were comparable to data from a previous study using similar size fish (Nilsson et al., 2007b), indicating that the elevated $\dot{M}O_{2max}$ under near-future CO_2 did not arise from a comparison to control fish that were underperforming.

The physiological mechanism(s) underlying the increased $\dot{M}O_{2max}$ of *P. amboinensis* under near-future seawater CO_2 conditions remain to be elucidated. However, the consistent increase of $\dot{M}O_{2max}$ exhibited by *P. amboinensis* across the three experimental protocols, two of which constituted an acute exposure to near-future CO_2, suggests that the phenomenon did not arise from physiological acclimation to the elevated CO_2, but rather from the consequences of an acute exposure to increased CO_2 on the physiology of the fish. One explanation for the increased $\dot{M}O_{2max}$ of *P. amboinensis* when exposed to near-future CO_2 is that the maximum swimming speed of the fish was greater. Indeed, it is well established that $\dot{M}O_{2max}$ of fishes is positively correlated with swimming speed (Smith, 1965; Fry, 1971; Torres and Childress, 1983; Bushnell et al., 1984; Lee et al., 2003). A greater swimming speed could arise from a number of possibilities.

A greater maximal swimming speed could stem from a change in the motivation of the fish to swim fast. Recent studies have revealed that

tropical reef fish exhibit an array of behavioral and sensory disruptions when exposed near-future CO_2 for several days. These range from reversal or loss of olfactory and auditory preferences, loss of behavioral lateralization to increased boldness and activity levels (Dixson et al., 2010; Munday et al., 2010; Simpson et al., 2011; Domenici et al., 2012; Nilsson et al., 2012). The behavioral changes are believed to arise from alterations of the normal flow of Cl^- and HCO_3^- through GABA-A receptors caused by disruptions of transmembrane Cl^- and HCO_3^- ion gradients (Nilsson et al., 2012). Nevertheless, we find it unlikely that the increased $\dot{M}O_{2max}$ of *P. amboinensis* arises from a motivational drive to swim faster due to the effects of CO_2 exposure on neurotransmitter function because the previously documented behavioral alterations only occurred after several days' exposure to increased CO_2 (Dixson et al., 2010; Munday et al., 2010; Simpson et al., 2011; Domenici et al., 2012; Nilsson et al., 2012). Short-term fluctuations in CO_2 did not induce behavioral effects (Munday et al., 2010). In comparison, in the present study, *P. amboinensis* displayed an elevated $\dot{M}O_{2max}$ immediately (within minutes) of exposure to near-future CO_2. Moreover, a recent study investigating acid-base balance of the gulf toadfish (*Opsanus beta*) during exposure to near-future seawater CO_2, reported that full compensation of the respiratory acidosis and elevation of plasma HCO_3^- did not occur until after two hours of exposure (Esbaugh et al., 2012). Again, the time course of physiological change is at odds with alacritous increase of $\dot{M}O_{2max}$ displayed by *P. amboinensis* in the present study.

Alternatively, a greater swimming speed could arise from an increased oxygen delivery to the swimming muscles and oxygen uptake at the gills. Recent *in vitro* and *in vivo* studies have revealed that moderate acidosis can serve to increase the delivery of oxygen to the red muscle of teleost fish (Rummer and Brauner, 2011; Rummer et al., 2013). Briefly, in fish exposed to a stressor or mild acidosis, catecholamine release stimulates the activation of Na^+/H^+ exchange across the erythrocyte membrane, thereby increasing red blood cell intracellular pH relative to the plasma and thus facilitating hemoglobin-O_2 binding at the gills (Boutilier et al., 1986; Nikinmaa, 1986). However, if plasma accessible carbonic anhydrase, which catalyzes the reversible conversion of HCO_3^- and H^+ to CO_2, is present in muscle capillaires, it serves to short-circuit the Na^+/H^+ exchange, reduce red blood cell pH and hemoglobin-O_2 affinity and enhance O_2 unloading. For rainbow trout (*Oncorhynchus mykiss*) exposed to less than 1% CO_2, the mechanism increased red muscle pO_2 by 65% (Rummer et al., 2013). In the present study, the possibility exists that the combined exposure of *P. amboinensis* to near-future CO_2 and maximal exercise led to catecholamine release. If carbonic anhydrase is present in the red muscle of *P. amboinensis*, an increased oxygen delivery to the swimming muscles would have also likely ensued. Moreover, external CO_2 can rapidly induce cardiorespiratory responses *via* gill chemoreceptors (Reid et al., 2005). In particular, environmental CO_2 elicits increased ventilation in most water-breathing fishes. An increased oxygen delivery to the muscles combined with an increased ventilation rate could translate to the observed greater oxygen uptake.

In this regard, the species-specific responses to near-future CO_2 in terms of $\dot{M}O_{2max}$ may indicate different response times for catecholamine release, regulatory capacities or tolerance to changes in blood H^+ and/or differences among the species with regards to the presence of plasma accessible carbonic anhydrase in the red muscle. The lack of increase of $\dot{M}O_{2max}$ of *P. amboinensis* when the fish were exposed to pH 7.7 (corresponding to approximately 1400 μatm CO_2) or pH 7.5 (corresponding to approximately 2400 μatm CO_2) could arise from negative physiological effects of acidosis at the more extreme lower pH levels (Brauner and Baker, 2009) nullifying the enhanced physiological capacity to swim faster. This possibility could explain why many previous studies that exposed fish to much higher CO_2 levels (3000–60000 μatm) than those employed in the present study did not report any positive effects on $\dot{M}O_{2max}$ or aerobic scope (McKenzie et al., 2003; Melzner et al., 2009a). The juvenile damselfish and adult *P. fuscus* studied were much too small to enable blood sampling to test the above hypotheses.

An alternate explanation for the increased $\dot{M}O_{2max}$ of *P. amboinensis* under near-future CO_2 conditions is that maximal swimming speed was unchanged at $\dot{M}O_{2max}$, but an additional demand for oxygen to maintain homeostasis arose that was not apparent at $\dot{M}O_{2rest}$ or during maximal swimming under control conditions. For example, the 'osmo-respiratory compromise' almost doubles with exercise (Randall et al., 1972; Nilsson, 1986). In this scenario, *P. amboinensis* would have incurred a greater cost to swim at its maximum speed under near-future CO_2 conditions. Clearly, future studies incorporating the measurement of swimming speed at $\dot{M}O_{2max}$ are needed to differentiate the possible explanations for the increased $\dot{M}O_{2max}$ of *P. amboinensis* under near-future CO_2. The use of a swimming flume was not feasible in the present study because such a system would have required a much larger volume of water than appropriate to accurately measure oxygen consumption of the extremely small juvenile fish (Steffensen, 1989). Rather, a cylindrical swimming chamber with a small volume, but with the capacity to swim the fish at their maximal swimming speed (Nilsson and Östlund-Nilsson, 2004; Fisher et al., 2005; Nilsson et al., 2007a; Munday et al., 2009a; Nilsson et al., 2009; Gardiner et al., 2010; Nilsson et al., 2010) was utilized to obtain reliable measurements of oxygen consumption.

Regardless of the possible mechanistic determinant(s) of the elevated $\dot{M}O_{2max}$ under near-future CO_2, the differing response to near-future CO_2 between *P. amboinensis* and its congener *P. moluccensis* could foreseeably have consequences for ecological interactions and the relative abundance of species within coral reef fish communities. With a higher aerobic metabolic capacity (and potentially maximum swimming speed), *P. amboinensis* would have the potential for increased individual performance in any energetically demanding behavior, such as swimming against a current, repaying O_2 debt after repeated anaerobic burst-swimming escapes from a threat, foraging or digesting (*i.e.*, specific dynamic action). Concurrently, *P. amboinensis* should still be able to enter and remain in the hypoxic waters found deep inside coral colonies at night in order to escape predation (Nilsson et al., 2007a). The unchanged O_{2crit} of *P. amboinensis* after exposure to near-future CO_2 suggests that no trade-off exists for this species between its higher aerobic capacity and its hypoxia tolerance. In addition, in face of warming ocean surface temperatures, the enhanced $\dot{M}O_{2max}$ of juvenile *P. amboinensis* under near-future CO_2 may enable it to maintain its thermal tolerance window, and perhaps geographical distribution, as thermal tolerance is thought to be guided by aerobic scope in many species (Pörtner and Knust, 2007). On the contrary, if *P. amboinensis* incurs a greater metabolic cost while swimming at its maximum speed under near-future CO_2 conditions, energy expenditure for any other energetically demanding process would be reduced. Consequently, individual performance would be decreased in any energetically demanding behavior, leading to potentially negative consequences for the species.

Interestingly, the results of a recent experiment that examined mortality rate of a number of juvenile damselfish species when facing *P. fuscus* in a mesocosm after a four-day exposure to CO_2-acidified water found that *P. amboinensis* showed a similar mortality rate to *P. moluccensis*, as well as to other damselfish species (Ferrari et al., 2011b). The findings suggest no benefit or disadvantage of the increased $\dot{M}O_{2max}$ displayed by *P. amboinensis* under near-future CO_2 conditions. It may be that larger scale and longer term studies that encompass a variety of other variables such as temperature, current, life stages and the presence of other predators are required to reveal the implications of species-specific $\dot{M}O_{2max}$ responses to near-future CO_2 on fitness and mortality rate. Another recent study reported that in CO_2 acidified water, *P. fuscus* had a slower response to prey detection than in control water, but higher activity levels (Cripps et al., 2011). The higher activity level had been suggested to compensate for slower prey detection by increasing the chance of prey encounter (Cripps et al., 2011). The present findings indicate that a higher activity level of *P. fuscus* in CO_2-acidified water is not linked to a higher aerobic performance (aerobic scope). The higher activity levels of *P. fuscus* might be one of the consequences of increased neural excitation in CO_2-acidified water (Nilsson et al., 2012).

5. Concluding remarks

In summary, the present study reports an increased aerobic capacity of a juvenile marine teleost, *P. amboinensis*, in response to near-future pCO_2, but no effect on its congener *P. moluccensis* or on their predator *P. fuscus*. While the mechanistic basis for the species-specific responses and potential for differences in the swimming performance of the fish under near-future pCO_2 remains to be investigated, the results emphasize that being of the same genus, sharing similar ecology and life history, or living in the same environment, does not necessarily imply similar physiological responses to near-future CO_2. The results highlight that understanding interspecific variability is an important component of predicting the consequences of ocean acidification on marine communities and ecosystems. Additional studies assessing the effects of a number of other environmental factors in conjunction with near-future CO_2 exposure are required to more fully understand the interesting finding of the increased $\dot{M}O_{2max}$ of *P. amboinensis* in response to near-future seawater CO_2. Most importantly, future experiments should assess how maximum swimming ability is affected and if species-specific responses to elevated CO_2 persist with elevated temperature. Elevated pCO_2 in the future will not occur independently of temperature. Likewise, investigations into the effects of near-future CO_2 and temperature on different life stages of tropical coral reef species, as well as the capacity of both prey and predators to adapt to ocean acidification over the long-term, are needed to understand key ecological interactions and ultimately how the structure of ecological communities will be affected by climate change variables.

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