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RESEARCH ARTICLE

Maximum thermal limits of coral reef damselfishes are size dependent and resilient to near-future ocean acidification

Timothy D. Clark[1],*, Dominique G. Roche[2], Sandra A. Binning[2], Ben Speers-Roesch[3] and Josefin Sundin[4]

ABSTRACT

Theoretical models predict that ocean acidification, caused by increased dissolved CO_2, will reduce the maximum thermal limits of fishes, thereby increasing their vulnerability to rising ocean temperatures and transient heatwaves. Here, we tested this prediction in three species of damselfishes on the Great Barrier Reef, Australia. Maximum thermal limits were quantified using critical thermal maxima (CT_{max}) tests following acclimation to either present-day or end-of-century levels of CO_2 for coral reef environments (~500 or ~1000 µatm, respectively). While species differed significantly in their thermal limits, whereby *Dischistodus perspicillatus* exhibited greater CT_{max} (37.88±0.03°C; N=47) than *Dascyllus aruanus* (37.68± 0.02°C; N=85) and *Acanthochromis polyacanthus* (36.58±0.02°C; N=63), end-of-century CO_2 had no effect (*D. aruanus*) or a slightly positive effect (increase in CT_{max} of 0.16°C in *D. perspicillatus* and 0.21°C in *A. polyacanthus*) on CT_{max}. Contrary to expectations, early-stage juveniles were equally as resilient to CO_2 as larger conspecifics, and CT_{max} was higher at smaller body sizes in two species. These findings suggest that ocean acidification will not impair the maximum thermal limits of reef fishes, and they highlight the critical role of experimental biology in testing predictions of theoretical models forecasting the consequences of environmental change.

KEY WORDS: Carbon dioxide, CO_2, Critical thermal maximum, CT_{max}, Great Barrier Reef, Climate change, Climate warming, Thermal tolerance, Fish, Ontogeny

INTRODUCTION

Climate change is causing concomitant increases in average temperature and carbon dioxide partial pressure (P_{CO_2}) in aquatic environments, as well as increasing the frequency of transient heatwaves (Hughes et al., 2003; IPCC, 2013; Pearce and Feng, 2013; Wernberg et al., 2013; Popova et al., 2016). Tropical organisms tend to have narrower thermal performance curves than temperate species and they are forecasted to be among the most sensitive to climate warming, either because they have evolved under relatively stable thermal conditions (Tewksbury et al., 2008; Lough, 2012) or because rate processes (e.g. metabolism) change proportionally faster in tropical systems as a result of warmer ambient temperatures (Payne and Smith, 2017). Regardless of what

may cause heightened thermal sensitivity of organisms in tropical systems, little is known of how increased P_{CO_2} (i.e. acidification) may interact with thermal stress in these or other environments. Theoretical models, appearing in the latest report from the Intergovernmental Panel on Climate Change (Pörtner et al., 2014), predict that elevated P_{CO_2} reduces the aerobic metabolic scope of aquatic ectotherms at a given temperature and consequently narrows the species-specific thermal window over which organisms can function (Fig. 1A; Pörtner, 2008; Pörtner and Farrell, 2008). Thus, these models predict that elevated P_{CO_2} will decrease thermal tolerance limits. While some aspects of these models are hotly debated, namely the assertion that thermal tolerance, niche patterns and fitness are governed by the temperature dependence of aerobic metabolic scope (Clark et al., 2013b; Jutfelt et al., 2014; Ern et al., 2016; Lefevre, 2016), insufficient attention has been focused on testing the prediction that thermal limits will be negatively affected by end-of-century levels of P_{CO_2}. Indeed, even in the absence of a change in aerobic metabolic scope, there could be other mechanisms such as cellular acid–base disequilibria that cause a negative impact of P_{CO_2} on thermal limits. Prior to extensive investigations into putative mechanisms, it is prudent to first quantify whether elevated P_{CO_2} does indeed impact thermal limits.

Thermal limits of aquatic organisms such as fishes can vary greatly across latitudinal scales, yet interspecific differences at regional scales are much less understood. This is particularly true for tropical systems, where the research effort has been small compared with temperate systems (but see Mora and Ospína, 2001; Ospína and Mora, 2004; Eme and Bennett, 2009). Moreover, relatively little is known about factors (beyond thermal acclimation) that drive thermal limits at an intraspecific level, although available evidence suggests that disease state, ontogenetic stage (Fig. 1B) and body size might be influential determinants (Vaughan and Coble, 1975; Brewer, 1976; Hutchison, 1976; Daufresne et al., 2009; Rijnsdorp et al., 2009; Forster et al., 2012; Cheung et al., 2013). In general, early life stages are thought to be the most sensitive to environmental stressors because their physiological/regulatory systems may not be fully developed (Fig. 1B; Brewer, 1976; Pörtner et al., 2005; Rijnsdorp et al., 2009; Freitas et al., 2010), yet much of the support for this idea stems from comparisons across studies/species rather than dedicated investigations across an ontogenetic range within a study/species (but see Komoroske et al., 2014). These knowledge gaps impede our capacity to identify sensitive species or life stages that may be of particular conservation concern.

With a rich history in comparative physiology (Becker and Genoway, 1979; Paladino et al., 1980; Lutterschmidt and Hutchison, 1997), the critical thermal maximum (CT_{max}; the upper temperature at which an animal loses motor function) has emerged as a potentially useful metric for understanding species' distribution ranges and resilience to heatwaves and climate warming

[1]University of Tasmania (Institute for Marine and Antarctic Studies) & CSIRO Agriculture and Food, Hobart, TAS 7000, Australia. [2]Institute of Biology, University of Neuchâtel, 2000, Neuchâtel, Switzerland. [3]Department of Biology, University of New Brunswick, Saint John, NB, Canada E2L 4L5. [4]Department of Neuroscience, Uppsala University, SE-751 24 Uppsala, Sweden.

*Author for correspondence (timothy.clark.mail@gmail.com)
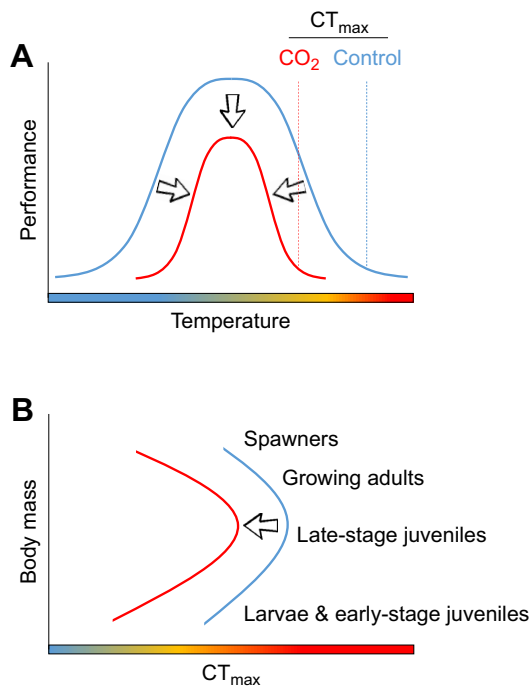
T.D.C., 0000-0001-8738-3347

Fig. 1. Theoretical models of the effects of temperature and elevated P_{CO_2} on ectotherms. (A) Theoretical changes in the performance (i.e. fitness) of ectothermic animals as a function of temperature, where animals under present-day levels of P_{CO_2} (blue curve; e.g. 400 µatm) have higher performance and a broader thermal performance curve than animals under future projected levels of P_{CO_2} (red curve; e.g. 1000 µatm). Putative mechanisms to explain this hypothetical response include cellular acid–base disequilibria and/or a reduction in the scope for oxygen transport (i.e. aerobic metabolic scope) of the animal. Such a response to P_{CO_2} would lower the critical thermal maximum (CT_{max}) of animals in an elevated P_{CO_2} environment (leftward shift in dashed vertical line). (B) Thermal limits (e.g. CT_{max}) have been proposed to increase throughout ontogeny and reach peak values in late-stage juveniles, subsequently declining as animals approach reproductive maturation and spawning. Increased environmental P_{CO_2} is predicted to decrease the thermal limits of all life stages (shift from blue to red curve) (adapted from: Pörtner, 2008; Pörtner and Farrell, 2008).

(Bennett and Beitinger, 1997; Sunday et al., 2012, 2014). While measurements of CT_{max} are typically conducted using thermal ramping rates that are relevant to heatwave scenarios or habitats such as tide pools, emerging evidence suggests that qualitative trends from CT_{max} measurements may also have relevance to the slower warming rates associated with progressive climate warming (Mora and Maya, 2006; Sunday et al., 2012). The controlled nature of CT_{max} measurements offers an avenue for investigating the influence of P_{CO_2} on the maximum thermal limits of animals, yet we are aware of only two studies on only two species of fish (one published during the peer review of the present paper) that have taken a similar approach. Pimentel et al. (2014) exposed larvae of the subtropical Senegalese sole (*Solea senegalensis*) for 30 days post-hatching to an extreme ocean acidification scenario of pH 7.51 (estimated P_{CO_2} ∼1600 µatm; *N*=30 per group). They reported that CT_{max} decreased (at a starting temperature of 18°C) or remained the same (at a starting temperature of 22°C) compared with a control group maintained at pH 8.02 (P_{CO_2} ∼400 µatm). Ern et al. (2017) measured CT_{max} in adults of the tropical black-axil chromis (*Chromis atripectoralis*) following exposure to pH 8.23 (estimated P_{CO_2} ∼340 µatm) or pH 7.81 (P_{CO_2} ∼1086 µatm; *N*=5 per group) and did not detect any differences between treatment

groups. The impacts of near-future levels of ocean acidification (end-of-century P_{CO_2} ∼1000 µatm in a business-as-usual scenario; IPCC, 2013) on the thermal limits of fishes, including those in highly sensitive tropical systems, remain poorly understood.

Here, we studied three species of site-attached coral reef damselfishes (*Acanthochromis polyacanthus*, *Dascyllus aruanus* and *Dischistodus perspicillatus*) in a near-equatorial location to test the prediction that end-of-century levels of P_{CO_2} reduce the maximum thermal limits (CT_{max}) of aquatic ectotherms. These species were chosen because they are co-occurring, abundant on the Great Barrier Reef, spread out across the damselfish phylogeny (Frédérich et al., 2013), and they were available in a wide size range (early-stage juveniles to adults) at the time of the study. Indeed, we were able to additionally examine whether CT_{max} and the impacts of P_{CO_2} differ within species as a function of body size (i.e. ontogeny). While levels of P_{CO_2} on coral reefs can vary across diurnal and seasonal cycles (Shaw et al., 2012), daily means are often around 450–550 µatm and an increase to ∼1000 µatm is predicted for the end of the century in a business-as-usual emissions scenario (IPCC, 2013). Using direct comparisons of species that co-exist in abundance across the tropical Western Pacific, we aimed to highlight interspecific and intraspecific patterns that may underlie susceptibility to the single and combined effects of acute warming and elevated P_{CO_2}.

MATERIALS AND METHODS
Animal collection and holding conditions
Animal collection and experiments were conducted under permits from the Great Barrier Reef Marine Park Authority (G13/35909.1) and with approval from the Animal Ethics Committee of James Cook University in association with the Australian Institute of Marine Science (A2314). Spiny chromis [*A. polyacanthus* (Bleeker 1855), *N*=63], humbug dascyllus [*D. aruanus* (Linnaeus 1758), *N*=85] and white damsel [*D. perspicillatus* (Cuvier 1830), *N*=47] of mixed sex were collected in the austral summer from around Lizard Island in the northern Great Barrier Reef, Australia (14°40′S, 145°28′E). Fish were collected on SCUBA either using a barrier net (10 mm stretch monofilament) or using hand nets and diluted clove oil spray. Body mass and standard length (SL) ranges were 0.10–24.08 g and 14.1–80.8 mm for *A. polyacanthus*, 0.02–9.45 g and 7.4–53.0 mm for *D. aruanus*, and 0.14–5.65 g and 14.9–53.6 mm for *D. perspicillatus*. Fish were moved from underwater holding cages into aerated buckets and transported within 90 min of capture to the aquarium facilities at the Lizard Island Research Station, where they were placed in tanks with flow-through seawater at ambient temperature (∼29°C; range 28.0–30.7°C). Species were kept separate and fish were divided into approximately even numbers between 22 holding tanks (10–30 l each, depending on fish size; 1–3 l min⁻¹ flow-through). At 24–48 h post-capture, the P_{CO_2} of half of the holding tanks (*N*=11) was gradually increased to 1089±326 µatm (mean±s.d., encompassing daily fluctuations) over 24 h using a CO_2 dosing system (pH stat Computers, Aqua Medic, Bissendorf, Germany) connected to solenoid valves regulating administration of 100% CO_2 gas into four aerated flow-through (∼10 l min⁻¹) header tanks (30 l each). The remaining holding tanks (*N*=11) received water from four additional header tanks kept at ambient P_{CO_2} levels (520±94 µatm, mean±s.d.). Water samples (*N*=9) collected from different reefs around Lizard Island confirmed that ambient levels of P_{CO_2} ranged from 540 to 660 µatm in the morning of commencement of the CT_{max} experiments, values which are typical for coral reef environments (Shamberger et al., 2011). The P_{CO_2} levels of the holding tanks were checked at least

daily using a handheld CO_2 meter (GM70, Vaisala, Helsinki, Finland) connected to a submerged gas-permeable PFTE probe (Qubit systems, Kingston, ON, Canada), as described previously (Jutfelt and Hedgärde, 2013). The Vaisala CO_2 meter was factory calibrated in Vantaa, Finland, prior to experiments. The experimental design and CO_2-dosing system followed best practices for ocean acidification research (Riebesell et al., 2010; Moran, 2014; Cornwall and Hurd, 2015). Fish were given 9–18 days to acclimate to the treatment conditions before trials commenced, which is sufficient time for individuals to establish new acid–base equilibria (Toews et al., 1983; Cameron and Iwama, 1987) and more than double the modal acclimation time used in previous P_{CO_2} studies on coral reef fishes (Heuer and Grosell, 2014). Fish were fed to satiation with commercial pellet food once per day, but food was withheld for 15–16 h prior to experiments. Tanks were cleaned 2–3 times per week. Negligible mortality was observed during the acclimation period.

CT_{max}

The same CT_{max} protocol was repeated on two consecutive days to achieve desired sample sizes (D. aruanus: control $N=41$, high P_{CO_2} $N=44$; A. polyacanthus: control $N=31$, high P_{CO_2} $N=32$; D. perspicillatus: control $N=24$, high P_{CO_2} $N=23$). The CT_{max} of each individual was measured in two rectangular tanks (L×W×D, 60×38×39 cm; water depth 27 cm) supplied with seawater from individual sumps (60×38×39 cm; water depth 28 cm). Water was continuously pumped from the sumps into the respective CT_{max} tanks (3.3 l min^{-1}; Eheim submersible pumps), draining through a standpipe back into the sumps below. One sump contained control water (ambient P_{CO_2}) while the other contained water with elevated P_{CO_2} (same as holding tanks) regulated by a dosing computer and solenoid valve (Aqua Medic, as above). Each sump received additional flow-through seawater throughout the trial to maintain water quality. P_{CO_2} was measured >4 times throughout each trial to ensure the differential between the treatment groups was maintained. Each sump contained an identical 1 kW heater with a digital controller that maintained the set-point temperature within ±0.1°C, and these were supplemented with smaller aquarium heaters (300 W, Aqua One) at the higher temperatures during the trials (i.e. >36°C). Each sump was aerated to ensure oxygen levels remained at >90% air saturation at all times.

Fish of a broad size range that had been acclimated to either control or elevated P_{CO_2} treatments were placed into the corresponding CT_{max} tanks in the evening and left to settle overnight (6–31 fish per species per treatment per day). Two pieces of opaque PVC piping (diameter 10 cm) were placed vertically in each CT_{max} tank during the whole trial to provide structure for the fish. Water temperature was kept at ambient conditions for the Lizard Island region (29–30°C) during the overnight settling period (~12 h), and P_{CO_2} was 530–620 µatm (control) or 970–1010 µatm (elevated P_{CO_2}) at the commencement of trials the following morning. At approximately 07:00 h, the 1 kW heater in each sump was set to 30.5°C and the temperature was subsequently increased 0.5°C every 30 min until all fish had reached their CT_{max}, which was defined as the temperature at which loss of equilibrium occurred uninterrupted for 10 s (Lutterschmidt and Hutchison, 1997). The temperature of each CT_{max} tank was logged continuously [0.125°C resolution using calibrated (with a mercury thermometer) iButton data loggers; Maxim Integrated, San Jose, CA, USA] and monitored manually with a handheld meter (0.1°C resolution; HQ40D, Hach Lange, Loveland, CO, USA). When an individual reached CT_{max}, the time, temperature (from the handheld meter), treatment (control

or elevated P_{CO_2}), mass (to 0.01 g) and standard length (with callipers to the nearest 0.1 mm) were noted before the fish was placed in a recovery tank containing seawater at ambient temperature (~29°C). Sixth-order polynomial regressions were plotted through the iButton temperature data as a function of time at the conclusion of each trial (R^2 range 0.989–0.994) and used to calculate precise CT_{max} temperatures (i.e. interpolating between logged values).

Statistical analyses

The effect of P_{CO_2} exposure on CT_{max} was tested in the three species using a general linear model (LM). We controlled for mass and trial number by specifying these terms first in the model and computing Type I sequential sum of squares. Mass was centred by subtracting the grand mean from all values to facilitate the interpretation of parameter estimates. We allowed a four-way interaction and all three-way interactions between predictors and performed model simplifications using two likelihood ratio (LR) tests. The four- and three-way interactions were subsequently removed (LR test, $P>0.05$ in both cases), and the final model included trial, mass, species and treatment as fixed factors, and all two-way interactions (in this order). We verified model assumptions using diagnostic plots (i.e. plot {base}, residualPlots {car} and qqPlot {car} functions in R v3.2.0; http://www.R-project.org/). Excluding five outliers considerably improved these diagnostics (Figs S1 and S2), hence results herein are from models where outliers have been excluded. Analysing the entire dataset (i.e. including the five outliers) produced qualitatively similar results (i.e. significance and direction of effects unchanged), as did a mixed-effects model with trial specified as a random factor. We conducted post hoc comparisons using the functions 'lsmeans' and 'contrast' in the R package 'lsmeans'. The R script and data used for the analyses are available on figshare (https://doi.org/10.6084/m9.figshare.3408310.v1), allowing readers to assess and visualize the outputs of models with and without outliers.

RESULTS

Overall, our statistical model explained 96.5% of the variance in CT_{max} (adjusted R^2). Species differed in their maximum thermal limits ($F_{2,175}=2412.10$, $P<0.001$; Fig. S3A): D. perspicillatus had the highest CT_{max} (mean±s.e.=37.88±0.03°C), followed by D. aruanus (37.68±0.02°C) and A. polyacanthus (36.58±0.02°C). CT_{max} values were slightly higher for all species (0.28±0.02°C) in the first versus the second trial but the magnitude of this difference varied among species (species×trial: $F_{1,175}=24.92$, $P<0.001$; Fig. S3B).

The effect of P_{CO_2} exposure on CT_{max} differed among species (species×treatment: $F_{2,175}=7.87$, $P<0.001$; Fig. 2). P_{CO_2} exposure did not influence CT_{max} in D. aruanus (estimate±95% CI=0.043±0.028, $P=0.123$), whereas it increased CT_{max} by 0.21°C in A. polyacanthus (estimate±95% CI=0.208±0.032, $P<0.001$) and by 0.16°C in D. perspicillatus (estimate±95% CI=0.161±0.037, $P<0.001$).

The relationship between body mass and CT_{max} also differed among species (species×mass: $F_{2,175}=29.47$, $P<0.001$; Fig. 3), irrespective of P_{CO_2} treatment (3-way interaction not significant, LR test $P>0.05$): smaller A. polyacanthus and D. perspicillatus tolerated higher temperatures (CT_{max}~mass slope±95% CI=−0.074±0.026 and −0.048±0.031, respectively), whereas the opposite trend existed for D. aruanus (CT_{max}~mass slope±95% CI=0.021±0.012). Nevertheless, a single, very small D. aruanus (0.02 g) was excluded from the analyses to satisfy model assumptions but had an exceptionally high CT_{max} (38.38°C; small outlier in Fig. 3A).
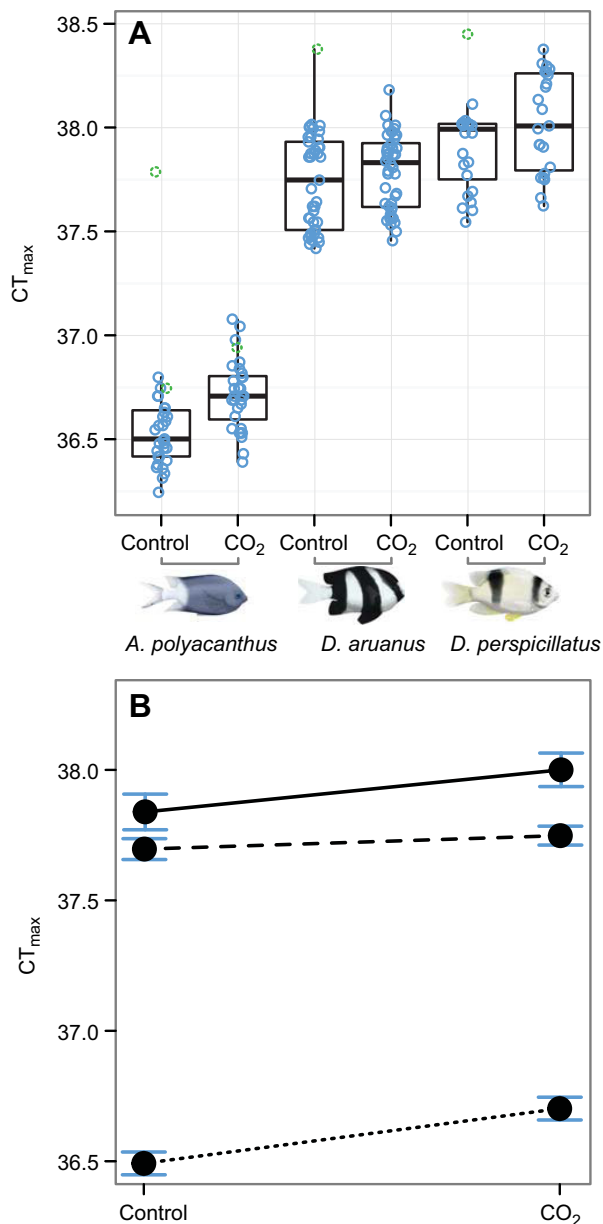
Fig. 2. CT$_{max}$ of three damselfish species maintained in present-day (control; ~500 μatm) or end-of-century (CO$_2$; ~1000 μatm) P_{CO_2} conditions. (A) Box-and-whisker plots (median and interquartile range) showing the distribution of the raw data, where five outliers (excluded from the model) are indicated with dotted green circles. (B) Model predictions for *Acanthochromis polyacanthus* (dotted line; N=63), *Dascyllus aruanus* (dashed line; N=85) and *Dischistodus perspicillatus* (solid line; N=47). Error bars are 95% confidence intervals.

DISCUSSION

Our study shows that end-of-century levels of P_{CO_2} do not compromise the maximum acute thermal limits of coral reef damselfishes, providing empirical evidence against theoretical models that predict detrimental effects of ocean acidification on the maximum thermal limits of aquatic ectotherms (Pörtner, 2008; Pörtner and Farrell, 2008). We also show clear interspecific differences in CT$_{max}$ among common damselfish species that co-exist in identical reef environments across the Western Pacific, and reveal differences in maximum thermal limits within species as a function of body mass (i.e. ontogeny). It is notable that our CT$_{max}$

protocol utilized fish in communal tanks to avoid any anomalies that may arise when keeping social animals in isolation, and therefore our measures of CT$_{max}$ are likely to be more representative of what may occur on natural reefs if the heating rate was to match the rate used here. While the mechanism underlying thermal tolerance is often assumed to be a mismatch between oxygen supply and demand (Pörtner and Knust, 2007; Pörtner and Farrell, 2008), existing evidence seems to refute this idea and highlights a need for exploration of alternative mechanisms, including the thermal dependence of protein denaturation, neural function and mitochondrial membrane integrity (Clark et al., 2013a,b; Iftikar and Hickey, 2013; Wang et al., 2014; Brijs et al., 2015; Ern et al., 2016). Even so, the new knowledge derived from our study has broad implications for understanding interspecific and intraspecific variability in responses to ocean acidification and acute thermal challenges.

Interspecific differences in environmental resilience

The three damselfish species we examined differed in their maximum thermal limits, despite residing in the same habitat. *Dischistodus perspicillatus* had the highest CT$_{max}$, followed by *D. aruanus* and *A. polyacanthus* (Fig. 2). The effect of P_{CO_2} exposure on CT$_{max}$ also differed among species, with high CO$_2$-exposed *D. perspicillatus* and *A. polyacanthus* having a higher CT$_{max}$ than control fish, while there was no effect of elevated P_{CO_2} on CT$_{max}$ of *D. aruanus* (Fig. 2). Although statistically significant, the increases in CT$_{max}$ with P_{CO_2} were small (0.16°C in *D. perspicillatus* and 0.21°C in *A. polyacanthus*). The biological significance of these differences may thus be negligible, particularly in light of a difference in CT$_{max}$ of similar magnitude within species across trials (0.28°C).

To our knowledge, only two previous single-species studies on fish have examined CT$_{max}$ in the context of ocean acidification. Pimentel et al. (2014) reported that a shift in pH from 8.02 (estimated P_{CO_2} ~400 μatm) to pH 7.51 (~1600 μatm) caused a decline in the CT$_{max}$ of *S. senegalensis* when individuals were initially held at 18°C, but did not affect CT$_{max}$ when individuals were initially held at 22°C (heating rate of 0.033°C min^{-1} in both cases). While it is possible that the ~1.5°C decrease in CT$_{max}$ reported for *S. senegalensis* held at 18°C could be due to the young age of the individuals (up to 30 days post-hatching), our study does not lend support to the idea that elevated P_{CO_2} (i.e. lower pH) disproportionately affects early life stages (Figs 2 and 3). Our conclusions are corroborated by a very recent study by Ern et al. (2017), in which one species of coral reef fish (*C. atripectoralis*) exhibited a CT$_{max}$ of ~38.1°C regardless of whether it was exposed to pH 8.23 (estimated P_{CO_2} ~340 μatm) or pH 7.81 (P_{CO_2} ~1086 μatm). Our results and those of Ern et al. (2017), combined with a lack of influence of pH on CT$_{max}$ at the higher test temperature used by Pimentel et al. (2014), suggest that the concomitant increases in temperature and P_{CO_2} occurring in the world's oceans may not compromise CT$_{max}$ in most fishes.

One of our study species (*D. aruanus*) was previously reported to have a CT$_{max}$ of 40.0°C when individuals were acclimated to similar temperatures to those used here (~29°C) (Eme and Bennett, 2009). The higher CT$_{max}$ in Eme and Bennett (2009) compared with that of *D. aruanus* in our study (mean CT$_{max}$ 37.7°C) is probably a consequence of faster heating rates (0.31°C min^{-1} versus 0.017°C min^{-1} in our study), a smaller average size of the fish (16 mm SL versus 34 mm SL in our study), and differences in the duration constituting loss of equilibrium ('at least 1 min' versus 10 s in our study). Comprehensive analyses of the positive relationship
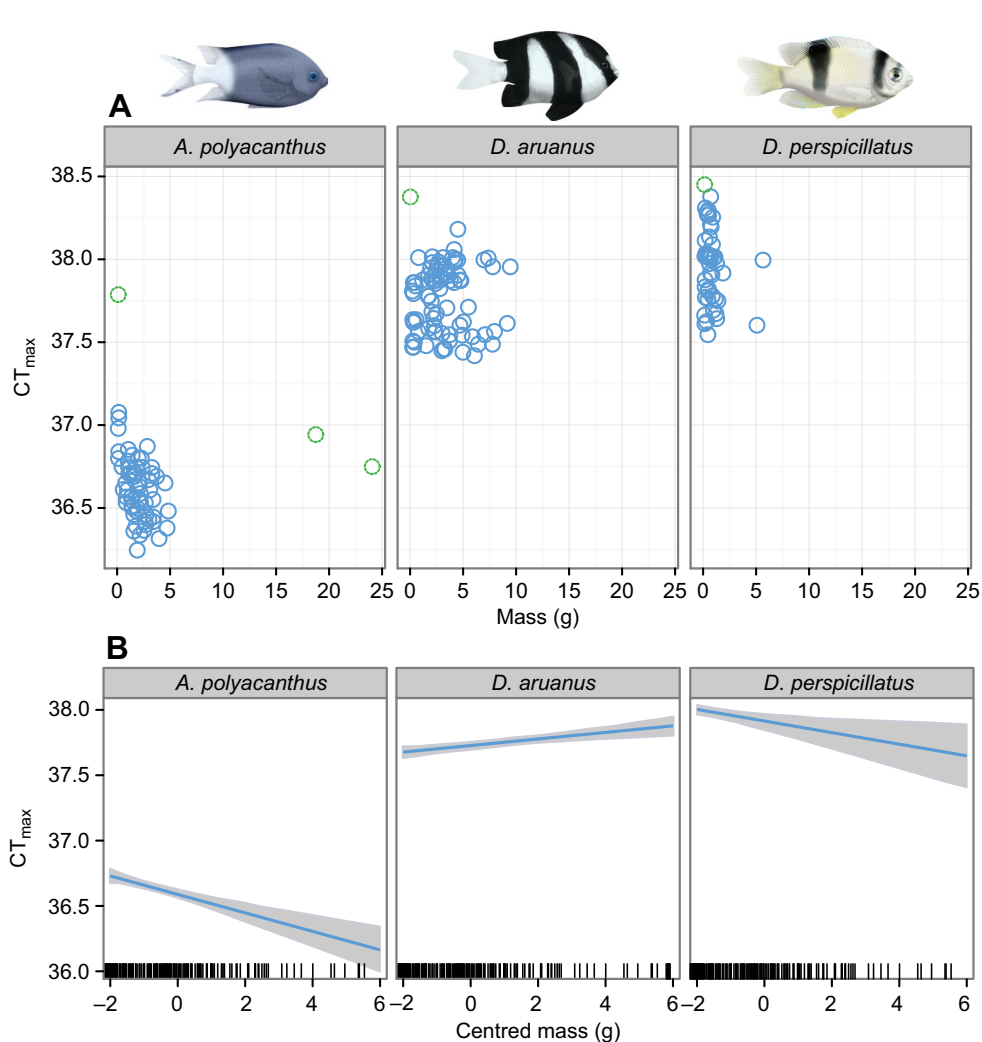
Fig. 3. Effects of body mass on the CT_{max} of three damselfish species maintained in present-day (control; ~500 µatm) or end-of-century (CO₂; ~1000 µatm) P_{CO_2} conditions. Sample sizes are given in Fig. 2. (A) Plots of the raw data with five outliers (excluded from the model) indicated with dotted green circles. (B) Model predictions with 95% confidence intervals indicated in grey; mass was centred (mean subtracted from each value) so that zero represents the mean mass across species.

between heating rate and CT_{max} have been presented previously (Mora and Maya, 2006; Peck et al., 2009), and this is likely to be an underlying factor in the difference in CT_{max} of *A. polyacanthus* reported here (~36.7°C when heated at 0.017°C min^{-1}) versus that in Ern et al. (2017) (~38.4°C when heated at 0.033°C min^{-1}). Indeed, CT_{max} for seven reef fish species investigated by Ospína and Mora (2004) ranged from 35.0 to 37.5°C when heating rates were 0.017°C min^{-1} (starting temperature 26.5°C), while CT_{max} of 24 reef fish species investigated by Menasveta (1981) ranged from 36.7 to 42.0°C when heating rates were 0.25°C min^{-1} (starting temperature 28.0°C). Notably, some reef-associated species such as the white mullet (*Mugil curema*) display exceptional thermal limits (CT_{max} 40.8°C; starting temperature 26.5°C) even when heating rates are relatively low (0.017°C min^{-1}) (Mora and Ospína, 2001).

It should be emphasized that CT_{max} represents a thermal limit for acute, not chronic exposures. For example, Eme and Bennett (2009) found that *D. aruanus* did not survive an acclimation period of 14 days when temperatures exceeded 34–35°C, despite having a CT_{max} of 40°C. Even so, meta-analyses on CT_{max} data have shown that the differences identified among species have relevance for understanding thermal distribution patterns and the potential impacts of climate warming on fish populations (Sunday et al., 2012, 2014). Thus, the lower CT_{max} of *A. polyacanthus* compared with that of the other two species studied here is likely to have

functional significance in the natural environment. Specifically, an increase in the frequency of coastal heatwaves (e.g. Wernberg et al., 2013) is likely to impact *A. polyacanthus* to a greater extent than *D. aruanus* or *D. perspicillatus*. Such interspecific differences should be considered not only in the context of mortality but also in relation to sub-lethal impacts that are known to arise at temperatures much lower than CT_{max}. For example, anomalously high temperatures can cause cessation of feeding (e.g. Ortubay et al., 1997) and delayed reproductive maturation (e.g. Jeffries et al., 2012) in fishes.

A notable difference in the life history of *A. polyacanthus* compared with that of most other damselfishes, including *D. aruanus* and *D. perspicillatus*, is the lack of a pelagic larval stage (Miller-Sims et al., 2008). Whether the lack of open ocean exposure of *A. polyacanthus* larvae underpins the lower CT_{max} in this species is unknown, although this idea is hard to reconcile given that coral reefs are generally warmer than the open ocean because of their shallow depth and proximity to land. Perhaps a more likely explanation for the lower thermal limits observed in *A. polyacanthus* is local adaptation to the temperature profiles experienced around Lizard Island. Gene flow among populations of *A. polyacanthus* is low compared with that of other damselfish species as a result of their limited dispersal (Doherty et al., 1994; Bay et al., 2006); juveniles only disperse away from their parents after approximately 3 months, and some related individuals remain together as adults (Miller-Sims et al., 2008). As a result, most *A. polyacanthus* are

likely to experience similar thermal conditions to their parents, possibly selecting for narrower thermal limits compared with species such as *D. perspicillatus* and *D. aruanus*, which spend 2–3 weeks as pelagic larvae (Wellington and Victor, 1989). As pelagic larvae can disperse hundreds of kilometres across a large latitudinal range (e.g. Victor, 1987), the ability to withstand a broad temperature range should be favoured in species like *D. perspicillatus* and *D. aruanus*. Further study of the life history characteristics and physiological processes that underlie differences in CT_{max} across damselfish species may prove fruitful for deciphering the underlying mechanisms of the findings presented here.

Intraspecific/ontogenetic differences in environmental resilience

Our finding that small, early-stage juvenile damselfishes generally have a higher CT_{max} compared with that of larger conspecifics conflicts with the established belief that larval and early-stage juvenile fishes are especially sensitive to environmental stressors as a consequence of underdeveloped physiological/regulatory systems (Hutchison, 1976; Pörtner and Farrell, 2008). While the enhanced thermal limits of small individuals were statistically apparent in *A. polyacanthus* and *D. perspicillatus*, where several early-stage juveniles were represented in the dataset, the observation of exceptionally high CT_{max} in the single, early-stage juvenile *D. aruanus* is suggestive of a similar phenomenon in this species (small outlier in Fig. 3A).

Previous attempts to examine ontogenetic or size differences in thermal limits of reef fishes have failed to identify obvious trends. Ospína and Mora (2004) reported that CT_{max} was poorly related to body size in seven reef fish species, but they noted significant negative slopes in regressions of CT_{max} versus body size for rainbow wrasse (*Thalassoma lucasanum*; SL range 30–125 mm) and chameleon wrasse (*Halichoeres dispilus*; SL range 20–65 mm). Based on our findings, it is possible that the absence of early-stage juveniles from previous datasets has masked significant ontogenetic differences in thermal limits. That said, temperate delta smelt (*Hypomesus transpacificus*) generally exhibit a decline in CT_{max} with successive ontogenetic stages (Komoroske et al., 2014). Interestingly, we found some evidence that mature *A. polyacanthus* may have higher thermal limits than late-stage juveniles (but not as high as early-stage juveniles; Fig. 3A). Future experiments encompassing the entire mass range from larvae to full-sized adults are required to determine whether damselfishes may exhibit a U-shaped CT_{max} curve as a function of body mass, which would be the opposite to theoretical predictions (Fig. 1).

The underlying mechanisms associated with superior thermal limits in early-stage juvenile reef fishes remain unknown, but existing evidence suggests that CT_{max} is unlikely to be governed by a mismatch between oxygen supply and demand (Wang et al., 2014; Brijs et al., 2015; Ern et al., 2016). Our *a priori* assumption based on available data and theoretical models of climate change resilience was that late-stage juveniles and sub-adults would possess the highest thermal limits because they have fully developed physiological systems (unlike early-stage juveniles) but are not yet challenged by investment in reproduction (unlike mature adults) (Fig. 1B; Stewart and Allen, 2014). We also anticipated that if P_{CO_2} influenced CT_{max}, the effects would be most pronounced in early-stage juveniles as a result of less-developed acid–base regulation (Brown and Sadler, 1989; Pörtner and Farrell, 2008). While intuitive, the assumption that larvae and early-stage juveniles are more sensitive to elevated P_{CO_2} is primarily based on comparisons across unrelated studies/species (e.g. Pörtner et al.,

2005, and references within) rather than single studies that compare different life stages using consistent approaches [but see Ishimatsu et al. (2004) for ontogenetic comparisons at very high P_{CO_2} levels]. In this context, the present study is, to our knowledge, the first to quantify the interactive effects of elevated P_{CO_2} and high temperature on fishes across a broad range of sizes and life stages.

Implications and conclusions

Our study demonstrates the existence of interspecific and ontogenetic differences in maximum thermal limits of coral reef damselfishes. Importantly, we show that end-of-century levels of P_{CO_2} do not compromise acute maximum thermal limits. The heightened thermal limit and equivalent P_{CO_2} tolerance of smaller individuals provide evidence of an unexpected capacity to maintain physiological homeostasis from a young age, at least over temporal scales of hours (high temperature) to weeks (high P_{CO_2}).

It is apparent that existing theoretical frameworks regarding the temperature and P_{CO_2} sensitivities of fishes must be assessed with empirical evidence from robust experimental studies. While ongoing ocean acidification may influence coral reef fishes indirectly through detrimental effects on the calcification capacity of habitat-forming corals, we found no direct effects of end-of-century levels of P_{CO_2} and acidification on upper thermal tolerance limits. Given the rapid rate of warming compared with the rate of increase in P_{CO_2}, we propose that thermal stress via heatwaves and chronic warming will be the primary concern to the conservation of most coral reef organisms. Nevertheless, major reductions in CO_2 emissions are necessary to curtail the rate of warming (Hughes et al., 2017).

Data availability
The R script and raw data used for the analyses are publicly archived following best practices (Roche et al., 2015): https://doi.org/10.6084/m9.figshare.3408310.v1.

Supplementary information
Supplementary information available online at
http://jeb.biologists.org/lookup/doi/10.1242/jeb.162529.supplemental

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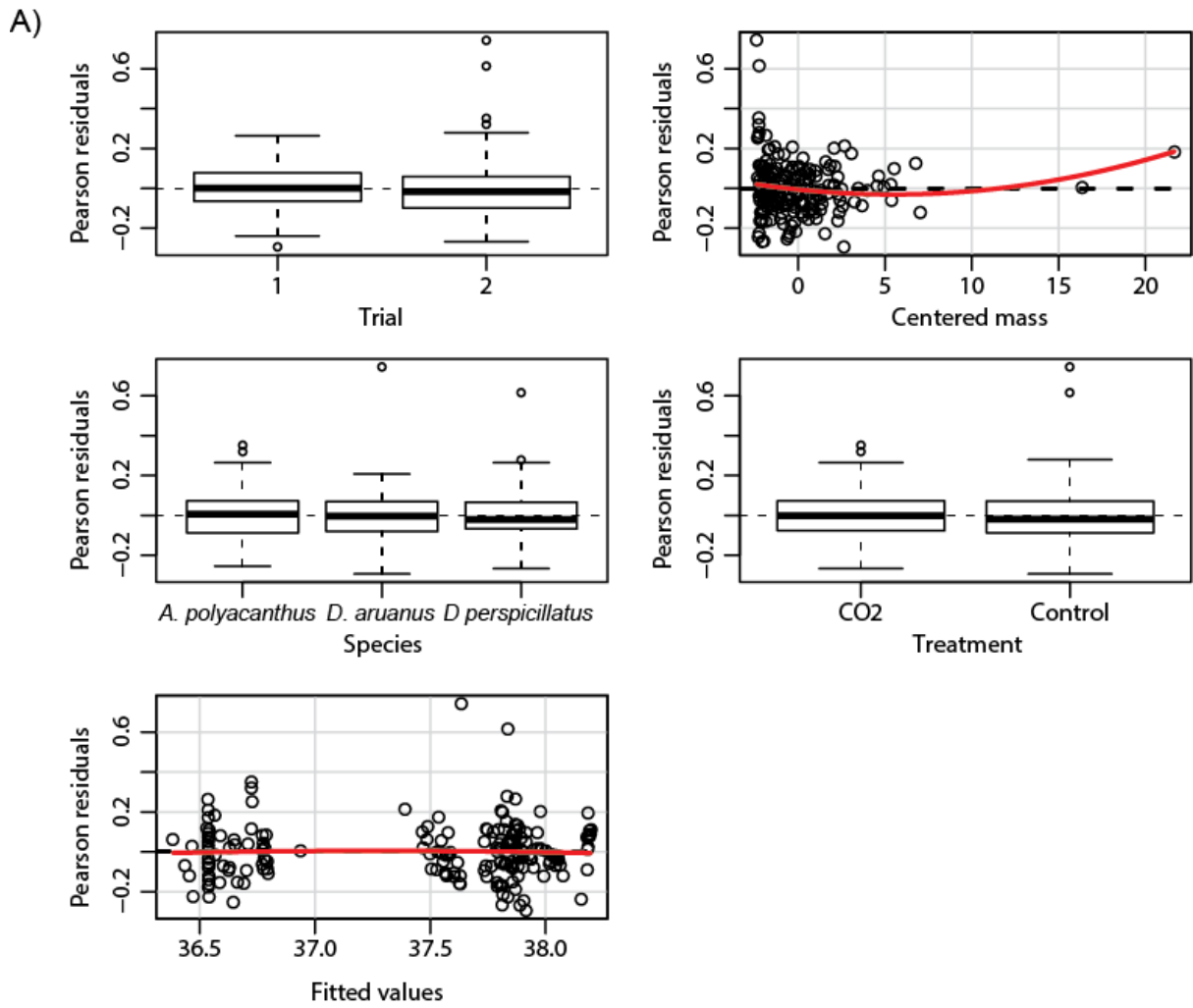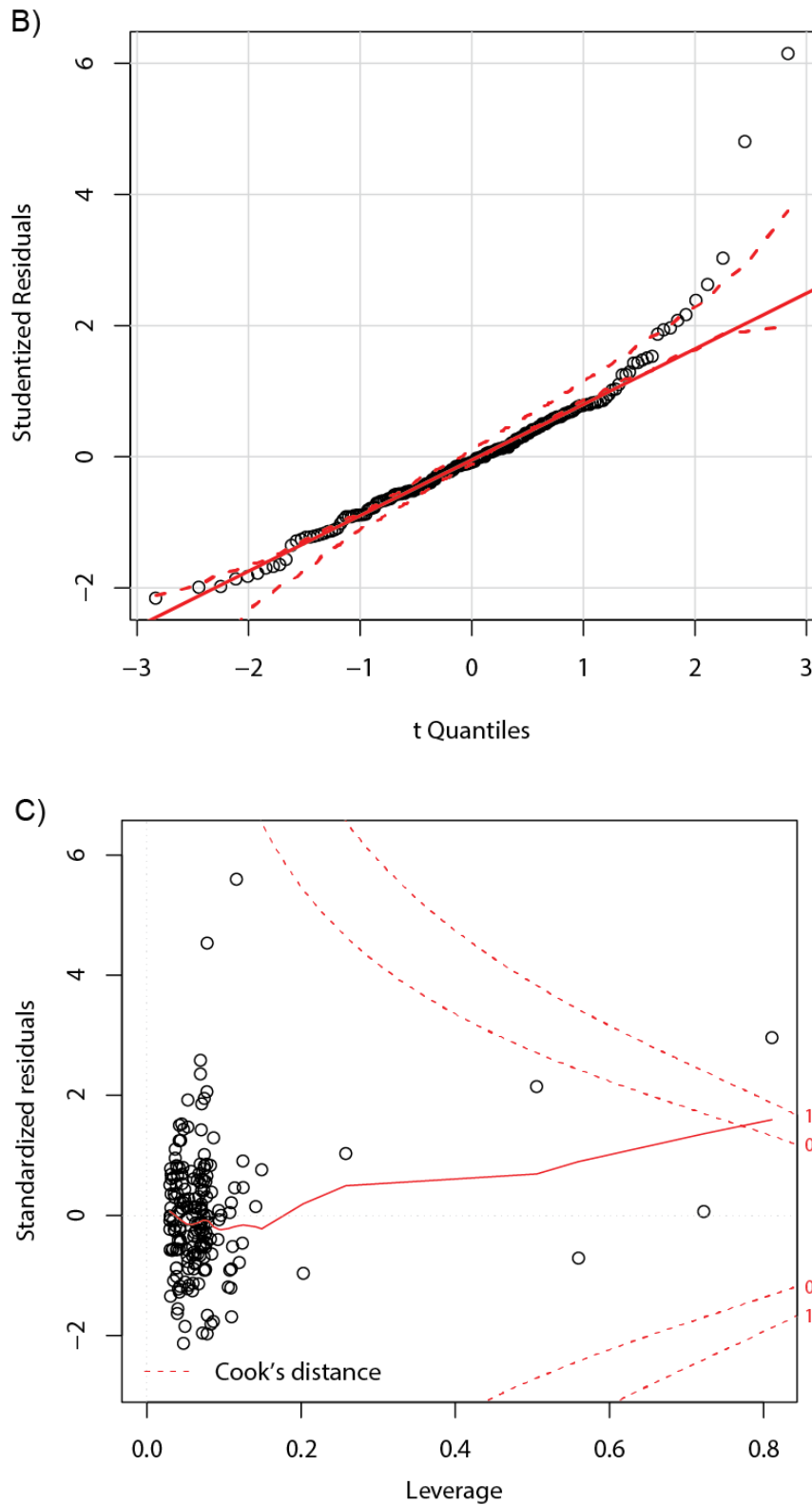
Supplementary figures

A)

Fig. S1A

Figs. S1B, S1C

Fig. S1 Diagnostic plots showing the distribution of residuals from the model CT_{max} ~ (trial + mass + species + treatment)^2 to assess (A) homoscedasticity (i.e., homogeneity of variance), (B) normality (via a quantile-quantile plot), and (C) leverage (i.e., the influence of values on model output). *All data points were included in the model.* T1 and T2 represent the two separate trials. For a discussion of these diagnostic plots and reasons for excluding data points from general linear models, see Kim (2015) and Prabhakaran (2016).
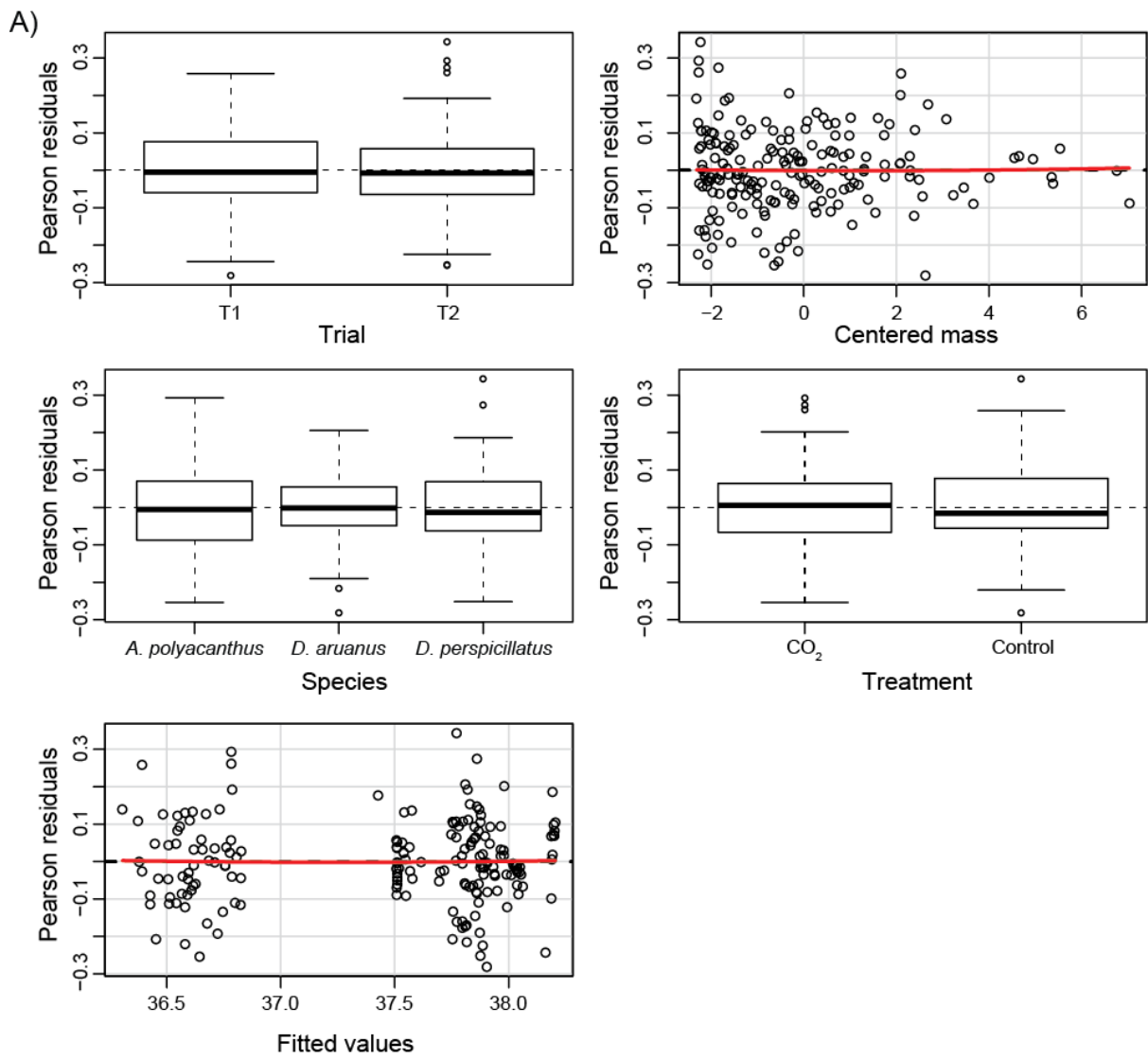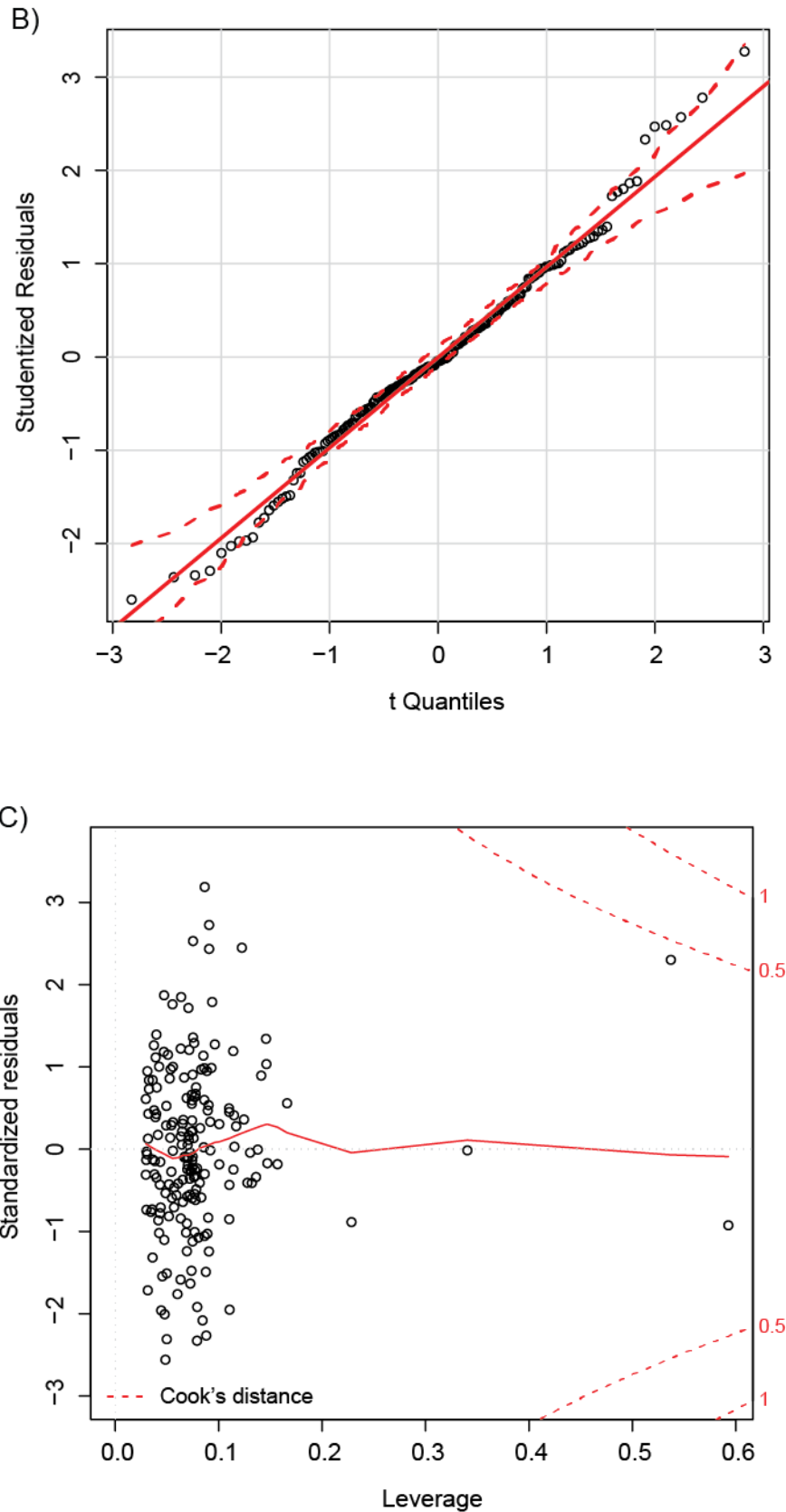
A)

Fig. S2A

Figs. S2B, S2C

Fig. S2 Diagnostic plots showing the distribution of residuals from the model $CT_{max} \sim$ (trial + mass + species + treatment)^2 to assess (A) homoscedasticity (i.e., homogeneity of variance), (B) normality (via a quantile-quantile plot), and (C) leverage (i.e., the influence of values on model output). *Five outliers were excluded from the model* (identified as dotted green circles in Figs. 2, 3 and S3). T1 and T2 represent the two separate trials.
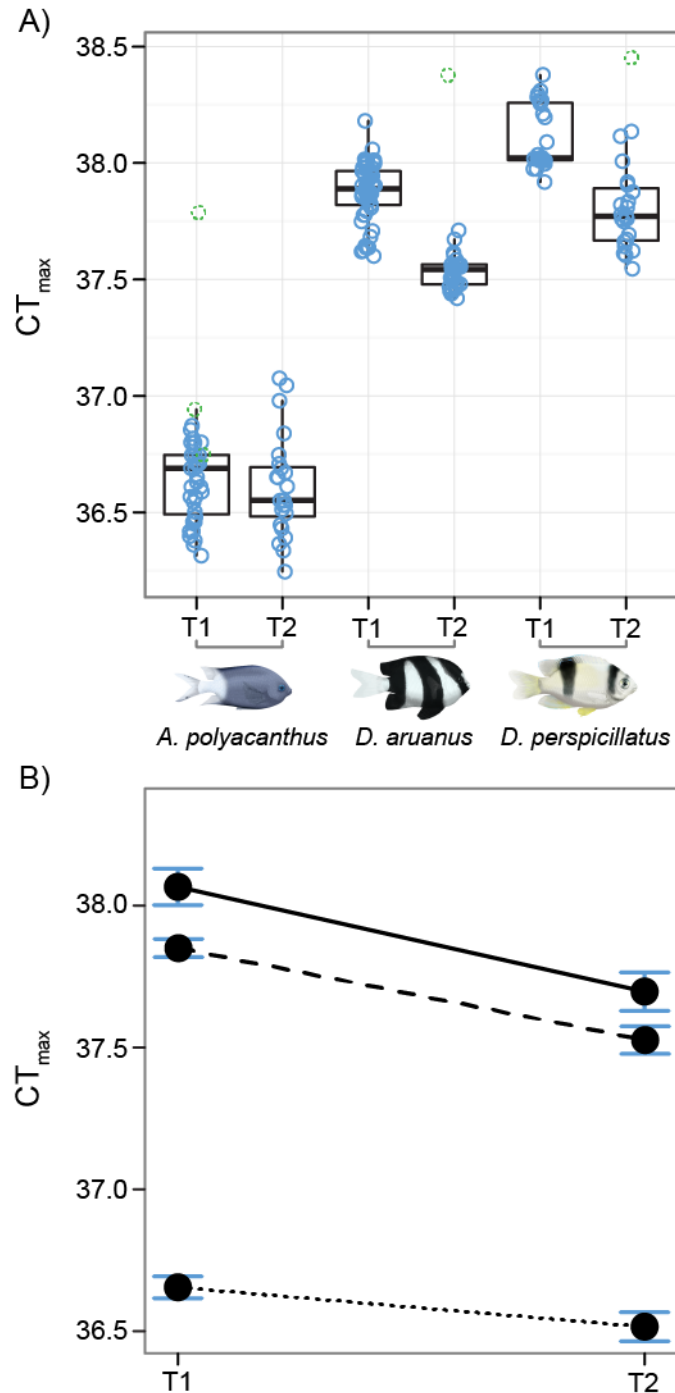
Fig. S3 CT_{max} of three damselfish species across two trials (T1 and T2). (A) Box-and-whisker plots showing the distribution of the raw data with five outliers (excluded from the model) indicated with dotted green circles. (B) Model predictions for *A. polyacanthus* (dotted line), *D. aruanus* (dashed line) and *D. perspicillatus* (solid line). Error bars are 95% confidence intervals.

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