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Warming has a greater effect than elevated CO_2 on predator–prey interactions in coral reef fish

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Ocean acidification and warming, driven by anthropogenic CO_2 emissions, are considered to be among the greatest threats facing marine organisms. While each stressor in isolation has been studied extensively, there has been less focus on their combined effects, which could impact key ecological processes. We tested the independent and combined effects of short-term exposure to elevated CO_2 and temperature on the predator–prey interactions of a common pair of coral reef fishes (*Pomacentrus wardi* and its predator, *Pseudochromis fuscus*). We found that predator success increased following independent exposure to high temperature and elevated CO_2. Overall, high temperature had an overwhelming effect on the escape behaviour of the prey compared with the combined exposure to elevated CO_2 and high temperature or the independent effect of elevated CO_2. Exposure to high temperatures led to an increase in attack and predation rates. By contrast, we observed little influence of elevated CO_2 on the behaviour of the predator, suggesting that the attack behaviour of *P. fuscus* was robust to this environmental change. This is the first study to address how the kinematics and swimming performance at the basis of predator–prey interactions may change in response to concurrent exposure to elevated CO_2 and high temperatures and represents an important step to forecasting the responses of interacting species to climate change.

1. Introduction

Atmospheric CO_2 has risen from approximately 280 ppm pre-industrially to 400 ppm in 2016, the highest level in at least 800 000 years [1]. CO_2 levels in the ocean are rising at the same rate as in the atmosphere, leading to a decline in seawater pH [2]. If the current rate of anthropogenic CO_2 emissions is maintained, climate models project that the partial pressure of CO_2 (pCO_2) in the surface ocean will exceed 900 µatm by the end of this century and ocean pH will decline by 0.14–0.43 units compared with current-day values [1,3]. These changes in ocean chemistry are set against a background of warming, with average ocean temperatures projected to increase by up to 3°C [3]. This climate forcing can lead to positive feedback loops whereby increasing temperature can amplify the release of CO_2 from terrestrial and marine sinks, leading to further increases in temperature [1]. Consequently, marine organisms face complex environmental changes throughout this century. While there has been extensive research into the effects of ocean acidification and rising temperature on marine organisms, the majority have only tested the effects of these drivers in isolation, with fewer studies exploring their combined effects, although the importance of testing for combined effects of multiple drivers is increasingly recognized [4–7]. Understanding how multiple drivers affect key physiological or behavioural traits in marine organisms is critical, as extrapolations based on single driver responses could lead to incorrect predictions about future impacts [5,7].

In marine fish, some of the most marked effects of elevated CO_2 and warming reported to date are altered behaviours and physiology. Behavioural effects of elevated CO_2 include reduced anti-predator responses, altered olfactory and auditory preferences, loss of learning, changes in activity levels, altered behavioural lateralization and visual risk assessment [6,8,9]. These behavioural changes translate into consequences for survival in natural habitats [10,11]. Similarly, higher temperature strongly influences the physiological performance of fishes leading to reductions in aerobic scope (the difference between maximum and resting metabolic rate) in some species [12,13], reduced cardiac output [14], decreased muscle development [15] and changes to the contractile properties of the swimming muscles [16] and sensory performance [17]. Similar to exposure to elevated CO_2, higher temperatures also lead to changes in behaviours such as greater activity rates [18] and sensory responsiveness [19] and increased interactions between predators and their prey [20–22].

Emerging evidence suggests that the behaviour and physiology of fishes are sensitive to changes in simultaneous exposure to CO_2 and temperature. For example, there can be effects on predation rates and predator selectivity [23], directional changes in lateralization [24], foraging behaviour [25], activity rates [26], offspring quality [27] and aerobic scope [28], all of which can influence persistence of species into the future. However, the direction of reactions to multiple stressors is not always clear. For example, if multiple stressors affect similar pathways, an additive effect may be observed [29]. By contrast, antagonistic or synergistic effects may occur if the stressors affect different pathways, depending on the direction of the response to each stressor [30].

A critical stage in the life of reef fishes occurs at the end of the larval phase, when they settle to the benthic environment. Mortality rates during the first few days of settlement are especially high. It is at this stage that fishes undergo a severe predation-induced bottleneck [31]. Success at this life stage is dependent upon the size, growth rate and fast-start performance of new recruits [32–34]. Fast starts are short, high-energy swimming bursts that are driven by the rapid contraction of the white (anaerobic) muscle fibres [35,36]. The fast kinematics of escape responses are usually controlled by the large Mauthner neurons, which are triggered as a reaction to the fast approach of a predator, although other reticulospinal cells may also be involved [37]. Successful fast starts consist of finely tuned responsiveness and locomotor performance [38]. However, fast starts are sensitive to differences in ambient temperatures and CO_2 due to the interplay between temperature and muscle contraction rates [16], and elevated CO_2 exposure and sensory performance [17]. Fast-start performance can also differ between predators and their prey due to differences in their temperature tolerance and sensitivity to elevated CO_2 [20–22].

To date, only one study has investigated the combined effects of multiple climate change drivers on predator–prey dynamics in coral reef fishes [23]. Therefore, the aim of this study was to employ a multi-stressor approach to assess the independent and combined effects of both elevated CO_2 and high temperatures on the attack and escape performance of a common pair of coral reef fishes: the piscivorous dusky dottyback (Pseudochromis fuscus), and its prey, the juvenile of a common damselfish (Pomacentrus wardi). Previous work has shown that both the predator (Ps. fuscus) and prey

(Po. wardi) are sensitive to increases in ambient CO_2 and temperature [10,20,39]. Pomacentrus wardi also displayed behavioural impairment following simultaneous exposure to both stressors [24]. By exposing predators and prey to elevated CO_2 and high temperatures, both independently and simultaneously, we were able to determine the relative effects of each stressor on the attack and escape performance that forms the basis of predator–prey interactions. To date, this is the first study examining how the swimming kinematics of an attacking predator and the reactivity and swimming performance of its escaping prey may change following concurrent exposure to elevated CO_2 and temperature.

2. Material and methods

(a) Study species

Pomacentrus wardi (Pomacentridae) is a small planktivorous fish commonly found on Indo-Pacific coral reefs and was chosen as our prey organism. Newly metamorphosed Po. wardi larvae (range 11.2–15.5 mm, average 13.2 ± 1.2 mm standard length (SL) $\pm$ standard deviation (s.d.)) were collected using light traps moored 100 m from the fringing reef off Lizard Island in the northern Great Barrier Reef, Australia. On the morning of capture, larvae were transferred into 30 l aquaria at either control (approx. 405 µatm) or elevated CO_2 (approx. 930 µatm) and cross-factored with control (approx. 27°C) or high temperature (approx. 30°C). Control conditions were ambient for the study site at the time of the study (October 2012). Larvae were split into four different treatment groups in a 2 temperature $\times$ 2 CO_2 design: control temperature–control CO_2 ($n = 17$); high temperature–control CO_2 ($n = 19$); elevated CO_2–control temperature ($n = 18$); elevated CO_2–high temperature ($n = 18$). For fish in the high (+3°C) temperature treatments, the temperature was raised by 1°C every 8 h until the final temperature of approximately 30°C was reached [20]. Following this time period, the fish were placed into three tanks per treatment where they remained for 7 days. Fish were fed four times daily ad libitum with newly hatched Artemia sp. but were starved for the 12 h prior to commencement of experimental trials to standardize for satiation.

The dottyback, Ps. fuscus (Pseudochromidae), was used as the predator. Pseudochromis fuscus is widely distributed throughout the Indo-Pacific and has been observed to prey upon newly settled coral reef fishes [40], including Po. wardi. Adult Ps. fuscus (range 63–99 mm, mean 78.2 ± 9.4 mm) were collected on SCUBA using a solution of clove oil diluted with ethanol and seawater from around the shallow fringing reef off Lizard Island. Immediately after collection, fish were placed within three 30 l aquaria per treatment. To avoid aggressive interactions, fish were housed individually in mesh breeding baskets (16.5 cm $L \times 12.5$ cm $W \times 12.5$ cm H) containing small lengths of PVC pipe to be used as shelter. Predators were maintained in treatment tanks for 7 days (following the same treatment exposure as Po. wardi) and were fed two juvenile reef fish morning and night and then not fed for the last 24 h prior to the interaction trial to standardize for satiation. A 12 L : 12 D regime was used. We used 72 Ps. fuscus in total: $n = 17$ for control temperature–control CO_2, $n = 19$ for high temperature–control CO_2, $n = 18$ for elevated CO_2–control temperature and $n = 18$ for high temperature–elevated CO_2.

(b) CO_2 and temperature treatments

Tanks were heated with 300 W bar heaters and insulated to ensure stability of the experimental temperatures (27 and 30°C). CO_2 treatments were maintained by CO_2 dosing to a set

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pH. Seawater was pumped from the ocean into 60 l sumps where it was infused with ambient air (control) or CO_2 to achieve a pH_{NBS} of 7.87. The pH value was selected to achieve the approximate CO_2 conditions required, based on preliminary observations of total alkalinity, salinity and temperature of seawater at Lizard Island. A pH-controller (Aqua Medic, Germany) was attached to the CO_2 treatment sump to maintain pH at the desired level. A solenoid injected a slow stream of CO_2 into a powerhead at the bottom of the sump whenever the pH of the seawater rose above the set point. Equilibrated seawater from each sump was supplied at a rate of approximately 720 ml min^{-1} to each of the aquaria. Temperature and pH_{NBS} of each aquarium were measured daily with a temperature probe (C22, Comark, Norwich, UK) and a pH meter (HQ40d, Hach, CO, USA) calibrated with fresh pH_{NBS} buffers. Seawater CO_2 in the treatment tanks was confirmed with a portable CO_2-equilibrator and non-dispersive infrared (NDIR) sensor (GMP343, Vaisala, Helsinki, Finland) [41,42]. The CO_2-equilibrator consisted of a coil of CO_2-permeable silicone tubing that was submerged in water and connected in a closed loop of CO_2 impermeable tubing to the Vaisala GMP343 sensor. Air in the closed loop was circulated between the CO_2-equilibrator and the Vaisala GMP343 by a small pump. The pCO_2 of the air in the closed loop was equilibrated with the pCO_2 in the treatment water and measured by NDIR in the Vaisala GMP343 sensor. This cross-validation confirmed that pCO_2 calculated from water chemistry (pH_{NBS} and total alkalinity) was within 5.1–56.0 µatm of pCO_2 measured by NDIR ($N = 9$). Total alkalinity of seawater was estimated by Gran titration (888 Titrando, Metrohm, Switzerland) from water samples taken twice weekly from control and treatment tanks. Alkalinity standardizations achieved accuracy within 1% of certified reference material from Dr A. Dickson (Scripps Institution of Oceanography). Seawater pCO_2 was calculated from seawater parameters in the program CO2SYS [43] using the constants of Mehrbach et al. [44], refit by Dickson & Millero [45]. Seawater parameters are shown in table 1.

(c) Laboratory assays

Predator–prey interactions were evaluated using a standard protocol established by Allan et al. [32]. Predators and prey that had experienced the same treatment were paired together, i.e. control temperature–control CO_2-exposed predators and prey were paired together. Briefly, one predatory Ps. fuscus was placed into the experimental arena and one Po. wardi was released into an opaque PVC tube (11 cm diameter, 15 cm high) placed upright in the middle of the experimental arena (38 cm × 58 cm × 10 cm water height). Both fish were allowed to acclimate for 30 min. The PVC tube was then carefully raised and removed from the tank using a wire connected to the top of the tube; this allowed the predator and the prey to start the interaction. Trials were only used when the predator was at the opposite end of the tank to the prey at the start of the interaction to standardize for predator position. The ensuing interaction was filmed at high speed (420 fps) for 10 min or until the prey had been consumed. Maximum predator attack speed and maximum prey escape speed were measured based on the centre of mass (COM) of the fish when stretched straight based on Webb [46]. COM was assumed to be at 35% of the body length from the tip of the snout, as is the case for generalist fish [47]. Predator attacks were measured only when a predator showed a fast-directed burst towards the prey (at a speed greater than 3 body lengths s^{-1}). All variables with the exception of number of prey caught were measured using only the first attack that occurred within a trial. This was done to control for any stress either the predator or prey may have experienced due to prolonged attacks. Both predators and prey were used only once regardless of the trial outcome. Prey suffering was minimal

because prey were consumed immediately after a successful strike. Trials were conducted over 5 days in a temperature-controlled room at the same water temperature as the relevant temperature treatment. Because of the difficulties in producing enough CO_2-enriched seawater to be used in the experimental arena, control CO_2 seawater was used. Previous work has shown that the influence of CO_2 on behaviour lasts for at least 24 h after return to normal CO_2 conditions [10] and that there is no effect of using either control or elevated CO_2 water in the test arena when testing the escape performance of prey that have been exposed to control or elevated CO_2 for a similar duration to that used here [48]. Trials were randomized to account for any potential temporal or day effect.

The following variables were measured:

Predator

(1) Capture success: percentage of trials in which the predator ingested the prey within the 10 min filming period, out of the total number of trials for each treatment.

(2) Attack rate: number of attacks occurring within the 10 min filming period or until the prey was consumed.

(3) Predation rate: number of captures divided by the number of attacks within the 10 min filming period.

(4) Predator attack distance (m): the straight-line distance between the predator COM at the time the attack commenced and at the end of the attack (end is defined as when the predator came to a halt).

(5) Predator attack speed (m s^{-1}): was measured as the average speed occurring over the attack.

Prey

(6) Prey reaction distance (RD, measured in metres): the distance between the prey COM and the tip of the predator's snout at the onset of the escape response to a predator attack. A large RD indicates high reactivity to a predator attack.

(7) Apparent looming threshold (ALT): the apparent looming threshold for prey escape responses to a predatory strike. This is a measure of the reactivity of the prey to the perceived threat of predation. The higher the perceived threat, the lower the ALT (in radians per second) measured at the onset of the escape response and measured as the rate of change of the angle (α) subtended by the predator's frontal profile as seen by the prey. Previous work has shown that fish tend to react to an approaching stimulus (a predator) when a given threshold of $d\alpha/dt$ (i.e. ALT) is reached. The ALT is calculated as $(4US)/(4D^2 + S^2)$, based on Dill [49] and Domenici et al. [50], where U is the predator speed, calculated as the speed of the predator in the frame prior to the prey's response; S is calculated based on the morphological characteristics of the predator, i.e. S = (maximal depth + maximal width)/2, whereby both maximal depth and maximal width are at 0.25 lengths of the predator (personal observation); and D is the distance, measured at the onset of the escape response, between the prey COM and the point on the predator where its maximal width is located. Hence, D = RD + 0.25 lengths of the predator. For any given speed, a large RD will result in a low ALT; hence, a low ALT indicates high reactivity to a predator attack.

(8) Directionality: this was measured by dividing escape responses into 'away' and 'towards' responses based on the first detectable movement of the head relative to the approaching predator [50].

(9) Prey escape distance (m): the straight-line distance between the prey COM at the onset of the escape response and at the end of the escape response (i.e. when the prey came to a halt).

(10) Mean prey escape speed (m s^{-1}): this was measured as the distance covered within a fixed time (24 ms). This fixed

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Table 1. Mean ($\pm$ s.e.) seawater parameters in the experimental system. Temperature, pH, salinity and total alkalinity (TA) were measured directly. pCO_2 was estimated from these parameters using CO2SYS.

CO₂ treatment	temp treatment	temp (°C)	salinity	pH$_{NBS}$	total alkalinity (μmol kg^{-1} SW)	pCO_2 (μatm)
control	control	26.7 ($\pm$ 0.1)	35.2	8.18 ($\pm$ 0.01)	2274 ($\pm$ 6)	396 ($\pm$ 8)
control	high	29.6 ($\pm$ 0.1)	35.2	8.17 ($\pm$ 0.01)	2274 ($\pm$ 6)	415 ($\pm$ 9)
elevated	control	26.8 ($\pm$ 0.1)	35.2	7.87 ($\pm$ 0.01)	2257 ($\pm$ 6)	921 ($\pm$ 19)
elevated	high	29.8 ($\pm$ 0.1)	35.2	7.87 ($\pm$ 0.01)	2257 ($\pm$ 6)	935 ($\pm$ 19)

duration was based on the average duration of the first two flips of the tail (22.8 ms; the first two axial bends, i.e. stages 1 and 2) defined based on Domenici & Blake [35], which is the period considered crucial for avoiding ambush predator attacks and is typical for fish within the size range used.

(d) Statistical analyses

To test the null hypothesis that capture success is independent of predator and prey temperature and elevated CO_2 exposure, capture success was compared using 4×4 contingency table analysis. Predation rate data did not meet the assumption of homogeneity of variance, so we rank transformed the data and performed a non-parametric two-way ANOVA to explore differences in predation rates between the four treatments. The effects of temperature and CO_2 exposure on performance kinematics were tested separately for prey and predators using two-factor MANOVAs with temperature and CO_2 as the fixed factors and the multiple dependent variables being: (predator) attack rate, attack distance, attack speed and (prey) RD, ALT, escape distance and escape speed. The MANOVA assumption of homogeneity of variance–covariance matrices was tested using a Box's M test of equality of covariance and was non-significant for the three experimental treatments (temperature, $p = 0.002$; temperature/CO_2, $p = 0.02$ and CO_2, $p = 0.3$). Univariate two-factor ANOVAs with Tukey's HSD post hoc tests were then performed to determine the nature of any differences found by the MANOVAs. Assumptions of normality and homogeneity of variance were examined using residual analysis and Levene's test. Attack rate, RD, escape speed and ALT data did not meet the assumption of normality, so we performed square root (for attack rate, RD and escape speed) and log$_{10}$ (for ALT) transformations, respectively, to normalize the data. Two-tailed binomial tests were performed to determine whether CO_2 or temperature exposure had an effect on the directionality of the escape response, comparing the proportion of individuals that moved away from or towards the stimulus and testing the null hypothesis of a 0.5 probability of turning either way.

3. Results

The overall capture success was significantly associated with the treatments experienced by the predator and prey ($\chi^2 = 7.82$, d.f. $= 3$, $p = 0.04$) (figure 1a). Capture success was greatest in the high temperature–control CO_2 treatment, with 47.3% of prey caught. Under elevated CO_2 conditions, high temperature exposure had a much-reduced effect on the probability of capture, with only 33.3% of prey captured under this treatment regime. Capture success was moderate (22%) in the elevated CO_2–control temperature treatment group and the lowest capture success occurred under current-day control temperature and control CO_2 conditions (5.8%).

The performance characteristics of the predator were unaffected by the combined effect of high temperature and elevated CO_2 (MANOVA, Pillai's trace$_{3,66} = 1.3070$, $p = 0.2$). There was a significant trend for predators exposed to high temperature and control CO_2 to have a higher attack rate, with more than double the attacks compared with the control temperature–control CO_2 treatment (table 2; $F_{1,67} = 4.38$, $p = 0.04$) (figure 1b). There was no effect of control temperature–elevated CO_2 and no interaction between elevated CO_2 and high temperature exposure on attack rates ($p > 0.05$). Predation rate increased following exposure to the high temperature–control CO_2 treatment, with this group displaying a marked increase in predation rate compared with the control temperature–control CO_2 treatment (table 2; $F_{1,67} = 5.99$, $p = 0.01$) (figure 1c). There was no effect of elevated CO_2 alone or an interaction effect ($p > 0.05$) on predation rate. Predator attack distance and attack speed were unaffected by the elevated CO_2 or high temperature treatments ($p > 0.05$).

The performance characteristics of prey were affected by the combined effect of high temperature and elevated CO_2 (MANOVA, Pillai's trace$_{8,61} = 2.233$, $p = 0.03$), and there was a significant main effect of high temperature (MANOVA, Pillai's trace$_{8,61} = 3.271$, $p = 0.003$). There was no multi-variate effect of elevated CO_2 independent of temperature ($p > 0.05$). Univariate two-factor ANOVAs detected significant differences between treatments in four of the tested performance attributes: two measures of reactivity (RD, whereas a large RD indicates high reactivity to a predator attack) and ALT (a high ALT value is indicative of reduced prey responsiveness) and two measures of kinematic performance (prey escape distance and prey escape speed).

There was a strong effect of high temperature on the RD of the prey (table 2; $F_{1,67} = 15.7$, $p = 0.0001$) (figure 1d). Specifically, prey exposed to high temperatures and control CO_2 allowed the predator to get twice as close to them before undertaking an escape response (0.08 m compared with 0.04 m). There was little effect of elevated CO_2–control temperature exposure on the RD of the prey, with values nearly identical to the control temperature–control CO_2 treatment (0.08 m compared with 0.075 m). When prey were exposed to the combined effect of elevated CO_2 and high-temperature treatment, RD decreased to 0.034 m; however, this was not significantly different from the other treatments.

ALT increased from 1.39 rad s^{-1} in the control temperature–control CO_2 treatment to 14.52 rad s^{-1} in the high temperature–control CO_2 treatment (table 2; $F_{1,67} = 16.4$, $p = 0.0001$) (figure 1e). There was a non-significant increase in ALT in response to elevated CO_2–control temperature (3.27 rad s^{-1}) and when prey were exposed to the
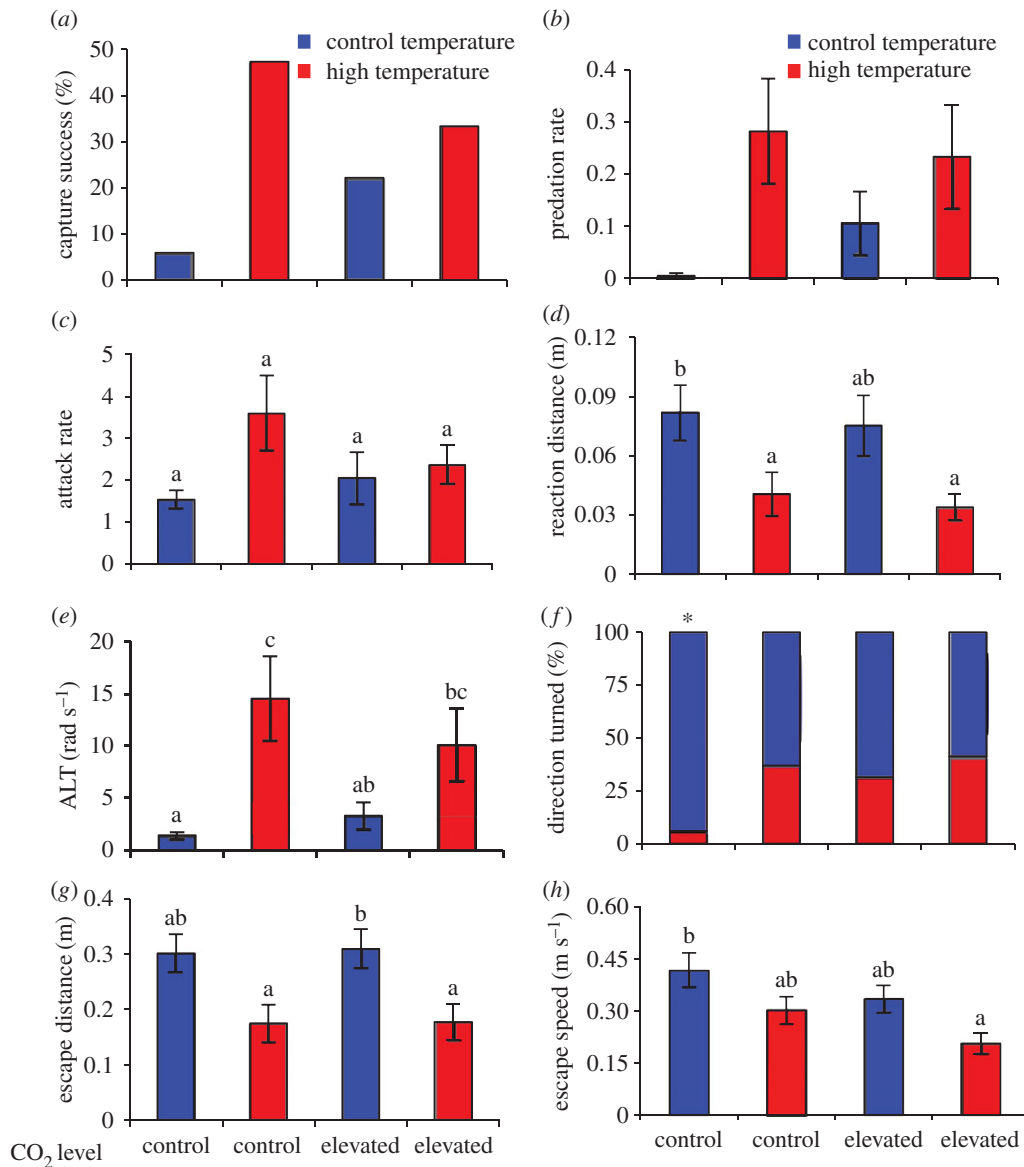
Figure 1. Comparison of the effects of elevated CO_2 (approx. 405 and 930 µatm) and temperature (control—26.7°C and elevated—29.6°C) on interactions between a predator (*Ps. fuscus*) and prey (*Po. wardi*), on eight performance characteristics: capture success (*a*); attack rate (within the 10 min filming period) (*b*); predation rate (within the 10 min filming period) (*c*); prey RD (*d*); prey ALT (*e*); directionality (*f*); prey escape distance (*g*); and prey escape speed (*h*). $n = 17$ for control temperature–control CO_2, $n = 19$ for high temperature–control CO_2, $n = 18$ for elevated CO_2–control temperature and $n = 18$ for high temperature–elevated CO_2. Error bars are s.e.m. Letters above bars represent Tukey's HSD groupings of means. Asterisk above the panel indicates that the proportion of responses directed away from (blue portion) and towards (red portion) the stimulus differed significantly from random.

combined effect of elevated CO_2–high-temperature treatment, ALT increased to 10.06 rad s^{-1}, suggestive of a strong trend. However, this was not statistically significant.

Directionality did not differ among treatments ($\chi^2 = 6.25$, d.f. = 3, $p = 0.1$) (figure 1*f*). However, when treatments were individually compared with the control temperature–control CO_2 treatment, there were significant differences (elevated CO_2–high temperature treatment: $\chi^2 = 9.21$, d.f. = 2, $p = 0.01$; high temperature–control CO_2, $\chi^2 = 7.37$, d.f. = 2, $p = 0.02$ and the elevated CO_2–control temperature, $\chi^2 = 5.99$, d.f. = 2, $p = 0.03$). Specifically, in the control temperature–control CO_2 treatment, most of the prey (94%) turned away from the predator. By contrast, only 58% of prey turned away from the predator following combined exposure to elevated CO_2 and high temperature. The direction turned by the prey in the high temperature–control CO_2 treatment and the elevated CO_2–control temperature treatment was intermediate to the control temperature–control CO_2 treatment and combined

effect of elevated CO_2 and high temperature treatment group. The proportion of responses directed away from and towards the predator differed significantly from random (50 : 50) for the control temperature–control CO_2 treatment ($n_{norm} = 17$, $p = 0.001$), but not for the other three treatments (high temperature–control CO_2: $n_{norm} = 19$, $p = 0.09$; elevated CO_2–control temperature; $n_{norm} = 16$, $p = 0.06$ or the combined effect of elevated CO_2 and high-temperature treatment: $n_{norm} = 17$, $p = 0.1$).

There was a strong effect of high temperature on the total prey escape distance (table 2; $F_{1,67} = 14.8$, $p = 0.0002$) (figure 1*g*), with prey exposed to high temperature–control CO_2 having 50% lower escape distances compared with prey from control temperature–control CO_2 (0.30 versus 0.17 m). By contrast, elevated CO_2 exposure–control temperature had no significant effect on prey escape distances (0.31–0.30 m; $p > 0.05$) and there was no significant interaction between the two treatments ($p > 0.05$).

Table 2. Effects of water temperature (approx. 27, 30°C) and CO_2 (approx. 405, 930 μatm) on the behavioural interaction between a predator (*Ps. fuscus*) and its prey (*Po. wardi*) on six performance variables: attack rate, predation rate, prey RD, ALT, prey escape distance and prey escape speed (n = ambient; n = 17; high temperature; n = 19; elevated CO_2; n = 18; high temperature and elevated CO_2; n = 18).

behaviour	source of variation	d.f.	MS	F	p-value
attack rate	temp	1	1.9700	4.3823	0.0401
	CO_2	1	0.2235	0.4971	0.4832
	temp × CO_2	1	0.5780	1.2858	0.2608
	error	67	0.4495		
predation rate	temp	1	18.282	5.992	0.0169
	CO_2	1	0.7952	0.2606	0.6113
	temp × CO_2	1	5.7200	1.8747	0.1754
	error	67	3.0511		
reaction distance	temp	1	0.1436	14.785	0.0002
	CO_2	1	0.0026	0.2741	0.6023
	temp × CO_2	1	0.0003	0.0338	0.8547
	error	67	0.0009		
ALT	temp	1	8.7191	16.402	0.0001
	CO_2	1	0.0193	0.0363	0.8494
	temp × CO_2	1	0.4005	0.7535	0.3884
	error	67	0.5315		
escape distance	temp	1	0.3145	14.829	0.0002
	CO_2	1	0.0001	0.0050	0.9437
	temp × CO_2	1	0.0005	0.0267	0.8707
	error	67	0.0212		
escape speed	temp	1	0.0034	0.0877	0.7680
	CO_2	1	0.1897	4.8022	0.0318
	temp × CO_2	1	0.3769	9.5418	0.0029
	error	67	2.6862		

There was a significant interaction between the high temperature and elevated CO_2 treatment for the mean prey escape speed (table 2; $F_{1,67} = 9.54$, $p = 0.002$) (figure 1h). Prey from the control temperature–control CO_2 treatment escaped significantly faster than those from the combined elevated CO_2 and high-temperature treatments (0.4 versus 0.26 m s^{-1}).

4. Discussion

The oceans are warming and seawater pCO_2 is rising due to increasing anthropogenic CO_2 emissions, with these environmental changes potentially having antagonistic, additive or synergistic effects on the physiology and behaviour of marine animals [4,6,9]. In this study, we show that short-term exposure to elevated CO_2 and high temperature led to pronounced changes in predator–prey interactions of reef fishes. In terms of the prey, high temperature had the strongest effect on reactivity (i.e. RD and ALT) and locomotory performance. However, the interaction between CO_2 and temperature was complex, resulting in a mildly additive response with CO_2 exposure slightly increasing the effect of temperature for some performance traits.

Prey exposed to high temperatures displayed a decrease in reactivity with an attenuation of RD and an increase in ALT. The fast kinematics of escape responses are likely to be under the control of Mauthner cells, which are triggered as a reaction to the fast approach of a predator [37], with reactivity a crucial factor for predator evasion [38]. Our findings are in line with those of Webb & Zhang [19], who found that the RD and ALT of prey (goldfish, *Carassius auratus*) to an attacking predator (rainbow trout, *Oncorhynchus mykiss*) deteriorated following acute exposure to high temperatures, contributing to increased predator success. Exposure to high temperatures can lead to changes in the physiology of the Mauthner cell circuit, resulting in differences in the balance between the excitatory and inhibitory transmission onto the Mauthner cell [17].

Our results also showed that directionality was impaired in prey fish exposed to high temperatures. Directionality is an important trait, with an early mistake—such as turning towards a predator—likely to be costly [38]. Furthermore, the score of directionality is a good indicator of whether there is neural impairment associated with a treatment. The direction of the fast-start indicates whether the right or left Mauthner cell has been fired [37]. Typically, if the stimulus is on the right, the Mauthner cell that fires is also on the right, meaning that the muscle that contracts will be the contralateral one (i.e. on the left), leading to an 'away' response. In this study, prey exposed to higher temperatures tended to

show lower directional discrimination of the predator by exhibiting directionality that was not different from random (i.e. 50% away and 50% towards the stimulus). By contrast, individuals that were exposed to control conditions exhibited directionality that was different from random and significantly away from the threat. We also observed both an independent effect of elevated CO_2 and an effect due to the combined exposure (CO_2 and temperature) on directionality, with both groups turning away from or towards the predator randomly. Directionality was one of the few escape variables impaired by CO_2 exposure. The increase in capture success from 5% in the control treatment to 22% in the elevated CO_2 treatment, suggests that directionality is a fundamental factor facilitating successful escapes.

Interestingly, we saw little influence of elevated CO_2 on prey reactivity with both RD and ALT at similar levels as in fish under control CO_2. This is a surprising result as earlier studies have demonstrated numerous behavioural effects of elevated CO_2 on coral reef fishes, including *P. wardi* [10,51,52]. It has been suggested that behavioural abnormalities associated with CO_2 exposure involve a malfunction of the $GABA_A$ neurotransmitter, an impairment that is restored following treatment with the $GABA_A$ antagonist gabazine [52]. $GABA_A$ receptors are found throughout the Mauthner cell [53] and previous work with a closely related species (*Pomacentrus amboinensis*) hypothesizes that impairment of these receptors may be the cause of decreased prey reactivity and escape performance during a predator–prey interaction [32]. Alternatively, these conflicting responses may be due to species-specific differences; despite the close phylogenetic history with *P. wardi*, *P. amboinensis* is known to be particularly sensitive to increasing CO_2 [54,55]. Previous studies have found little effect of high CO_2 exposure on the physiological performance of most juvenile reef fishes [51,56]. The physiological tolerance of these species to high CO_2 may at least partly explain why there were limited effects of high CO_2 on escape kinematics in this study. Furthermore, recent work has shown that a change in behaviour in response to elevated CO_2 is not ubiquitous, with some temperate species showing little influence of elevated CO_2 exposure on behaviour [57].

We observed significant combined effects of temperature and CO_2 on prey escape speed. These changes may be due to reduced muscle power resulting from changes in the contractile properties of the swimming muscles [16] associated with increased temperature exposure, as well as changes in aerobic scope. Although not directly measured in this study, declining aerobic scope at higher temperatures may explain the reduction in locomotory performance. Despite fast starts being powered anaerobically, an energy debt has to be paid off by post-exercise oxygen consumption, requiring more energy than used initially, creating an energy deficit [58]. Fishes with reduced aerobic scope in high-temperature conditions may therefore show low performance escapes as an energy-saving strategy [13]. Similarly, previous work on fish exposed to hypoxia has shown that their escape (i.e. anaerobic) swimming performance is impaired [50].

The results for the predators are more complex to interpret, as capture success is determined by predator performance and motivation as well as the interplay between the predator and the prey. Predators exposed to high temperatures had a significant increase in capture success compared with the other three groups, which may have been due to two non-mutually

exclusive possibilities. First, prey responsiveness may have decreased, thereby making them more vulnerable. Second, there may have been an increase in the effectiveness of the predators, leading to an increase in attack and predation rates. Increasingly, high temperatures are known to increase metabolic rate [59], which in turn increases energetic demand. This may manifest as an increase in predator motivation to capture prey. As food availability was kept constant between the four treatment groups, this seems likely to be the cause of the increase in attack motivation. By contrast, Ferrari *et al.* [23] found that predation rates increased after the combined exposure to elevated CO_2 (995 µatm) and high temperatures (31°C), yet failed to find an increase in predation rates in response to high-temperature exposure alone [23]. Despite the increase in capture success, we saw little influence of temperature on the locomotory performance of the predator, suggesting that *P. fuscus* may be unaffected by temperature increases such as those used here, potentially as a result of its recent thermal experience or evolutionary history. Alternatively, there may have been differential acclimation occurring between the predator and the prey, despite the consistent time spent in treatment between the two species. In contrast with earlier work by Allan *et al.* [32], there was little influence of elevated CO_2 on the locomotory performance on the predator despite observing a fourfold increase in capture success compared with controls. This is consistent with the physiological tolerance to high CO_2 in *P. fuscus* reported by Couturier *et al.* [56].

We observed a strong effect of short-term temperature exposure on predator–prey interactions. This may lead to changes in the replenishment of recruits to coral reefs. The temperature used in our experiment was 3°C higher than the control October temperature of 27°C; however, both species (*Po. wardi* and *Ps. fuscus*) do experience temperatures around 30°C in the middle of summer. In Australia, October is the start of the breeding season and settling recruits experience a severe predation-induced bottleneck at this vulnerable time. Therefore, any changes in the numbers of fish that successfully settle could lead to changes in community structure. Examining interactions between species in response to environmental stressors can be complex due to behavioural subtleties that may not be captured. Only focusing on the responses of individual species is likely to provide an insufficient basis from which to gain an understanding of long-term responses to climate change in complex ecological environments, such as coral reefs [60] due to differing tolerances to environmental perturbations [20,22].

While this study examined short-term exposure to elevated temperature, the timeframe is relevant to exposure of new recruits on the reef. Furthermore, there is little evidence to suggest that reef fishes acclimate to elevated temperatures over longer durations (7–22 days) [61]. However, there is evidence for transgenerational thermal acclimation in reef fishes [62] and evidence for heritable variation in the metabolic responses of juvenile reef fishes to higher temperatures that could aid them in adapting to future warming [63]. Consequently, future studies will also need to consider the potential for adaptation. Our results, combining escape performance, attack behaviour and mortality rates, suggest that increasing CO_2 and warming may lead to complex changes in the predator–prey relationships of coral reef communities. The novelty is that this study examines a crucially important process that is fundamental to all food-webs: predation by a predator, and avoidance by a prey. To date, most studies have only examined physiological or performance aspects of

isolated species and have not explored the interactions among species. Understanding how organisms and ecosystems respond to key environmental drivers concurrently remains a priority for science, management and conservation, and this study provides the foundation for studies of other predator–prey interactions.

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