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Interactive effects of ocean acidification and rising sea temperatures alter predation rate and predator selectivity in reef fish communities

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Abstract

Ocean warming and acidification are serious threats to marine life. While each stressor alone has been studied in detail, their combined effects on the outcome of ecological interactions are poorly understood. We measured predation rates and predator selectivity of two closely related species of damselfish exposed to a predatory dottyback. We found temperature and CO_2 interacted synergistically on overall predation rate, but antagonistically on predator selectivity. Notably, elevated CO_2 or temperature alone reversed predator selectivity, but the interaction between the two stressors cancelled selectivity. Routine metabolic rates of the two prey showed strong species differences in tolerance to CO_2 and not temperature, but these differences did not correlate with recorded mortality. This highlights the difficulty of linking species-level physiological tolerance to resulting ecological outcomes. This study is the first to document both synergistic and antagonistic effects of elevated CO_2 and temperature on a crucial ecological process like predator–prey dynamics.

Keywords: climate change, elevated temperature, foraging rate, global change, predator selectivity, predator–prey interaction, routine metabolic rate

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Introduction

Ocean warming and acidification have the potential to dramatically change the structure and function of marine ecosystems (Harley *et al*., 2006; Hoegh-Guldberg & Bruno, 2010; Doney *et al*., 2012). Many studies have tested the effects of either elevated temperature or higher CO_2 concentrations on individual performance, measuring key physiological or life-history traits such as metabolism, development and growth, calcification, behaviour and reproduction (Doney *et al*., 2009; Kroeker *et al*., 2010; Briffa *et al*., 2012). Some of these studies show a surprisingly large variation in the effects of those stressors among closely related species (Ferrari *et al*., 2011a; Johansen & Jones, 2011). Fewer studies, however, have examined the consequences of such environmental change on the resulting ecological interactions among species (Diaz-Pulido *et al*., 2011; Ferrari *et al*., 2011b; Connell *et al*., 2013; Mccormick *et al*., 2013). While the effects of projected future temperatures and CO_2 concentrations have been studied individually for many years, both stressors will act simultaneously in the future. Consequently, it is necessary to consider how they interact to reliably predict their impacts on marine ecosystems (Wernberg *et al*., 2012; Kroeker *et al*., 2013). However, rare are the studies that focused on ecological endpoints. One study has looked at the combined effects of warming and acidification on the foraging efficacy of a predator: Landes & Zimmer (2012) investigated the combined effects of acidification and warming on the handling time of a green crab predator (*Carcinus maenas*) feeding on periwinkles (*Littorina littorea*) but failed to find any interactions between the two stressors. More studies on ecological processes, such as competition and predation, are needed to understand how species-level sensitivity to warming and acidification will manifest themselves in the ecosystem. Not all sensitivities at the species level may be ecologically meaningful, and

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inversely, small species-level alterations in some conditions may be exacerbated by the addition of other stressors. More integration between species-level and ecological-level effects are needed, and this integration needs to focus on multiple stressors (Russell *et al.*, 2012).

The first goal of this study was to investigate the interactive effects of increased CO_2 and temperature on the outcome of predator–prey encounters, as predation is a key process shaping communities. We used a simplified community of two common damselfish species, *Pomacentrus amboinensis* and *P. nagasakiensis*, and one mesopredator, the dottyback *Pseudochromis fuscus*. Fishes were exposed to ambient (440 μatm) or elevated (995 μatm) CO_2 conditions crossed with ambient or elevated temperature (+3 °C), in a full factorial design, to examine both the individual and combined effects of the stressors on trophic interactions. Elevated CO_2 and temperature were chosen to match the end-of-century projections for the tropical oceans based on the RCP8.5 emission scenario (Stocker *et al.*, 2013).

Ectotherms, whose performances are tightly linked to environmental temperature, will be particularly sensitive to ocean warming (Gilbert *et al.*, 2014). Tropical species are known to live near their thermal optimum, and as temperature rises, their capacity for oxygen transport or aerobic capacity is reduced, affecting behaviour, growth and swimming abilities (Portner & Farrell, 2008; Johansen & Jones, 2011). Increased CO_2 levels have also been shown to provide physiological constraints on these same traits (Kroeker *et al.*, 2010; Briffa *et al.*, 2012). These constraints at the species level are thought to be somewhat translatable into changes at the ecological level (Portner & Farrell, 2008). Hence, our second goal was to identify the possible prey-related physiological mechanisms responsible for the changes we observed. We did this by investigating any alterations in routine metabolic rates of prey under the four combinations of temperature and CO_2. Metabolic costs could either increase or decrease in the presence of the stressors, and the energy surplus or deficiency would then indicate the individual's capacity to exhibit costly but ecologically relevant traits such as activity level, habitat choice, aggression or predator avoidance (Portner & Farrell, 2008). Increased temperature should increase foraging demands as fish have to bridge the energetic gap caused by increased routine metabolic rate (Gilbert *et al.*, 2014). In turn, this may lead to increased risk-taking behaviour to have access to more or higher value food items. Recent studies showed that elevated CO_2 conditions resulted in altered risk assessment and antipredator behaviour via neurotransmitter interference (Nilsson *et al.*, 2012; Chivers *et al.*, 2014) but also affect metabolic scopes, although the direction of this change is variable among species (Munday *et al.*, 2009; Rummer *et al.*, 2013). Hence, predictions on the interactive effects of the two stressors are difficult to make. In addition, the differential effects of the stressors on the two species may matter more than the absolute level of handicap they provide.

We focused on coral reef communities for three reasons. First, coral reef ecosystems may be among the most threatened by changes in temperature, via habitat loss (Hoegh-Guldberg & Bruno, 2010). Second, some reef fishes are living close to their thermal optimum and may be particularly vulnerable to relatively small increases in temperature (Nilsson *et al.*, 2009). Damselfish species have become a model organism for the study of temperature and CO_2 effects on fish and much information is already known on their response to both CO_2 and temperature for a number of endpoints, including physiology, growth, behaviour, cognitive function and even *in situ* survival. Finally, our juvenile prey are tested just at their transition from the open ocean to coral reef habitat, a time when the population is subject to a severe predation-induced bottleneck; this transition is likely to be the period when most of the CO_2 tolerance phenotypic selection will occur (Munday *et al.*, 2010). Our two prey species are known to exhibit different sensitivities to elevated CO_2 conditions (Ferrari *et al.*, 2011a), but share the same ecological niche, compete for the same food items and share the same mesopredators, including *Pseudochromis fuscus*. The predator, *P. fuscus* has altered food preference (Cripps *et al.*, 2011) and decreased strike success (Allan *et al.*, 2013) in high CO_2 conditions. Elevated CO_2 levels can alter trophic interactions in a coral fish system (Ferrari *et al.*, 2011b), but it is unknown how the addition of elevated temperature might influence these dynamics.

Materials and methods

Fish maintenance and CO_2 and temperature treatments

Wild-caught presettlement juvenile *P. amboinensis* and *P. nagasakiensis* (16–21 days old) were maintained in the laboratory (<2 weeks) until needed and were then transferred into 35-l aquaria and assigned to one treatment combination of the following 2 × 2 design: fish were exposed to control or elevated CO_2 (440 vs. 995 μatm) crossed with control or elevated temperature (28 vs. 31 °C). Control CO_2 and temperature matched ambient summer conditions at Lizard Island, where the study was conducted (see Appendix S1 for details on location and fish capture). The fish were fed freshly hatched *Artemia* nauplii three times a day. Wild-caught adult *P. fuscus* were kept individually in mesh baskets placed in flow-through tanks and fed daily with squid pieces and fish pellets. They underwent the same CO_2 and temperature treatment protocols as the damselfishes, but were kept separated from the prey.

The treatment consisted of two phases: a 6-day temperature acclimation period and a 4-day CO_2 treatment period. The first phase consisted of slowly acclimating the fish to elevated temperature, by increasing the temperature at a maximum rate of 0.5 °C per day, while the controls were maintained in identical conditions at ambient temperature. The fish were then transferred into their respective treatment containers, which were either maintained at ambient or elevated temperature and ambient or elevated CO_2 levels for 4 days. Previous experiments demonstrated that the behavioural effects of elevated CO_2 are manifest within 4 days of exposure to relevant CO_2 treatments, but longer durations of exposure do not further alter behavioural responses (Munday *et al.*, 2014). Descriptions of the CO_2 treatments can be found in the Appendix S1. Water chemistry for different treatments is summarized in Table 1.

As in previous CO_2 studies limited to short-terms exposures, our exposure to CO_2 was rapid. However, damselfish larvae exposed to elevated CO_2 over a few days showed identical behavioural impairment as larvae raised under the same CO_2 levels from hatching (Munday *et al.*, 2010) or those occurring at natural CO_2 seeps (Munday *et al.*, 2014), indicating that alterations in behaviour were not due to a sudden CO_2 exposure. In addition, our larval fish naturally experience a change in CO_2 environments as they transition from the open ocean to coral reef habitats and experience natural fluctuating CO_2 concentrations on a daily basis (Shaw *et al.*, 2013).

Mesocosm experiment

Mesocosm set-up. All mesocosms were placed outdoors under a shade cloth, so the fish would experience natural light cycles. Each mesocosm consisted of an insulated 368-l circular food-grade pool (111 cm diameter, 45 cm high) containing a 1-cm deep sand substrate, air stone and a high-precision water heater. The heater could potentially provide shelter from predator attacks, so every pool was equipped with one, although only the heaters in pools at elevated temperature were functioning. Average treatment temperatures were: ambient temperature: 28.3 °C (range: 25.7–30.1 ± 1.0 °C – SD); elevated temperature: 31.1 °C (range: 29.4–32.3 ± 1.0 °C), based on 4 daily measurements of each replicate mesocosm (see Fig. S1). Two pieces of clean dead bushy hard coral (*Pocillopora damicornis*) were placed beside each other in the middle of each pool, forming a coral patch of ~90 cm in circumference and ~20 cm in height. Live corals provide a better quality habitat, but dead coral were used to avoid coral stress and bleaching in high temperature pools. To allow a better control of temperature, there was no inflow of water in the pools during the trials.

Experimental set-up. Six prey (3 per species) of matching treatment were randomly chosen and placed in a mesocosm of matching temperature and left to acclimate to their new environment. One hour later, a predator of matching CO_2 and temperature treatment was introduced, which marked the start of the trial. After 22 h, all the fish were removed from the pool and we recorded the number and species of damselfishes that survived the predation trial. Each day, two replicates from each of the 4 treatments were performed and we switched the treatment associated with each pool the next day to avoid position bias. Sea water was maintained at control CO_2 levels due to the difficulty of dosing mesocosms with CO_2 and because previous experiments have shown the effects of CO_2 levels similar to those used here last for at least 3 days. Indeed, we know that fish exposed to high CO_2 exhibit the same behavioural responses when tested in either control or elevated CO_2 conditions (Munday *et al.*, 2010). Each day, the coral patches from all the pools were mixed and rebuilt randomly to avoid patch structure biases in predation success/failure. The fish were fed twice daily (1100 and 1700 h) with 60 ml of a solution of freshly hatched *Artemia sp* (~250 per ml). We performed 17–18 replicates in each of the four treatment groups. Each animal was only used once in the experiment. The mean (±SD) standard length of *P. amboinensis, P. nagasakiensis* and *P. fuscus* were 1.26 (±0.08), 1.40 (±0.07) and 6.75 (±0.73) cm, respectively.

Respirometry

Intermittent-flow respirometry was used to determine routine O_2 consumption rates ($O_{2\mathrm{Routine}}$), as it has been found a reliable estimate of resting and routine metabolic rates in fish (Roche *et al.*, 2013). The O_2 consumption rates were measured for a total of 32 individual fish for each of the two damselfish species, which included 8 replicates per species in each of the four treatments. Prior to the start of each trial, all fish had been fasted for 24 h (Mcleod *et al.*, 2013; Rummer *et al.*, 2013). The methodology for the respirometry technique can be found in the Appendix S1. Unfortunately, our system was not adequate for larger species like our predators.

Table 1 Mean (±SD) sea water parameters in the experimental system. Temperature, salinity pH and total alkalinity were measured directly. pCO_2 was estimated from these parameters using CO2SYS

Treatment	Temperature (°C)	Salinity (ppt)	pH_{NBS}	Total alkalinity (μmol kg^{-1} SW)	pCO_2 (μatm)
Control CO_2 control temperature	27.8 (±0.1)	35.2	8.15 (±0.01)	2272.9 (±13.8)	436.2 (±13.6)
Control CO_2 elevated temperature	31.1 (±0.1)	35.2	8.15 (±0.01)	2272.9 (±13.8)	441.9 (±13.9)
Elevated CO_2 control temperature	27.8 (±0.1)	35.2	7.85 (±0.004)	2265.2 (±5.2)	984.7 (±10.6)
Elevated CO_2 elevated temperature	31.0 (±0.1)	35.2	7.85 (±0.004)	2265.2 (±5.2)	1006.7 (±10.6)

Statistical analyses

Fish size. Our two prey species differ naturally in their size. However, differences in size variation within species or variation in size between species among treatment groups could influence the results. Thus, we used 2-way ANOVAs to test whether the size of each species, or their difference, differed among CO_2 and temperature groups. We also tested if the size of the predators did not differ among our treatment groups.

Predation rate. For each trial, we computed a predation rate (#fish eaten/6). These were used as raw data in a 2-way ANOVA, testing the effect of CO_2 (control vs. elevated) and temperature (control vs. elevated) on predation rate.

Prey selectivity. We computed a prey selectivity index for *P. fuscus* following Chesson (1983):

$$\hat{\alpha}_i = \frac{r_i/n_i}{\sum_{j=1}^{m}(r_j/n_j)},\ i = 1,\ldots,m$$

Where n_i represents the number of prey type i at the beginning of the experiment, r_i the number of prey type i consumed by the predator and j the number of different prey types. This selectivity can be interpreted as the preference of the predator for a prey type relative to the average preference for alternative prey types. The selectivity value ranges from 0 (total avoidance of prey type) to 1 (only prey type selected). If both prey species are selected equally by the predator, the selectivity for each prey species is 0.5. Trials where predators ate none of the prey (n = 7 across all treatments without treatment bias) were removed, given that no selectivity could be computed, leaving us with n = 15–16/treatment for this response variable. Using the two indexes as response variables, we performed a 3-way RM MANOVA to test the effect of CO_2, temperature and species on the selectivity value for each trial. The repeated-measures approach accounted for the dependency of the selectivity among species (the score for the two species are related), while still allowing us to compare selectivity among species (Ferrari *et al*., 2011b).

Respirometry. We used a 3-way ANOVA, followed by subsequent 2-way ANOVAs to determine the effect of CO_2, temperature and species on the metabolic rate of the fish. The data for all tests described above met parametric assumptions.

Results

Fish size

The size of the fish did not differ among treatment groups, for either *P. nagasakiensis*: (CO_2: $F_{1,114}$ = 0.6, P = 0.4, temperature: $F_{1,114}$ = 0.1, P = 0.9, CO_2*temp: $F_{1,114}$ = 0.2, P = 0.6) or *P. amboinensis* (CO_2: $F_{1,141}$ = 0.1, P = 0.9, temperature: $F_{1,141}$ = 0.6, P = 0.4, CO_2*temp: $F_{1,114}$ = 0.1, P = 0.9). Further, the size difference between the two species did not vary among treatment groups (CO_2: $F_{1,102}$ = 2.2, P = 0.12, temperature: $F_{1,102}$ = 0.2, P = 0.6, CO_2*temp: $F_{1,114}$ = 2.6, P = 0.11). In addition, the size of the predator did not vary among treatment groups (CO_2: $F_{1,65}$ = 2.7, P = 0.1, temperature: $F_{1,65}$ = 0.1, P = 0.8, CO_2*temp: $F_{1,65}$ = 1.0, P = 0.3).

Predation rate

The 2-way ANOVA revealed a significant synergistic interaction between CO_2 and temperature on overall predation rate ($F_{1,65}$ = 4.9, P = 0.03, Fig. 1). Elevated temperature alone ($F_{1,33}$ = 0.1, P = 0.69) or CO_2 alone ($F_{1,33}$ = 0.1, P = 0.99) did not affect the overall predation rate compared to controls, but the rate increased from 30% to 70% when both stressors were present ($F_{1,33}$ = 10.5, P = 0.003).

Selectivity

The 3-way RM ANOVA revealed a significant antagonistic interaction of CO_2 and temperature on predator selectivity (species*CO_2*temperature: Pillai's trace: $F_{1,58}$ = 12.9, P = 0.001, Fig. 2). An increase in temperature alone reversed the selectivity patterns of the two species (Pillai's trace: species*temp: $F_{1,28}$ = 8.3, P = 0.007) and so did an increase in CO_2 alone (Pillai's trace: species*temp: $F_{1,29}$ = 11.5, P = 0.002). However, when both stressors were present, the selectivity pattern disappeared and both species were selected equally ($F_{1,15}$ = 0.1, P = 0.99).

Respirometry

The 3-way ANOVA revealed a significant interaction among CO_2, temperature and species ($F_{1,56}$= 5.2,

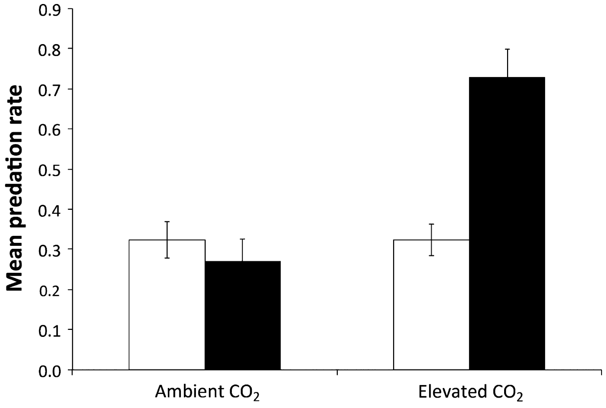

Fig. 1 Mean (±SE) predation rate (proportion of prey consumed in 22 h) when prey and predator were maintained under ambient (440 μatm) or elevated (995 μatm) CO_2, crossed with either ambient temperature (28 °C, light bars) or elevated temperature (31 °C, solid bars).

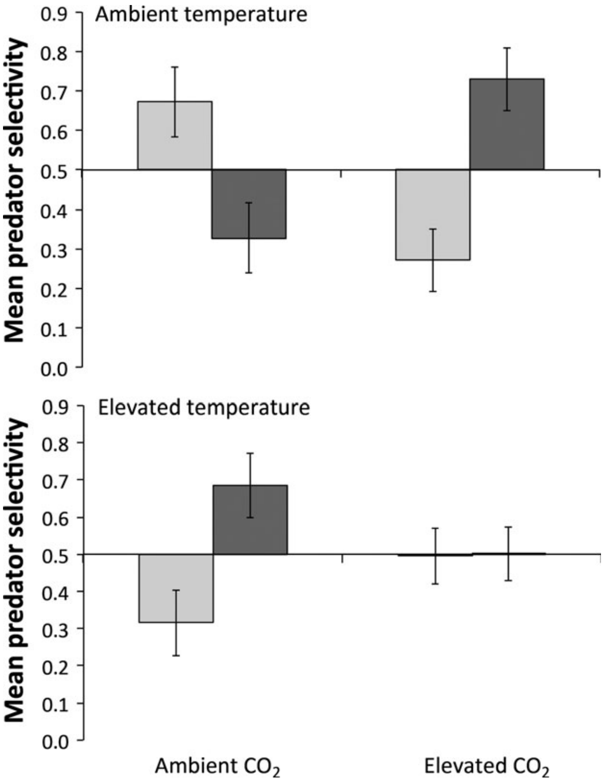

Fig. 2 Mean (±SE) predator selectivity for *Pomacentrus amboinensis* (light bars) and *P. nagasakiensis* (dark bars) when prey and predator were maintained under ambient or elevated CO_2 (440 vs. 995 μatm), and under ambient temperature (28 °C, top panel) or elevated temperature (31 °C, bottom panel). A selectivity of 0.5 indicates no preference for a particular prey species by the predator.

$P = 0.026$). We performed 2-way ANOVA on each species, and found that while the metabolic rate of *P. amboinensis* increased as a response to increased temperature only (temp: $F_{1,28} = 4.7$, $P = 0.04$; CO_2: $F_{1,28} = 0.001$, $P = 0.99$, temp*CO_2: $F_{1,28} = 0.27$, $P = 0.61$), the metabolic rate of *P. nagasakiensis* was affected by an interaction between temperature and CO_2 ($F_{1,28} = 9.0$, $P = 0.006$). The metabolic rate did not increase with increased CO_2 or temperature alone ($P = 0.5$ and 0.7, respectively), but increased when both CO_2 and temperature were elevated ($P = 0.003$) (Fig. 3).

Discussion

For the first time, we have been able to show a clear interactive effect of two climate change-related stressors on predator–prey interactions in a coral reef ecosystem, with a synergistic interaction on predation rate and an antagonistic interaction on selectivity. As an individual stressor, we found that elevated CO_2 concentrations completely reversed predator selectivity for *P. amboinensis* and *P. nagasakiensis* compared to ambient conditions, but did not cause a change in overall predation rate, results that are consistent with our previous work examining the effects of elevated CO_2 on trophic dynamics (Ferrari *et al.*, 2011b). Interestingly, an increase in water temperature had similar effects. However, when both stressors were present, a new pattern emerged. Together, these stressors caused total predation rate to increase dramatically from 30% to 70% and for any selectivity to disappear, with roughly equal numbers of each prey consumed by the predator. Our results also demonstrate that conclusions reached about the potential impacts of ocean acidification or warming may be inaccurate if these co-occurring stressors are not investigated together. Our results differ from those of Landes & Zimmer (2012) who failed to find an interaction between CO_2 and temperature on the foraging behaviour of crabs on periwinkles. However, their experiment did not allow to investigate the dynamics between predators and prey, given that the predator was given only one semi-sessile prey from which to choose. While the success of a predators on a prey may not be impacted, community-level effects may still appear in such systems if predators switch to easier alternate prey that are more affected by each or both of the stressors. Increasing the realism of such experiments will uncover patterns, such as switch in predator selectivity, that cannot be picked up by simple predator–prey systems.

To understand the ecological impact of a changing climate, studies designed to address the combined effects of increasing CO_2 and temperature are particularly valuable. So far, studies with reef fishes that have tested both stressors together have been limited to individual-level outcomes. Some demonstrated additive effects of the two stressors on some endpoints like metabolic rate (Munday *et al.*, 2009), while others showed interactive effects of the two stressors on behavioural lateralization (Domenici *et al.*, 2014) and foraging (Nowicki *et al.*, 2012). Our results provide the first ecological-level insights into the complex interactive effects of increased temperature and CO_2 on predator–prey dynamics in fishes. When both stressors were present, we saw that the overall rate of predation increased dramatically. We have considerable evidence from field observations that fish exposed to elevated CO_2 maintain a greater distance from shelter and are bolder (Munday *et al.*, 2010; Ferrari *et al.*, 2011a), taking greater risks to acquire food. If an increase in temperature were to shift the foraging/risk trade-off even further towards feeding, then we should expect much higher mortality with the combined stressors, consistent with our results. However, we cannot partition the role played by predator from that of prey. Ferrari *et al.* (2011a) documented

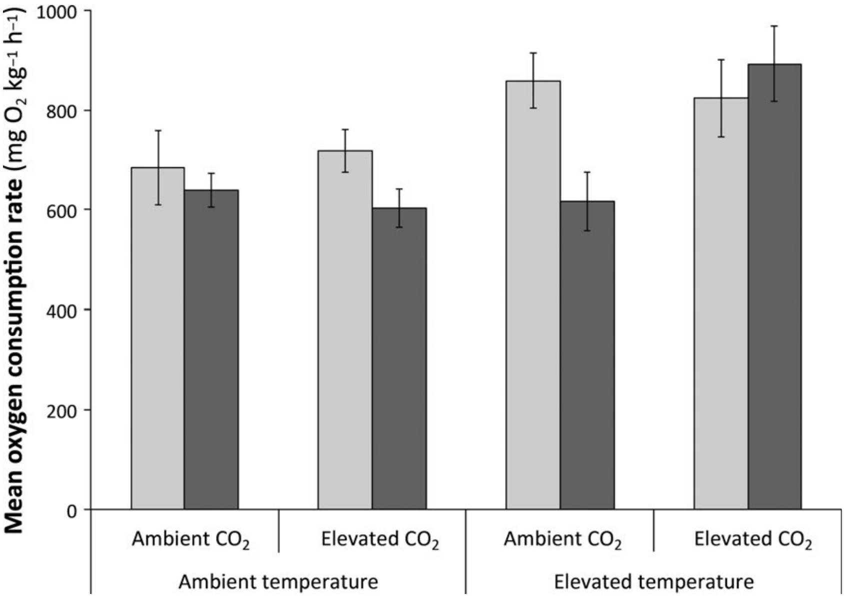

Fig. 3 Mean (±SE) routine metabolic rates (estimated from oxygen consumption rates) for *Pomacentrus amboinensis* (light bar) and *P. nagasakiensis* (dark bars) maintained under ambient or elevated temperature (28 vs. 31 °C) and ambient and/or elevated CO_2 (440 vs. 995 μatm).

that there is considerable variation in the tolerances of fish within the genus *Pomacentrus* to elevated CO_2, with *P. amboinensis* being more affected than *P. nagasakiensis*. However, our results suggest that such ranked sensitivities might not hold when additional stressors are added, and interspecific variation need to be re-evaluated in conditions of elevated temperature.

Our most striking finding is the effects on predator selectivity. Elevated CO_2 alone completely reversed predator selectivity, as did temperature alone. However, when elevated CO_2 and temperature were combined, the pattern of selectivity totally disappeared. This result highlights that predicting the outcome of predator–prey encounters under a changing climate will be challenging. To gain a full appreciation of how dynamics may change, it will be imperative that we consider both the relevant interacting species and the relevant co-occurring environmental stressors. There were clear effects of elevated CO_2 and temperature on resting metabolic rates of the two species; however, the species-level responses did not match the change in mortality of prey or the prey selectivity of the predator. The condition under, which the greatest discrepancy in basal metabolic rate was found, was for elevated temperature and ambient CO_2 levels, with *P. amboinensis* displaying a 15–20% increase in O_2 consumption compared to ambient conditions, while *P. nagasakiensis* still maintained the same metabolic rate as ambient conditions. Under these conditions, one could predict *P. amboinensis* to suffer higher predation rate, but this pattern did not emerge, indicating that other unmeasured factors are likely playing a more important role in dictating trophic outcomes. This demonstrates that it will be difficult to predict the outcome of ecological interactions, and thus the impacts of acidification and warming at the community and ecosystem level, simply based on species-level differences in physiological tolerances to these stressors.

A greater proportion of the predation was directed towards *P. nagasakiensis* when under high CO_2 than under ambient CO_2. The reason for this is unclear, however, a recent study by Mccormick *et al.* (2013) revealed that elevated CO_2 reverses the outcome of competition for space in juvenile damselfish, *P. amboinensis* and *P. moluccensis*. This outcome was mediated by changes in aggression levels, affecting the distribution and vulnerability of each species to predation risk. Our results could fit this hypothesis, given that our species also compete for habitat. An alternative hypothesis is that difference of these stressors on energy balance between the two species could alter their susceptibility to predation. Our results indicate that increased CO_2 conditions did not lead to a significant difference in routine metabolic rate, but we did not measure maximum metabolic rate. Two pomacentrids have been found to exhibit a higher maximum metabolic rate under elevated CO_2 (Couturier *et al.*, 2013; Rummer *et al.*, 2013) while others exhibited a lower one under similar situations (Munday *et al.*, 2009; Couturier *et al.*, 2013). Fish that are more 'active' or bold under elevated CO_2 could be easier targets for predators (Hamilton *et al.*, 2014). In contrast, fish exhibiting an enhanced maximum metabolic rate could escape a predator more efficiently, but data are not yet available to support this hypothesis.

Somewhat surprisingly, we did not find that an increase in temperature alone led to an increase in predation rate. Higher temperatures, up to a point, should be associated with higher metabolic rates – as found here for *P. amboinensis* – and hunger level for both predators and prey. From the prey's perspective, an increase in hunger level should promote greater risk taking, meaning that the fish should maintain a greater distance from the coral where food is more abundant and this should lead to higher predation rate (Mccormick & Weaver, 2012). It is possible that this mortality effect was not manifested in our study because of the relatively short duration of the test (22 h). An increase in temperature for a greater duration of time may be required to change the foraging/risk trade-off such that the fish move away from the coral and increase feeding (Lima & Bednekoff, 1999), and it may be that the prey's relatively high body condition may have buffered this trade-off in the short term. Perhaps most interesting was that an increase in temperature alone changed prey selectivity. Again *P. nagasakiensis* was more susceptible to predation under high temperature than under low temperature. This difference could be the result of differential foraging/risk trade-offs between the two species, changing competition for space or a difference in aerobic capacity.

The occurrence of multiple stressors may not simply intensify the patterns emerging from each stressor alone, but may, in some cases, change the patterns observed altogether. Carefully controlled studies integrating physiology, behaviour and trophic ecology hold promise to understand how a changing climate will influence trophic relationships. Moreover, these endpoints need to be studied and contrasted within the same studies, if we are to understand the potential integration between species-level sensitivities and the resulting ecological alterations.

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Author contributions

MF, DC, MIM, PM conceived the project; MF, DC, JLR and KC collected the data; MF and JLR analysed the data; PM and SAW provided water chemistry parameters and technical assistance with the CO_2 system; MGM and BA provided assistance with the implementation of the experiment. MF and DC wrote the initial draft of the manuscript; all authors contributed to the final version.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Methods.
Figure S1. Mean (±SE) temperature measured in the mesocosm pools during the experiment.