Article

Ocean acidification does not impair the behaviour of coral reef fishes

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The partial pressure of CO_2 in the oceans has increased rapidly over the past century, driving ocean acidification and raising concern for the stability of marine ecosystems[1-3]. Coral reef fishes are predicted to be especially susceptible to end-of-century ocean acidification on the basis of several high-profile papers[4,5] that have reported profound behavioural and sensory impairments—for example, complete attraction to the chemical cues of predators under conditions of ocean acidification. Here, we comprehensively and transparently show that—in contrast to previous studies—end-of-century ocean acidification levels have negligible effects on important behaviours of coral reef fishes, such as the avoidance of chemical cues from predators, fish activity levels and behavioural lateralization (left–right turning preference). Using data simulations, we additionally show that the large effect sizes and small within-group variances that have been reported in several previous studies are highly improbable. Together, our findings indicate that the reported effects of ocean acidification on the behaviour of coral reef fishes are not reproducible, suggesting that behavioural perturbations will not be a major consequence for coral reef fishes in high CO_2 oceans.

The partial pressure of CO_2 in the oceans has increased from average pre-industrial levels of around 280 µatm to present-day levels of approximately 410 µatm, driving a process known as ocean acidification. End-of-century levels of CO_2 in the oceans are expected to reach 900–1,000 µatm, exceeding what most marine species have experienced in the past 30 million years[1,2], and raising concerns over biodiversity loss and the stability of marine ecosystems[3].

Fishes have well-developed acid–base regulatory systems to maintain tissue pH, even when faced with partial pressure levels of CO_2 (p_{CO_2}) that exceed the end-of-century forecasts by 15 times (that is, 15,000 µatm)[6]. Therefore, physiologists have historically considered fishes to be robust to near-future CO_2 levels[7,8]. Notably, a number of highly publicized studies have reported detrimental effects of elevated CO_2 levels on the sensory systems and behaviours of fishes[4,9], with coral reef fishes appearing to be the most sensitive despite experiencing large daily and seasonal CO_2 fluctuations in nature (for example, 100–1,300 µatm)[7,10]. Indeed, CO_2 levels of around 1,000 µatm appear to alter or impair all of the sensory systems and associated behaviours of coral reef fishes studied to date[7,11]. Reported effects across a range of life stages include alterations in olfaction, hearing, vision, learning, behavioural lateralization, activity levels, boldness, anxiety and susceptibility to predation[11]. This literature has contributed to dire predictions for fish populations and marine ecosystems that are at risk of ocean acidification[12,13].

Although the reported effects of ocean acidification on the sensory systems and behaviours of fishes are considerable, there are substantial disparities among studies and species, even when methodological approaches are similar[14,15]. This discrepancy is surprising given that many of the most prominent studies that describe detrimental effects of ocean acidification on fish behaviour report exceptionally low variability and large effect sizes[4,5,9,16,17], which should maximize the probability of successful replication[18]. Moreover, the proposed mechanism that underlies the sensory impairments (interference with the function of the neurotransmitter $GABA_A$ (γ-aminobutyric acid) in the brain[17]) is reported to transcend animal phyla[11] and therefore should apply to all species of fish.

In response to the 'reproducibility crisis' that affects many scientific disciplines[19], the scientific community is demanding that studies are rigorously conducted and independently replicated before drawing broad conclusions and implementing management measures, particularly when describing widespread phenomena of global importance[20]. Establishing a robust and independently replicated database of the effects of ocean acidification on fishes is essential to gain a reliable understanding of the consequences of climate change on marine ecosystems[21].

To this end, we commenced a three-year research program in 2014 to quantify the effects of end-of-century ocean acidification on the sensory and behavioural ecology of coral reef fishes. Our objectives were to replicate and build on some of the most prominent studies in

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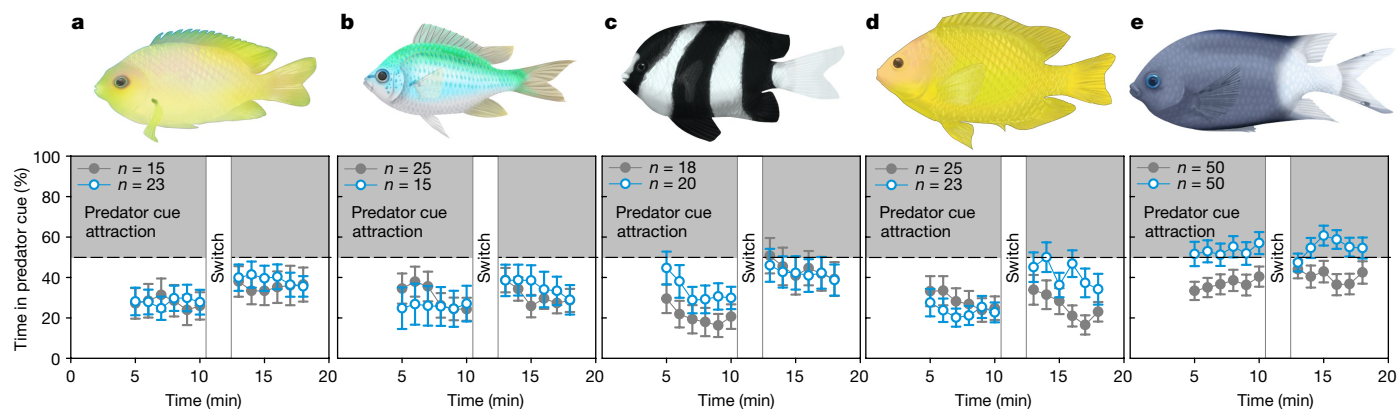
Fig. 1 | Widespread avoidance of predator chemical cues in coral reef damselfishes exposed to present-day and end-of-century levels of CO_2. **a**, *P. amboinensis*. **b**, *C. atripectoralis*. **c**, *D. aruanus*. **d**, *P. moluccensis*. **e**, *A. polyacanthus*. **a**–**e**, Percentage of time (mean ± s.e.m.) that fishes spent in water containing chemical cues of a predator (*C. cyanostigma* (**a**–**d**) or *C. urodeta* (**e**)) during two-current choice flume tests at the Lizard Island Research Station in 2014 (**a**–**d**, sub-adults and adults) and at the Australian Institute of Marine Science in 2015 (**e**, juveniles). Control fish (maintained in water containing around 410 μatm CO_2) in closed grey circles, CO_2-exposed fish (maintained in water containing around 1,000 μatm CO_2) in open blue circles (*n* of biologically independent animals are shown in the figure panels). Data were excluded between 10 min and 13 min for the predator cue switch. See Extended Data Table 2 for statistics. Fish illustrations by E. Walsh and S. Rowan.

this field to understand the diversity in behavioural responses within and across species. Notably, we aimed to enhance transparency and reduce methodological biases[22] by ensuring that our methods were fully documented and reproducible, and that raw data and videos of behavioural trials were publicly available and open to external review[23,24].

Responses to chemical cues from predators

In fishes, the reversal of chemical cue preferences is one of the most alarming effects of elevated CO_2 reported to date. Initial studies on this phenomenon used choice flumes and reported that larval clownfish (*Amphiprion percula*) and damselfish (*Pomacentrus wardi*) exposed to elevated CO_2 (850–1,050 μatm for 3–11 days) chose to spend a remarkable 90–100% of their time in water containing the chemical cues of predators (*Cephalopholis cyanostigma* or *Pseudochromis fuscus*) instead of avoiding these cues like conspecifics that were maintained at present-day CO_2 levels (0–10% of time in predator cues)[4,5]. These reports concluded that prey species will be attracted to their predators in a high CO_2 world. Many reports of cue preference reversal in coral reef fishes have since been published, including for fishes obtained from natural CO_2 seeps[16] and those experiencing transgenerational acclimation to elevated CO_2 under laboratory conditions[25].

Our experiments used established protocols in choice flume methodology (see Methods), including video footage of experiments (with pre-trial notes indicating the treatment history of each fish; see https://youtu.be/iH0w7Wqztjo) and the use of automated tracking software. We quantified the effects of elevated CO_2 on predator cue avoidance across 3 consecutive years in 560 individuals from 6 species of pomacentrid coral reef fishes (*Acanthochromis polyacanthus*, *Chromis atripectoralis*, *Dascyllus aruanus*, *Dischistodus perspicillatus*, *Pomacentrus amboinensis* and *Pomacentrus moluccensis*). Experiments covered a range of temperatures (Extended Data Table 1), CO_2 acclimation protocols were kept consistent with previous studies (4 or more days at around 1,000 μatm)[4,5,17] and four of our study species (*A. polyacanthus*, *D. aruanus*, *P. amboinensis* and *P. moluccensis*) have previously been reported to exhibit severe behavioural impairments following exposure to high CO_2 levels[16,25,26].

All four species of adult and sub-adult wild fishes tested in 2014 (*C. atripectoralis*, *D. aruanus*, *P. amboinensis* and *P. moluccensis*) significantly avoided the predator cue (*C. cyanostigma*) in both control and high CO_2 groups (Fig. 1a–d and Extended Data Table 2; pooled across all species, *n* = 164, all *P* > 0.21). The following year (2015), we detected a CO_2 treatment effect for *A. polyacanthus* juveniles reared in captivity (Extended Data Table 2; *n* = 100, *P* < 0.001): control fish spent 39 ± 2% (model estimate ± s.e.) of their time in the predator cue (*Cephalopholis urodeta*) whereas fish acclimated to high CO_2 levels spent 54 ± 3% of their time in the predator cue (Fig. 1e). This CO_2 treatment effect was not replicated in wild *A. polyacanthus* of any life stage in 2016 (Fig. 2a, b and Extended Data Table 2; *n* = 94, *P* = 0.86), nor were there any treatment effects for any of the life stages of *D. aruanus* (*n* = 83, *P* = 0.09) or *D. perspicillatus* (*n* = 119, *P* = 0.30) tested in that same year (Fig. 2c–e and Extended Data Table 2).

Overall, we detected a modest CO_2 treatment effect (no avoidance of predator cue) in one of six species in one of the two years in which that species was examined. These findings demonstrate that none of the coral reef fishes that we examined exhibited attraction to predator cues when acclimated to high CO_2 levels, in contrast to previous reports on the same and other species[4,5,16,27].

To investigate the marked disparity between our findings and previous reports for coral reef fishes, we took subsets of our choice flume data (*n* = 247 control, *n* = 239 high CO_2; 4 min per trial) to replicate the 4-min analysis approaches used previously (that is, around 9-min trials, using 2 min of data before and after the cue switch)[4,5,16,17,25,27]. We then used bootstrapping simulations to compare our data with previous datasets (Supplementary Information). On the basis of 10,000 bootstrap samples per scenario, we demonstrate using our large dataset that the results reported previously for coral reef fishes are highly improbable (probability of 0 out of 10,000): our frequency histograms of bootstrapping outputs show no evidence of CO_2 effects on chemical cue avoidance (Fig. 3a–c), and the within-group variance reported in previous studies is typically lower than what is statistically realistic (Fig. 3d–f).

Activity levels

Coral reef fishes exposed to end-of-century CO_2 levels have been stated to exhibit up to 90-fold higher levels of activity[27], prompting suggestions that these changes could underlie the higher mortality rates reported for fish that have been briefly exposed to high CO_2 and then placed onto patch reefs in the wild under present-day CO_2 conditions[5]. Notably, most activity measurements (for example, distances moved) from coral reef fishes have not used video footage but have been made
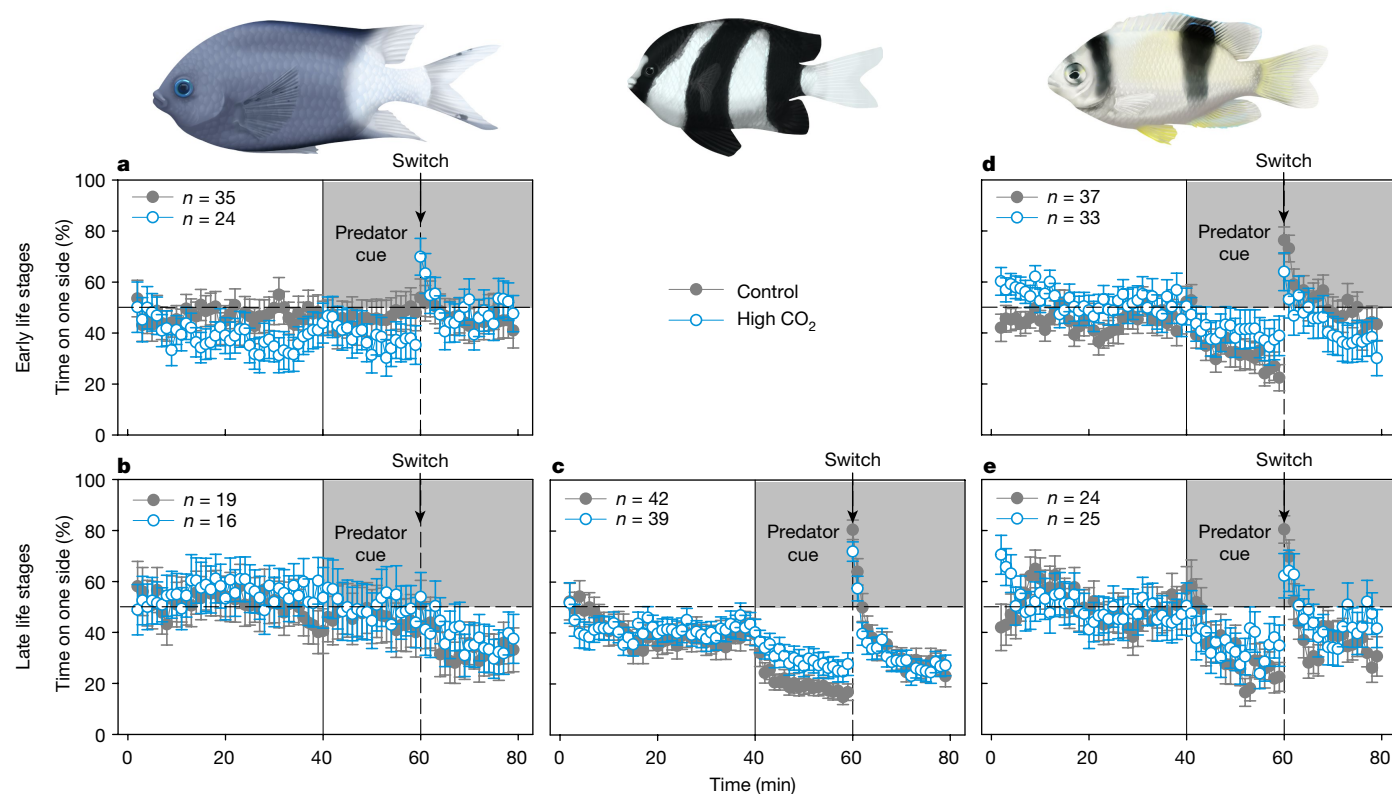
Fig. 2 | Damselfishes avoid predator chemical cues to the same degree when exposed to present-day or end-of-century CO_2 levels irrespective of life stage. a, b, *A. polyacanthus*. **c**, *D. aruanus*. **d, e**, *D. perspicillatus*. **a–e**, Percentage of time (mean ± s.e.m.) that fishes from early life stages and later life stages (including mid and late life stages) spent on one side of a two-current choice flume during experiments at the Lizard Island Research Station in 2016. Control fish (maintained at approximately 520 μatm) in closed grey circles, CO_2-exposed fish (maintained at around 1,090 μatm) in open blue circles. Fish were given 40 min to habituate to the flume (during which time their activity was quantified; see Fig. 4). Predator chemical cues (*C. cyanostigma*) were introduced to one side of the flume for 20 min and then switched to the other side for another 20 min. *n* of biologically independent animals are shown in the figure panels. See Extended Data Table 2 for statistics. Fish illustrations by E. Walsh.

using direct manual observations, either by SCUBA divers or by an observer counting the number of gridlines crossed by fish in aquaria[5,26].

We filmed 582 individuals from 6 species across 3 years and quantified swimming activity in behavioural arenas using automated tracking software. Activity levels were assessed in adults and sub-adults of 5 species in 2014, with 3 species showing no detectable effects of CO_2 treatment (*C. atripectoralis*, *P. amboinensis* and *P. moluccensis*; Fig. 4c–e and Extended Data Table 3; pooled across all species, *n* = 126, *P* > 0.08). We found some evidence that activity was affected by high CO_2 in *D. aruanus*, for which an interaction between CO_2 treatment and standard length suggested that activity was elevated by approximately 59–92% in smaller individuals (<37 mm standard length) in high CO_2 levels (Fig. 4b, Extended Data Fig. 1a and Extended Data Table 3; *n* = 46, *P* = 0.03). In *A. polyacanthus*, activity levels were increased by around 50% (*P* = 0.009) in fish acclimated to elevated CO_2 levels after controlling for a strong main effect of standard length (Fig. 4a, Extended Data Fig. 1b and Extended Data Table 3; *n* = 16, *P* < 0.001).

When we extended our experiments in 2015 using captive-reared juvenile *A. polyacanthus* with greater sample sizes and longer-duration trials (Supplementary Information), the effect of CO_2 on activity disappeared (Extended Data Table 3; *n* = 66, *P* = 0.1). There was, however, a weak interaction (*P* = 0.04); activity decreased in the high CO_2 fish (but not controls) with increasing body size (Fig. 4a, Extended Data Fig. 1c and Extended Data Table 3). In 2016, we conducted additional tests of activity in wild fish across various life stages and found no effects of CO_2 nor any interactions with body size in any of the three species (*n* = 122 *D. perspicillatus*, *n* = 112 *A. polyacanthus*, *n* = 94 *D. aruanus*; all CO_2 main effects *P* > 0.24; Fig. 4 and Extended Data Table 3).

Overall, we found that fish exposed to high CO_2 did not exhibit consistently elevated activity levels compared with conspecifics under control conditions (Fig. 4). Rather, we found that activity levels were highly variable among individuals, increasing the risk of type-I errors in experiments using small sample sizes[18], and possibly in large-sample experiments that rely on human observation rather than automated video analysis[22–24].

Behavioural lateralization

A tendency to favour the left or right side during behavioural activities (that is, behavioural lateralization) is thought to be an expression of functional asymmetries of the brain; this is important for tasks such as schooling and predator avoidance[28]. Elevated CO_2 has been reported to reduce or abolish behavioural lateralization in fishes[17,25], presumably as a result of brain dysfunction[17]. Population-level lateralization is present when a group of individuals collectively exhibits a side bias (the mean number of turns to one side significantly exceeds 50%), whereas individual-level lateralization is present when more individuals within a tested group exhibit a side bias than expected by chance (based on a binomial distribution with $\alpha = 0.5$). Both types of lateralization are independent of each other, but are not mutually exclusive (see Methods and Supplementary Information for details).

Using a standard detour test in a double T-maze, we quantified the effects of elevated CO_2 levels on behavioural lateralization using 175 fishes across four species in 2014 (*C. atripectoralis*, *D. aruanus*, *P. amboinensis* and *P. moluccensis*). None of the species exhibited population-level lateralization under control conditions (Extended
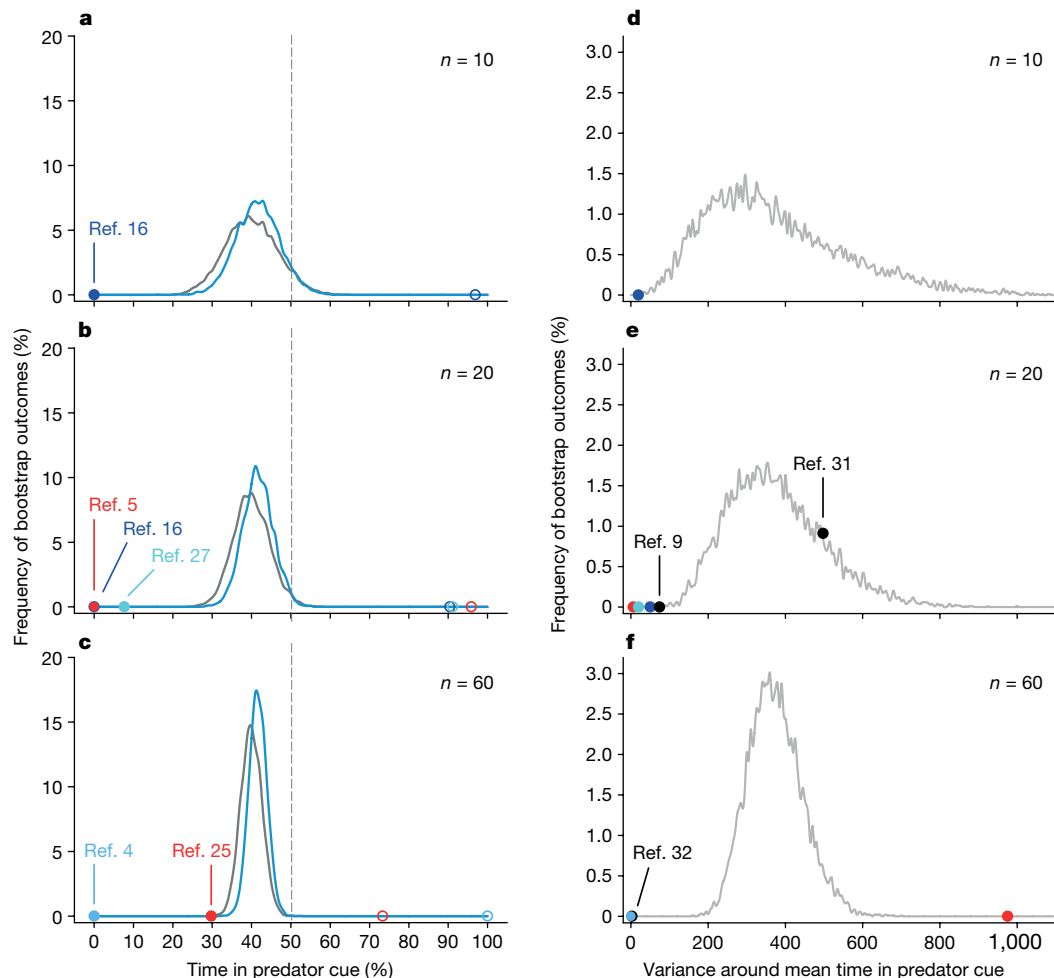
Fig. 3 | Bootstrapping data simulations of predator chemical cue avoidance and within-group variance. Bootstrapping data simulations reveal that fish avoid predator chemical cues regardless of whether they are acclimated to present-day or end-of-century CO_2 levels (**a**–**c**), and the within-group variance in many previous studies is lower than statistically reasonable (**d**–**f**). **a**–**c**, Frequency outputs from bootstrapping simulations of the mean percentage of time spent in water containing predator cues when $n = 10$ (**a**), $n = 20$ (**b**) or $n = 60$ (**c**) fish were sampled from each of the control (grey) and high CO_2 (blue) treatment groups (total n in sampled dataset: 247 control, 239 high CO_2; sample sizes represent biologically independent animals). The frequency distributions fall to the left of 50% (dashed vertical line) in both treatment groups, indicating similar avoidance of predator chemical cues under control and high CO_2 conditions. This is markedly different from previous reports of major effects of high CO_2 levels on predator and alarm cue avoidance in coral reef fishes (examples presented in coloured circles, selected

to match the group sample sizes presented in figure panels: closed circles, control; open circles, high CO_2). **d**–**f**, Frequency histograms (light grey) of the associated variance around the means from bootstrapping simulations presented in **a**–**c** (control and high CO_2 fish pooled for simplicity). Also presented are results of previous studies of coral reef fish (variance around the group mean, where similar groups were combined for simplicity) that have used choice flumes to examine chemical cue preferences. **a**, **d**, Dark-blue circle, data from ref. [16]. **b**, **e**, Dark-blue circle, data from ref. [16] (this circle overlaps with the red circle in **b**); red circle, data from ref. [5]; light-blue circle, data from ref. [27]. **c**, **f**, Blue circle, data from ref. [4]; red circle, data from ref. [25]. **e**, **f**, Black circles indicate references that did not examine the effects of high CO_2 and/or predator or alarm cue avoidance and thus do not appear in **a**–**c**. **e**, Left black circle, data from ref. [9]; right black circle, data from ref. [31]. **f**, Black circle, data from ref. [32]. For additional details, see Supplementary Information.

Data Fig. 2a–d and Extended Data Table 4), and only *C. atripectoralis* showed slight population-level lateralization under high CO_2 ($P = 0.047$; Extended Data Table 4). Three species (*C. atripectoralis*, *D. aruanus* and *P. moluccensis*) exhibited no individual-level lateralization under control conditions, which remained unchanged under high CO_2 conditions (Extended Data Fig. 2a–c and Extended Data Table 4). A treatment effect was detected for individual-level lateralization in *P. amboinensis*, with the high CO_2 group displaying reduced individual-level lateralization compared with controls (Extended Data Fig. 2d and Extended Data Table 4). However, this effect was no longer present when a subset of the same individuals was retested 7–8 days later ($n = 15$ control, $n = 15$ high CO_2; Extended Data Fig. 2e and Extended Data Table 4). Although our sample sizes were comparable to many similar studies (for example, ref. [17]), our inconsistent findings for *P. amboinensis* are likely to be a

consequence of low statistical power in a behavioural test that exhibits high inter-individual variability[18] (Extended Data Fig. 2).

We increased statistical power in 2015 when behavioural lateralization was tested in wild and captive-reared *A. polyacanthus* ($n = 120$ control, $n = 104$ high CO_2), a species for which impairments in lateralization caused by high CO_2 levels have been reported[25]. In contrast to previously reported results, we found no effect of CO_2 levels on behavioural lateralization: *A. polyacanthus* exhibited individual-level lateralization and no population-level lateralization, both under control and high CO_2 conditions (Extended Data Fig. 2f and Extended Data Table 5). On the basis of the previous studies that have reported that elevated CO_2 levels impair visual acuity[26,29], we slightly offset the barrier at one end of the lateralization arena, creating a shorter path around the barrier to the left. We predicted that fish under high CO_2 levels would not visually
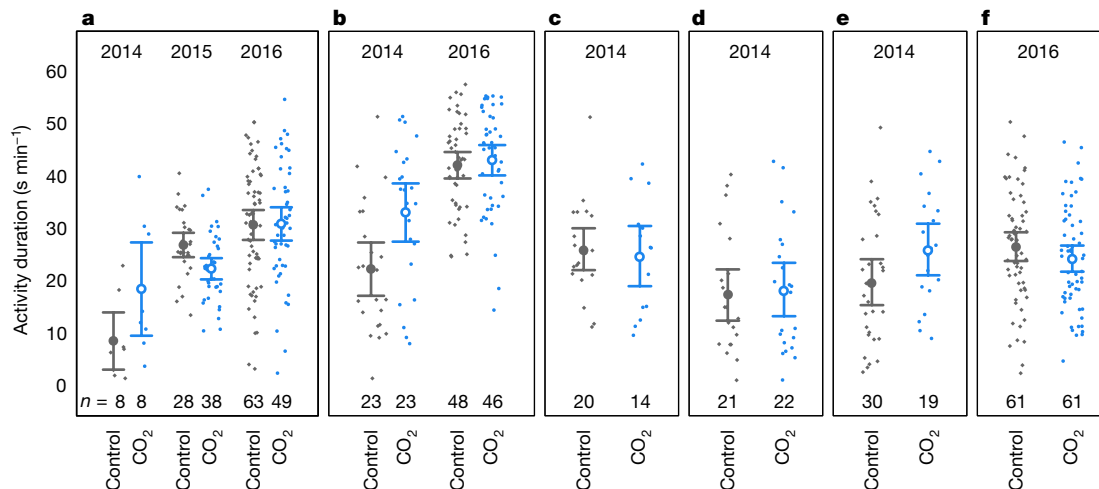
Fig. 4 | Widespread similarities in the activity levels of six species of coral reef damselfish regardless of whether acclimated to present-day or end-of-century levels of CO_2. a–f, Activity levels (s min^{-1}) after acclimation to control (around 450 µatm; closed grey circles) or end-of-century (about 1,000 µatm; open blue circles) levels of CO_2. Mean values for individual animals are shown (small symbols). The large symbols and error bars represent the mean ± 95% confidence intervals for each group. Data for *A. polyacanthus* (**a**) and *D. aruanus* (**b**) were collected across multiple years (indicated at the top of each panel), whereas data for *C. atripectoralis* (**c**), *P. amboinensis* (**d**) and *P. moluccensis* (**e**) were collected in 2014 and data for *D. perspicillatus* (**f**) were collected in 2016. *n* numbers along the bottom of the figure panels represent biologically independent animals. Note that there were some statistically significant (two-tailed tests), context-dependent effects of CO_2 treatment for *A. polyacanthus* and *D. aruanus*, including interactions with body size (see Extended Data Fig. 1; statistics are included in Extended Data Table 3).

detect the shortcut as strongly as control fish. By contrast, we found that fish from both treatment groups exhibited a preference for the shorter path (Extended Data Fig. 2g and Extended Data Table 5).

Conclusions and implications

Here we present a multi-species, multi-year and multi-life-stage examination of the sensory and behavioural impairments that have been reported for coral reef fishes under end-of-century levels of CO_2, thus answering an international call for comprehensive replication studies on issues of global importance[21]. Notably, we took great care to enhance transparency by systematically documenting our experiments and providing raw data and analysis code. In contrast to previous studies on the same and closely related species, we found no consistent detrimental effects of end-of-century CO_2 levels on the avoidance of predator chemical cues, activity levels or behavioural lateralization. Although CO_2 emissions are an environmental threat[3,30], the catastrophic projections for fish sustainability based on CO_2-induced behavioural impairments[12,13] must be reassessed in light of our findings.

We went to great lengths to match the species, life stages, location and season of previous studies, yet the discrepancies in findings were considerable. This was most apparent for the responses of fish to predator chemical cues, for which previous studies have reported extreme effect sizes (in which control fish spent <10% of their time in predator cues compared with >90% of time for fish under high CO_2; Fig. 3a–c) with exceedingly low variability around the group means (Fig. 3d–f). The research community in the field of ocean acidification and coral reef fish behaviour has remained small, and the study systems are often remote and expensive to access, both of which have precluded independent assessments of previous findings. Small sample sizes[18] and other methodological or analytical weaknesses[22] in previous studies could potentially explain the discrepancies between our results and the majority of articles that have reported minor impacts (small effect sizes) of CO_2 on fish behaviour. However, we cannot reconcile our findings with those that show extremely large effect sizes and small within-group variance in experiments with large sample sizes (Fig. 3). Inter-individual variation enables the persistence of populations and species and is a fundamental biological phenomenon on which selection acts; results

showing negligible variation (particularly for behaviours that are inherently variable) should be viewed with caution (see Supplementary Information).

On the basis of our findings on more than 900 wild and captive-reared individuals of 6 species across 3 years, we conclude that acclimation to end-of-century levels of CO_2 does not meaningfully alter important behaviours of coral reef fishes. Reasonably large sample sizes and consistent results across species, locations, life stages and years suggest that the probability of false-negative results (type-II errors) in our study is low. Given the importance of these issues to the management of coral reefs and other aquatic ecosystems[12,13], we encourage further replication of previous studies using the transparent and objective approaches described here (for example, video footage with pre-trial notes, complete data and code archiving)[22,23]. Only then will the research community be equipped to reach a consensus on whether end-of-century ocean acidification could have direct effects on the behaviour of fishes. Nonetheless, it should be firmly emphasized that there is strong evidence that increasing atmospheric CO_2 is causing ocean warming, which can profoundly affect marine fishes[30].

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41586-019-1903-y.

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Methods

Experiments were conducted across 3 years (2014–2016), at 2 locations in Australia (the Lizard Island Research Station (LIRS) and the Australian Institute of Marine Science (AIMS) in Townsville), and on a total of more than 900 individuals from 6 species across an ontogenetic range. The experimental designs and CO_2 dosing systems (described below) followed best practices for ocean acidification research[33–35]. For all experiments, fish were given at least 4 days to acclimate to the CO_2 treatment before trials commenced. Although an acclimation period of 4 days is short, this duration was chosen because it has been reported to be sufficient to maximize behavioural and/or sensory impairments in fishes[5,27]. Fish were placed in the two treatment groups at random. Other aspects of water chemistry (that is, the water supply used and the temperature it was kept at), lighting and feeding were kept constant among replicate tanks across the two acclimation treatments. Juvenile fish were mostly used in the experiments but when adult fish were used, we did not determine their sex in order to minimize handling. The sample sizes used in each experiment were based on previous studies and fish availability. Complete blinding regarding CO_2 treatment was not possible as the CO_2 dosing system was visible (both visually and auditory) to any observer physically present during the experiments. However, all activity and predator cue avoidance experiments were recorded on video and analysed using automated tracking software. Lateralization experiments could not be tracked using automated tracking software but were scored in real-time. A detailed description of the methods is included in the Supplementary Information. All experiments were conducted in compliance with relevant ethical regulations under approval from the James Cook University Animal Ethics Committee in association with the AIMS (permit A1924).

Animals and holding conditions

LIRS August 2014. Sub-adult and adult wild fishes (humbug dascyllus (*D. aruanus*), $n = 46$; Ambon damsel (*P. amboinensis*), $n = 43$; lemon damsel (*P. moluccensis*), $n = 49$; black-axil chromis (*C. atripectoralis*), $n = 43$; and spiny chromis (*A. polyacanthus*), $n = 16$) were collected from around Lizard Island at the northern end of the Great Barrier Reef, Australia (14° 40′ S, 145° 28′ E), by SCUBA divers using hand and/or barrier nets and spray bottles of clove oil anaesthetic (mixed 1:4 with ethanol). To produce predator chemical cues, predatory blue-spotted rock cods (*C. cyanostigma*; $n = 24$) were collected using hook and line. All fishes were transported in aerated seawater to LIRS, where they were placed in tanks with flow-through seawater (35 PSU) at ambient temperature (Extended Data Table 1). The damselfishes were divided in approximately even numbers between eight identical tanks (25 l each; 3 l min⁻¹ flow-through). *C. cyanostigma* were divided in even numbers between two identical tanks (200 l each; 12 l min⁻¹ flow-through) and fed pieces of sardine (*Sardinops sagax*) every 2–3 days.

After 1–2 days in captivity, the CO_2 of half of the tanks (including one of the *C. cyanostigma* tanks) was gradually increased to 945 ± 117 µatm (mean ± s.d.) (pH$_{total}$ of around 7.72, calculated using previously published constants[36,37]; Extended Data Table 1) over 24 h using a CO_2 dosing system (pH stat Computers, Aqua Medic) connected to solenoid valves that regulate the administration of 100% CO_2 gas (as previously described[38]). Although 24 h may seem a short duration over which to increase CO_2 to end-of-century levels, fish have a well-developed physiological capacity to endure much larger and/or quicker changes in p_{CO_2} levels[6,39]. In addition, some previous studies have reported that fish were simply transferred to end-of-century p_{CO_2} treatments rather than using a gradual change[27,40,41] and others did not report how fish were transferred to high p_{CO_2} levels[4,5,17]. The other half of the tanks remained at ambient CO_2 levels of 406 ± 21 µatm (pH$_{total}$ of approximately 8.04; Extended Data Table 1). Levels of CO_2 in each tank were checked twice daily using a handheld CO_2 meter (GMT 222, Vaisala) connected to an aspiration pump (Vaisala) and a submerged gas-permeable PFTE probe

(Qubit Systems) as described previously[42]. The CO_2 meter was factory-calibrated by Vaisala before experiments. Water samples (60-ml samples of water with 30 µl of mercury chloride to poison any microorganisms) were taken at 10 different points throughout the experiment for subsequent measurements of total alkalinity (Extended Data Table 1). Fish were fed to satiation 1–2 times per day with a commercial pellet food, but food was withheld for around 12 h before experiments. Tanks were cleaned every 3–4 days. Individual fish were reused for each of the three response variables that we measured (activity, behavioural lateralization and predator chemical cue avoidance) in a randomized order. At the end of the experiments, fish were released at their site of capture.

AIMS May and June 2015. Juvenile spiny chromis (*A. polyacanthus*) (age, 3–14 days after hatching, 0.019 ± 0.015 g (mean ± s.d.) initial wet weight, 9.1 ± 2.3 mm initial standard length) were obtained from the Reef HQ aquarium in Townsville, Australia (total $n = 1,494$). In addition, groups of wild *A. polyacanthus* juveniles (10–15 days after hatching) from four distinct schools (four breeding pairs) were corralled into clear containers by SCUBA divers at depths of 8–10 m at Davies Reef (18.8238° S, 147.6429° E) in April 2015 ($n = 481$ collected). Fish were transported in aerated seawater to AIMS, where they were placed in 25-l tanks with seawater recirculating (around 3.5 l min⁻¹) to one of four independent 200-l sumps, which themselves were continuously flushed with fresh seawater (4–7 l min⁻¹). Subsets of fish from Reef HQ were used for assessments of predator cue avoidance, activity levels and behavioural lateralization, whereas wild fish were only used in behavioural lateralization experiments. Four wild predatory fish (flagtail grouper (*C. urodeta*)) were freighted to AIMS and split evenly between two tanks after being caught from the northern Great Barrier Reef by Cairns Marine. The effluent water from the grouper tanks went straight to the drains to ensure that the *A. polyacanthus* did not habituate to predator chemical cues. *C. urodeta* were fed freshly killed juvenile *A. polyacanthus* every 1–2 days as previously described[43].

After at least 24 h to recover from transport, the CO_2 of half of the *A. polyacanthus* tanks ($n = 10$) and one of the C. urodeta tanks was gradually increased to $1,021 \pm 156$ µatm (mean ± s.d.) (pH$_{total}$ of around 7.70; Extended Data Table 1) over 24 h using a CO_2 dosing system (pH stat Computers, Aqua Medic) connected to solenoid valves that regulate the administration of 100% CO_2 gas into two of the partial-recirculation sump systems. The remaining tanks ($n = 10$ for *A. polyacanthus* and $n = 1$ for *C. urodeta*) were kept at ambient CO_2 levels (428 ± 13 µatm, pH$_{total}$ of around 8.03; Extended Data Table 1). Three large air stones in each sump ensured that the water remained well mixed and maintained dissolved oxygen at >90% air saturation. The CO_2 levels of the holding tanks were checked every 1–4 days using a LI-820 CO_2 Gas Analyzer (LI-COR). Fish were exposed to natural water temperatures for the region (quantified using thermal data-loggers sampling every 30 min; iButton, Maxim Integrated). Temperature decreased seasonally from 26.1 ± 0.2 °C during the first week of acclimation (May 2015) to 24.8 ± 0.5 °C during the final week of experiments (June 2015; Extended Data Table 1). Salinity was regulated through the AIMS SeaSim aquarium system (35.8 ± 0.15 PSU). Water samples for alkalinity were taken as described above for LIRS 2014 (five samples per treatment, Extended Data Table 1). Fish were fed ad libitum 1–2 times per day using commercial aquaculture pellets crushed to a powder and/or *Artemia* spp. nauplii, but food was withheld for 12–18 h before experiments. Tanks were cleaned weekly. Individual fish were used once; that is, for one of the three response variables that we measured (activity, behavioural lateralization or predator chemical cue avoidance). All fish used at AIMS in 2015 were euthanized with an overdose of tricaine methanesulfonate (MS-222, around 500 mg l⁻¹) at the end of the experiments, or at intermittent times during the experiments when they were euthanized to take precise length and weight measurements for another study[15].

LIRS January 2016. Wild fishes were collected from around Lizard Island, as described above for LIRS 2014. Adult predatory *C. cyanostigma*

($n = 15$) were caught using hook and line, and three damselfish species were caught using clove oil spray and hand or barrier nets (sub-adult and adult *D. aruanus* ($n = 96$); juvenile, sub-adult and adult *A. polyacanthus* ($n = 112$); sub-adult and adult white damsel (*D. perspicillatus*; $n = 50$)). Note that *A. polyacanthus* does not have a pelagic larval phase (see Supplementary Information). In addition, larval *D. perspicillatus* ($n = 72$) were caught near the end of their pelagic phase using established light-trapping techniques[44]. Fishes were placed in tanks with flow-through seawater at ambient temperature (Extended Data Table 1). The damselfishes were divided in approximately even numbers between 22 identical tanks that each received constant flow-through (one species per tank, 7–8 tanks per species; 10–25 l each and 1–3 l min^{-1} flow-through, depending on fish size). *C. cyanostigma* were divided in even numbers between four identical flow-through tanks (60 l each; 3 l min^{-1} flow-through) and fed sardine pieces and freshly killed adult damselfish every 2–3 days. All tanks were provided with pieces of PVC piping to act as shelter for the fish.

After 1–2 days in captivity, the CO_2 of half of the tanks ($n = 11$ damselfish tanks, $n = 2$ *C. cyanostigma* tanks) was gradually increased to $1,089 \pm 326$ µatm (mean ± s.d.) over 24 h using a CO_2 dosing system as described above for LIRS 2014, while the other half of the tanks remained at ambient CO_2 levels of 521 ± 93 µatm (Extended Data Table 1). Levels of CO_2 in each tank were checked twice daily using the handheld Vaisala as described above for LIRS 2014. Damselfishes were fed to satiation 1–2 times per day with a commercial fish flake–saltwater slurry (TetraMin Tropical Flakes, Tetra), but food was withheld for around 12 h before experiments. Tanks were cleaned every 3–4 days. Individual fish were used once; the two measured response variables were obtained from a single, continuous behavioural trial (activity followed by predator chemical cue avoidance). At the end of the experiments, fish were released at the approximate site of capture.

Response to predator chemical cues
LIRS 2014. Four species were examined for their responses to predator chemical cues (*P. amboinensis* (standard length range, 23–53 mm), *C. atripectoralis* (standard length, 15–43 mm), *D. aruanus* (standard length, 16–63 mm) and *P. moluccensis* (standard length, 19–34 mm); sample sizes are provided in Fig. 1 and Extended Data Table 2), using a two-current choice flume. The setup for the two-current choice flume followed established protocols[45] (for details, see Supplementary Information). The fish in the high CO_2 group had been acclimated to the CO_2 treatment for 5–16 days before commencement of experiments, while control fish had been held for 4–16 days. The choice flume was a custom-built, larger version ($L \times W \times H = 580 \times 260 \times 280$ mm^3; water depth, 80 mm) of a two-current choice flume used in previous studies[46]. Detailed information on the design and function of two-current choice flumes has been described previously[45] (for details, see Supplementary Information). *C. cyanostigma* was used to create predator chemical cues (see Supplementary Information for details). All trials in the choice flume were recorded using a computer with a webcam (Logitech HD Pro C920) positioned 45 cm above the choice arena. At the beginning of a trial, a paper note detailing the treatment history of the individual fish was placed in view of the camera before the fish was placed into the centre of the choice arena within a bottomless mesh cylinder (70-mm diameter) for 1.5–2 min. This step was included to ensure that the fish had the opportunity to receive sensory input from both sides of the choice flume—one side flowing with unmanipulated water and the other side flowing with water containing the predator cue. After the settling period, the mesh cylinder was carefully lifted and the fish was allowed to select its position within the flume. After a further 8 min, the configuration of flow through each side of the flume was switched using a series of valves such that water containing the predator cue now flowed through the opposite side of the flume. The valves were positioned near the secondary header tanks and could be adjusted without visually or physically disturbing the fish. The fish was given a

further 8 min to select its position in the flume with the new flow configuration before being removed and returned to its holding tank. The video files were analysed using tracking software (ViewPoint, Zebralab) to automatically quantify time spent in the flow of water (side of the flume) containing the predator cue.

AIMS 2015. The general flume setup used at AIMS followed the design described above, with some exceptions. Two choice flumes were used side-by-side under the view of a single camera (Microsoft LifeCam HD 5000, mounted around 45 cm above the tank) recording at 10 frames per second with a resolution of 1,280 × 720 pixels. To match the smaller size of the fish (compared with the fish of the LIRS 2014 dataset), we used choice flumes with an arena that was 90 mm long × 45 mm wide with a water depth of 22 mm (4.9 mm s^{-1} water speed, around 135 ml min^{-1} per current). We initially tested flumes built to the exact specifications of those used in previous papers[4,5,9,25]. However, we were unable to produce laminar flow using this setup; both incoming streams of water mixed in the test section of the flume, meaning that the fish would not be able to make a choice between the different currents (https://youtu.be/jrtyc-rLGWc?t=705, see Supplementary Information for details).

The fish (*A. polyacanthus* (standard length, 9–11 mm) from Reef HQ aquarium) were acclimated to their respective CO_2 conditions for 6–13 days before being used in choice flume trials. The predator chemical cue avoidance trials ($n = 50$ control, $n = 50$ high CO_2) followed the same protocol as at LIRS 2014 (see above; total duration of 18 min), including the presentation of an explanatory note in front of the camera before each trial. *C. urodeta* was used to create predator chemical cues (see Supplementary Information for details).

LIRS 2016. Three species across an ontogenetic range were examined for their responses to predator chemical cues at LIRS in January 2016 (*A. polyacanthus*, *D. aruanus* and *D. perspicillatus* from early life stages (7.5–14.5 mm standard length) and later life stages (15.0–51.0 mm standard length; sample sizes listed in Fig. 2 and Extended Data Table 2). Five two-current choice flumes were used in parallel (one 610 mm × 200 mm, two 290 mm × 93 mm, and two 235 mm × 45 mm, for details see Supplementary Information). All trials in the choice flumes were recorded using a computer with webcams (Logitech HD Pro C920, FireWire camera, Dragonfly 2, Point Gray; Microsoft LifeCam HD 5000 webcam) positioned 45–130 cm above the choice arenas (depending on camera type and flume size). Trials were executed in a similar manner as at LIRS in 2014. At the commencement of a trial, a paper note detailing the treatment history of each fish was placed in view of the relevant camera before the fish was placed into the centre of the choice arena (no mesh cylinder was used) of the flume. Unlike during the predator chemical cue trials described for LIRS 2014 and AIMS 2015, the fish were given 40 min to settle in the flumes with unmanipulated water running down both sides (that is, no predator cue) before the cue was added to one side for 20 min, before switching the predator cue to the other side for the final 20 min. *C. cyanostigma* was used to create predator chemical cues (see Supplementary Information for details). The video files were analysed using tracking software (ViewPoint, Zebralab) for subsequent analyses of activity levels (defined as seconds per minute spent swimming more than 0.5 standard lengths per second) and time spent in the side of the flume containing the predator cue. An example of a full day of flume trials can be found at https://youtu.be/iH0w7Wqztjo.

Activity levels
LIRS 2014. Eight tanks (2 × 4 arrangement) were used to monitor activity in five species (Extended Data Table 3). Each tank was 220 mm × 140 mm × 140 mm ($L \times W \times H$; water depth, 105 mm) and contained 3.2 l of flow-through water (70 ml min^{-1}, using the same header tank system as described above for LIRS 2014). Each tank was equipped with a halved piece of 50-mm diameter PVC pipe standing on its end (height 50 mm), which provided a vertical structure for the fish to use as

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shelter. A video camera (Panasonic HC-V130) was positioned 1 m above the tanks to monitor fish activity at all times. At the commencement of each trial, a paper note detailing the treatment history of the fish was placed in view of the camera before introducing individual fish into each tank. The fish were then video-monitored for activity levels for 27 min. Sample sizes for 2014 swimming activity trials are included in Fig. 4 and Extended Data Table 3.

AIMS 2015. The two choice flumes described above for use at AIMS in 2015 were also used for separate assessments of the activity levels of captive-reared *A. polyacanthus* for the two acclimation treatments ($n = 28$ fish from control; $n = 38$ fish from high CO_2; fish standard length, 11.7 ± 1.6 mm (mean ± s.d.); Extended Data Table 3) in unmanipulated acclimation water (that is, no predator cue). For these trials, fish were transferred from their home tank (without air exposure) into a flume and recorded for 2 h (Microsoft LifeCam HD 5000, mounted around 45 cm above the flume).

LIRS 2016. Activity trials were conducted in the choice flumes described above for LIRS 2016; activity levels were monitored for the first 40 min of the experimental trials before releasing any chemical stimulus into either side of the flume. Five flumes were used in parallel and the flume dimensions and water velocities are described above. Additional large adult *A. polyacanthus* ($n = 9$ control, 9 high CO_2) and *D. aruanus* ($n = 6$ control, 7 high CO_2) were tested in white opaque tanks (43×32.5 cm^2, water depth, 10 cm). Sample sizes are provided in Fig. 4 and Extended Data Table 3.

Behavioural lateralization
LIRS 2014. A double-ended opaque plastic T-maze ($39 \times 29 \times 20$ cm^3, $L \times W \times H$) was constructed to perform detour tests to examine behavioural lateralization in juveniles and adults of four species (*P. amboinensis*, control $n = 21$, high CO_2 $n = 22$; *C. atripectoralis*, control $n = 26$, high CO_2 $n = 17$; *D. aruanus*, control $n = 19$, high CO_2 $n = 21$; *P. moluccensis*, control $n = 29$, high CO_2 $n = 20$). The double T-maze was a modified version of those used in experiments that have been described previously[47,48]. Individual fish were netted from their tanks and transferred immediately to the double-ended T-maze. Fish were given 1 min to settle in the central channel of the T-maze before the trial commenced. Lateralization experiments consisted of an experimenter first manoeuvring the fish to the starting point of the channel and then coaxing it down the channel with perforated plastic paddles for 10 consecutive runs. Fish had to make a decision to turn left or right each time they reached the perpendicular barrier at the end of the channel. All lateralization tests were recorded on video (using an Olympus Tough TG1 or a Panasonic Lumix DMC-FT4 camera).

AIMS 2015. A double-ended T-maze ($31 \times 11 \times 13$ cm^3, $L \times W \times H$) similar to the maze described above was constructed to perform detour tests in juvenile *A. polyacanthus*. Wild-caught fish (10–33 mm standard length; control $n = 54$; high CO_2 $n = 42$) as well as captive-reared fish from Reef HQ aquarium (8–33 mm standard length; control $n = 66$; high CO_2 $n = 62$) were used. The lateralization trials at AIMS followed the method described above for LIRS with the exception that 20 rather than 10 consecutive turns were recorded and the fish were given 2 min rather than 1 min of settling time upon entrance to the arena. In addition, the barrier at one end of the central channel was offset by 5 mm to create a situation in which the path around the barrier was shorter if the fish turned left rather than right (rationale and further details are provided in the Supplementary Information).

Statistics
General analyses. Time spent in predator cue and activity levels were quantified for each min of the behavioural trial for each fish using tracking software, which meant many repeat observations for each individual. However, three limitations prevented us from analysing the data over time. First, the effect of time was nonlinear. Second, the data were temporally auto-correlated. Third, the data were bimodal around the minimum and maximum values (see Extended Data Fig. 3 for an example) and did not conform to any distribution readily available for use in generalized additive mixed models (with the mgcv package in R). For simplicity, we took a mean across the entire trial for each fish (for choice flume and activity data; see below), which resulted in data being normally distributed and without auto-correlated repeated measurements, allowing us to use general linear models (see Supplementary Information for additional details).

Response to predator chemical cues. General linear models were used to test for the effects of CO_2 treatment (present-day versus end-of-century levels) and fish size (standard length in mm) on the percentage of time that fish spent on the side of the flume that contained the predator cue. Among the six species, there were different sample sizes, size ranges and years (or locations, for details see Supplementary Information) in which the fish were tested. Therefore, we built separate models for each species–year combination ($n = 9$ models). We used backwards model selection, beginning by including an interaction between the two fixed effects (treatment, standard length): F-tests were used to assess the significance of removal of model terms on the Akaike information criterion (AIC) (using the 'drop1' function in R). For model selection, α was set to 0.05. We acknowledge that these (two-tailed) tests were repeated on multiple species and multiple response variables, inflating the potential for type-I errors; however see a previous study[49]. Therefore, in our interpretations, although we refer to effects with $P < 0.05$ as 'significant', we emphasize the strength and size of effects, recognizing that P values have limitations[18] and represent a continuum of statistical significance. Model assumptions were assessed with q–q plots of residuals and by plotting residuals against fitted values and against each of our predictor variables[50].

Bootstrapping. Most previous studies have used more rapid assessments of cue preferences than in the present study, in which 4 min of measurements have been taken during 9–11 min trials (typically a 2-min post-handling settling period, 2 min measurement, 3 min for side switch and post-switch settling, 2 min measurement)[4,5,9,16,25,27,32]. For direct comparisons with these studies in our bootstrapping simulations (see Supplementary Information), we averaged 2 min of data after a 2-min post-handling settling period and 2 min of data 3 min after the cue side switch (2014 and 2015), or we averaged 2 min of data 2 min after the predator cue was first introduced to the choice flume and 2 min of data 3 min after the cue side switch (2016). The bootstrapping results are presented in Fig. 3, with comparisons to seven papers[4,5,9,16,25,27,32]. Note that another study[31]—which is also included in Fig. 3—is included for comparative purposes. The extremely high variance in one paper[25] (Fig. 3f) was caused by an exceedingly high proportion of control individuals reported to have spent 0% of their time in the conspecific chemical alarm cue (grey solid bars in Extended Data Fig. 4a) and an equally high proportion of high CO_2 individuals reported to have spent 100% of their time in the cue (blue solid bars in Extended Data Fig. 4b). Additionally, control and high CO_2 data were pooled to calculate the associated variance around the group means for each of the sample size scenarios (Fig. 3d–f), similar to a previously published method[51]. For additional details on the bootstrapping, see Supplementary Information.

Activity levels. Time spent active (s) was calculated on a minute-by-minute basis (to give s min^{-1}). However, data were analysed as one value (mean of the trial for each fish) per individual, using the same general linear modelling procedures outlined above for 'Response to predator chemical cues'. See Supplementary Information for further details.

Behavioural lateralization. Data collected from each location and year were analysed separately owing to the differences in time of year, species used and exposure duration. Testing for lateralization is not straightforward because it involves multiple binomial experiments with structure; a description of issues with the statistical approaches used by previous studies to assess lateralization can be found elsewhere[52]. A test for detecting lateralization at the population level requires examining the mean lateralization score across all individuals in the sample as population-level lateralization is present when a group of individuals collectively exhibits a side bias. By contrast, a test for detecting individual-level lateralization requires examining the sample variance as individual-level lateralization is present when more individuals exhibit a side bias than expected by chance (irrespective of whether it is to the left or to the right). Explanations and examples of these two concepts have been published previously[48,53,54]. We tested population-level lateralization with a generalized linear mixed model (with glmer function in R) that sets the intercept equal to the grand mean of the data[52]. We tested individual-level lateralization with a χ^2 test comparing the observed variance (numerator) to the expected variance (denominator) assuming a normal approximation to the binomial distribution[52]. This is analogous to testing for overdispersion (that is, are there more observations in the tail ends of the distribution than expected by chance). See Supplementary Information for further details.

Reporting summary

Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

Data availability

The data necessary to reproduce figures and results in this study are publicly archived in Figshare following best-practice guidelines[55], and were made available to editors and reviewers at the time of submission: https://doi.org/10.6084/m9.figshare.7871522. We place no restrictions on data availability.

Code availability

Scripts for statistical analyses are available from Figshare (https://doi.org/10.6084/m9.figshare.7871522). We place no restrictions on code availability.

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Extended Data Fig. 1 | Interactions between CO$_2$ treatment and size for fish activity. a–c, Raw data points and fitted model estimates for activity in *D. aruanus* in 2014 (**a**), *A. polyacanthus* in 2014 (**b**) and *A. polyacanthus* in 2015 (**c**) as a function of acclimation treatment (grey diamonds, control; blue circles, high CO$_2$) and size (*x* axis), with shaded areas indicating 95% confidence intervals of model estimates. Model parameter estimates are included in Extended Data Table 3. **a**, *n* = 23 per treatment. **b**, *n* = 8 per treatment. **c**, Control, *n* = 28; CO$_2$, *n* = 38. Sample sizes represent biologically independent animals.
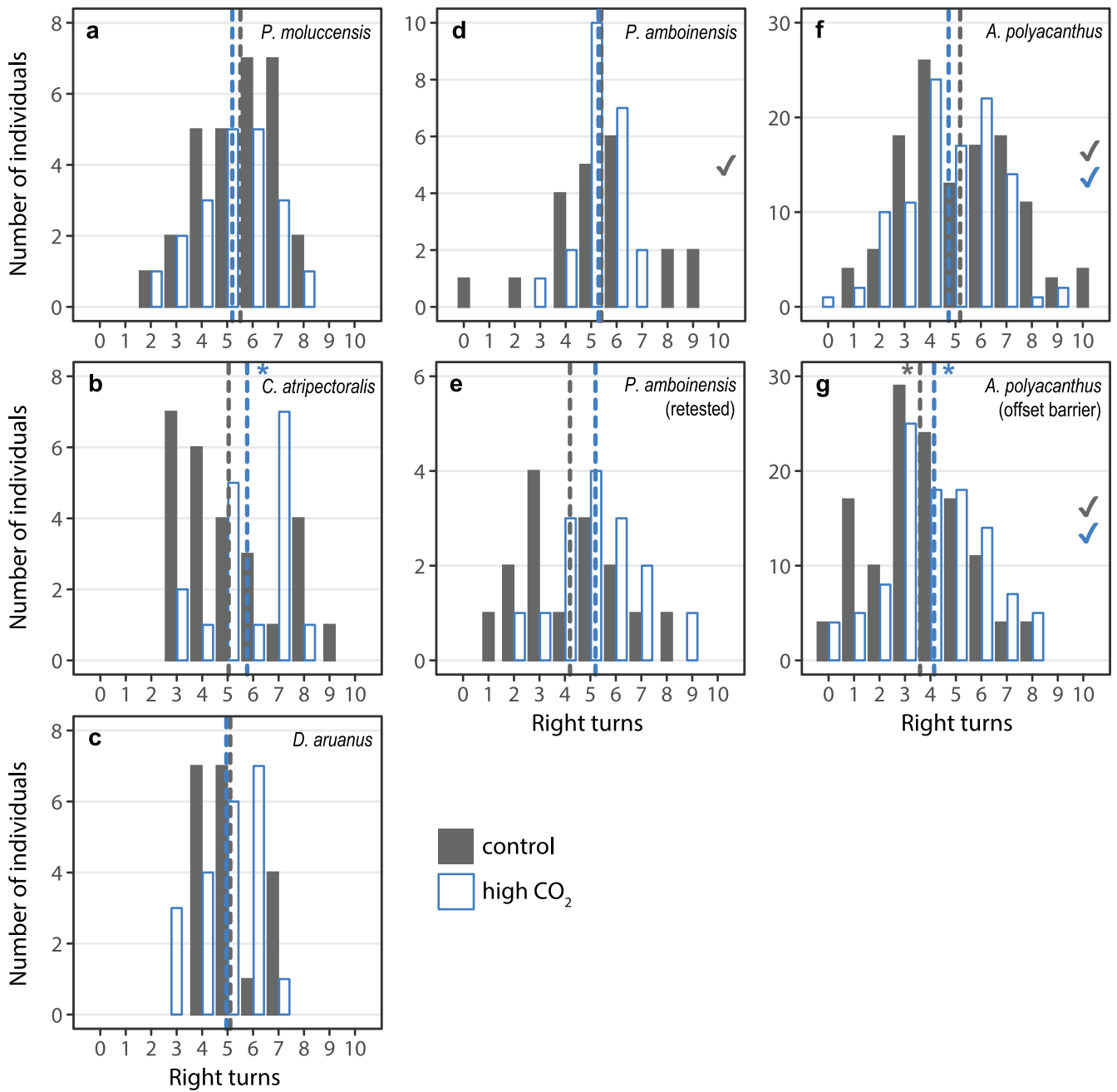
Extended Data Fig. 2 | Widespread resilience of behavioural lateralization in coral reef damselfishes when faced with end-of-century levels of CO_2.
**a–g**, Number of right turns (out of 10) under control (closed grey bars) and high CO_2 (open blue bars) conditions for fishes facing either a centred barrier at one end of the T-maze (**a–f**) or an offset barrier at the other end of the T-maze (**g**). Sample sizes represent biologically independent animals). **a**, *P. moluccensis*. Control, $n = 29$; CO_2, $n = 20$. **b**, *C. atripectoralis*. Control, $n = 26$; CO_2, $n = 17$. **c**, *D. aruanus*. Control, $n = 19$; CO_2, $n = 21$. **d**, *P. amboinensis*. Control, $n = 21$; CO_2,

$n = 22$. **e**, *P. amboinensis* retested. Control, $n = 15$; CO_2, $n = 15$. **f**, *A. polyacanthus*. Control, $n = 120$; CO_2, $n = 104$. **g**, *A. polyacanthus* (same sample sizes as in **f**). **a–e**, Data were obtained at the LIRS in 2014. **f**, **g**, Data were obtained at the AIMS in 2015. Dashed lines represent the mean number of right turns for each treatment group. A tick mark on the panel (coloured according to treatment) indicates significant individual-level lateralization, whereas an asterisk at the top of the panel indicates significant population-level lateralization. See Extended Data Tables 4, 5 for statistics.
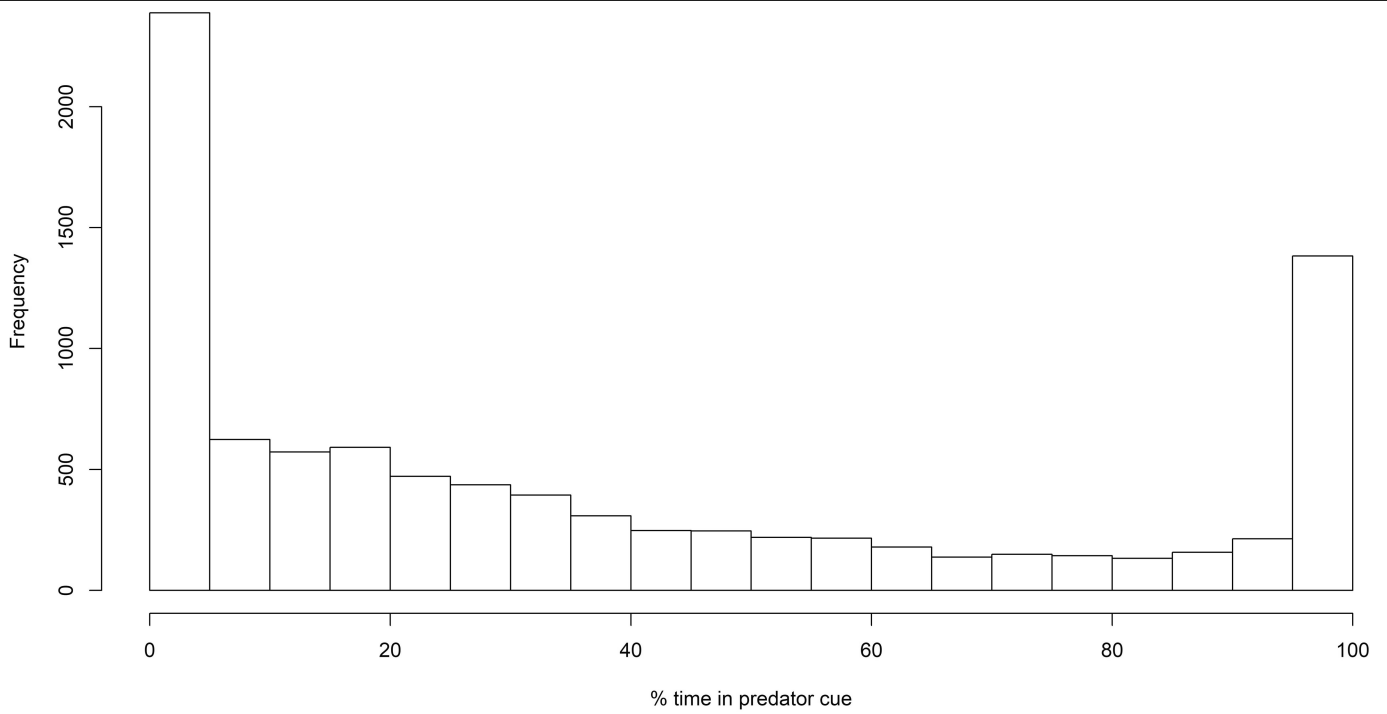
Extended Data Fig. 3 | Histogram of the percentage of time in predator cue data for fish used in choice flume trials at LIRS in 2016. Each data point included in this summary represents analysis of one minute of behavioural data for a fish; the plot contains many repeated measurements for each fish.
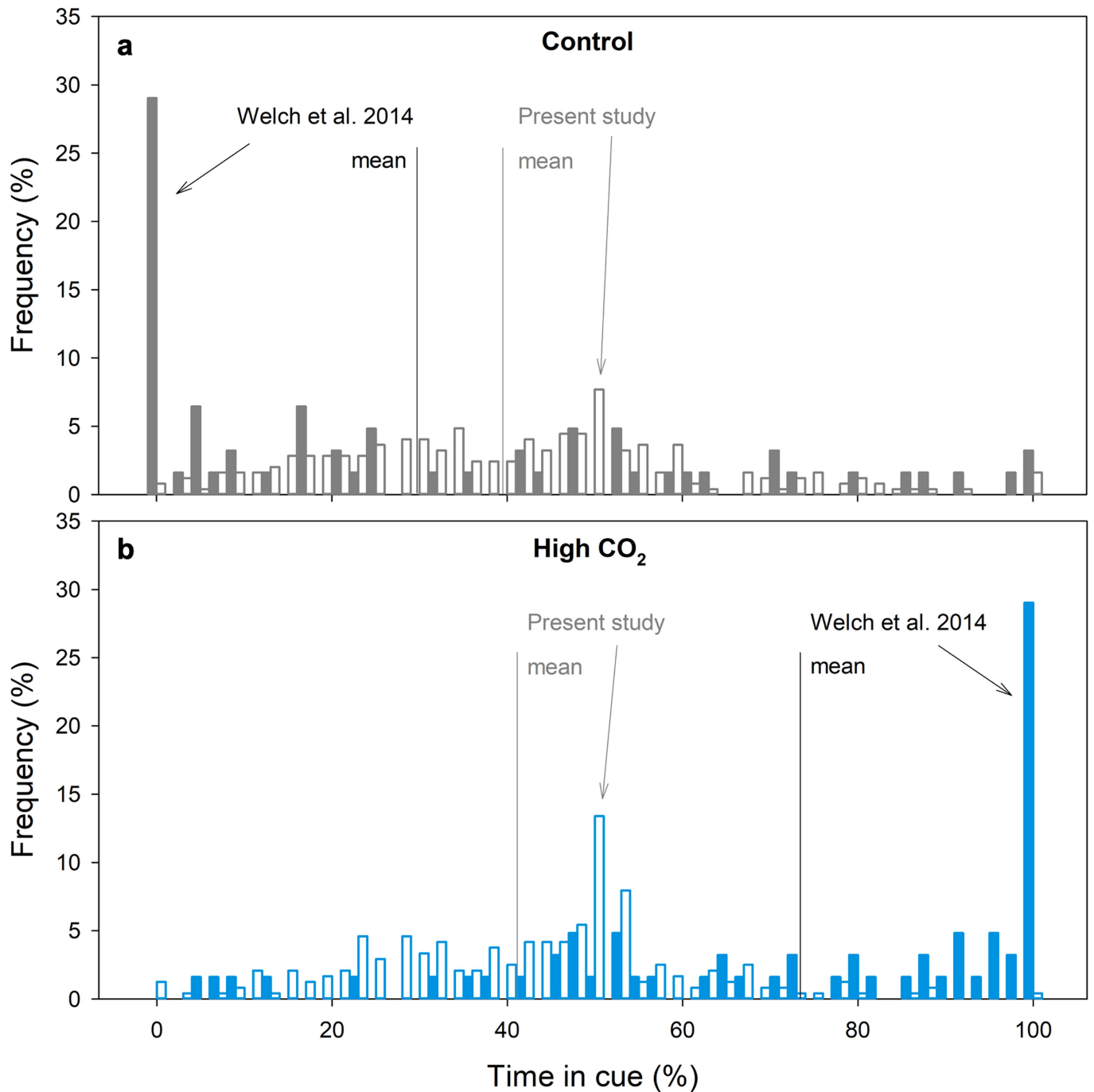
Extended Data Fig. 4 | Histogram of representative data for percentage of time spent in water containing predator cue or conspecific alarm cue. Histograms of representative data (4-min means) from a previous study[25] (solid bars) showing the disproportionate number of fish that were reported to spend 0% of time in conspecific chemical alarm cue when acclimated to control water (**a**) or 100% of time in the cue when acclimated to water with elevated CO_2 levels (**b**). The representative treatment groups[25] are juvenile *A. polyacanthus* in control water from parents acclimated to high CO_2 water (**a**, $n = 62$) and juvenile *A. polyacanthus* in high CO_2 water from parents acclimated to high CO_2 water (**b**, $n = 62$). Also presented are data (4-min means) from the present study (6 species, open bars; $n = 247$ control, $n = 239$ high CO_2) showing peak frequencies around 50% of time in predator cue for both control (**a**) and high-CO_2-exposed (**b**) fish. Sample sizes represent biologically independent animals. Mean values for each of the datasets are indicated with vertical lines, and arrows are directed at modal values in each of the datasets.

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Extended Data Table 1 | Water chemistry data for the two sites for the three years of the study

Location/year	Treatment	pCO$_2$ (µatm)	pCO$_2$ n	Temperature (°C)	Temperature n	Alkalinity (µmol kg^{-1})	Alkalinity n	pH$_{total}$ (calc.)
LIRS 2014	Control	406 (21)	19	23.1 (0.9)	2,048	2,292 (2.9)	10	8.04
	High CO$_2$	945 (117)	66	23.1 (0.9)	2,048	2,298 (4.7)	10	7.72
AIMS 2015	Control	428 (13)	1,907	25.8 (1.0)	5,747	2,356 (5.8)	5	8.03
	High CO$_2$	1,021 (156)	11,537	25.8 (1.0)	3,831	2,355 (5.5)	5	7.70
LIRS 2016	Control	521 (93)	8	29.4 (0.8)	147	-	-	-
	High CO$_2$	1,089 (326)	234	29.6 (0.8)	388	-	-	-

Data were obtained from the LIRS and AIMS between 2014 and 2016. p_{CO_2} was measured every 1–4 days, temperature was logged using iButton data-loggers (1 sample per 30 min), and alkalinity was measured from 10 samples at LIRS in 2014 and from five samples at AIMS in 2015. Data are presented as mean ± s.d. Sample sizes are included in the table. Some of the data for AIMS (2015)[15,43] and LIRS (2016)[56] have previously been published previously.

Extended Data Table 2 | Model predictions of time spent in predator chemical cue

Parameter name	Parameter estimate (± SE)	t	P
Humbug, *D. aruanus* (2014)			
n = 38, model R^2 = 0.03			
Model intercept	32.36 ± 3.59	9.02	<.001
Acclimation (CO_2)	5.67 ± 4.94	1.14	0.26
Humbug, *D. aruanus* (2016)			
n = 83, model R^2 = 0.13			
Model intercept	11.95 ± 4.07	2.93	0.004
Acclimation (CO_2)	4.23 ± 2.47	1.71	0.09
SL (mm)	0.44 ± 0.13	3.47	**<.001**
Spiny chromis, *A. polyacanthus* (2015)			
n = 100, model R^2 = 0.19			
Model intercept	38.57 ± 2.29	16.87	<.001
Acclimation (CO_2)	15.69 ± 3.23	4.85	**<.001**
Spiny chromis, *A. polyacanthus* (2016)			
n = 94, model R^2 = -0.01			
Model intercept	43.58 ± 1.93	22.56	<.001
Acclimation (CO_2)	-0.54 ± 2.96	-0.18	0.86
Ambon damsel, *P. amboinensis* (2014)			
n = 38, model R^2 = -0.02			
Model intercept	31.47 ± 3.51	8.98	<.001
Acclimation (CO_2)	1.95 ± 4.51	0.43	0.67
White damsel, *D. perspicillatus* (2016)			
n = 119, model R^2 = 0.04			
Model intercept	39.83 ± 1.73	22.98	<.001
Acclimation (CO_2)	2.26 ± 2.16	1.05	0.30
Size class (large)	-5.19 ± 2.19	-2.37	**0.02**
Lemon damsel, *P. moluccensis* (2014)			
n = 48, model R^2 = 0.01			
Model intercept	27.05 ± 3.04	8.90	<.001
Acclimation (CO_2)	5.50 ± 4.39	1.25	0.22
Black-axil chromis, *C. atripectoralis* (2014)			
n = 40, model R^2 = -0.03			
Model intercept	30.58 ± 4.99	6.13	<.001
Acclimation (CO_2)	-0.03 ± 6.31	-0.005	0.996

Parameters (± standard error) and their statistical significance for general linear models that predict the mean percentage of time spent on the side of the choice flume containing the predator chemical cue for an individual fish for six species of coral reef fishes tested in this study. We used backwards model selection using F-tests to compare the AIC of models with and without each predictor variable using the 'drop1' function in R. Only the parameter estimates for the final (best) models are given, although we always kept the main effect of acclimation treatment (that is, the effect of acclimation to elevated CO_2) in place because it was the key variable of interest. Note that for the *D. perspicillatus* model, the baseline factor level for size class was 'early-stage juveniles' (<15 mm standard length). Sample sizes represent biologically independent animals. Statistical significance is indicated in bold (α = 0.05).

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Extended Data Table 3 | Model predictions of mean activity levels

Parameter name	Parameter estimate (± SE)	t	P
D. aruanus (2014), *n* = 46, model R^2 = 0.31			
Model intercept	25.19 ± 7.05	3.57	<.001
Acclimation (CO_2)	31.83 ± 9.91	3.21	**0.003**
SL (mm)	-0.08 ± 0.19	-0.44	0.66
CO_2 × SL (mm)	-0.59 ± 0.26	-2.23	**0.03**
D. aruanus (2016), *n* = 94, model R^2 = 0.00			
Model intercept	41.14 ± 1.37	30.78	<.001
Acclimation (CO_2)	0.96 ± 1.96	0.49	0.63
A. polyacanthus (2014), *n* = 16, model R^2 = 0.71			
Model intercept	48.11 ± 7.81	6.16	<.001
Acclimation (CO_2)	9.66 ± 3.12	3.10	**0.009**
SL (mm)	-0.59 ± 0.11	-5.28	**<.001**
A. polyacanthus (2015), *n* = 66, model R^2 = 0.19			
Model intercept	23.33 ± 9.60	2.43	0.01
Acclimation (CO_2)	19.76 ± 11.81	1.67	0.10
SL (mm)	0.30 ± 0.79	0.38	0.71
CO_2 × SL (mm)	-2.11 ± 0.99	-2.13	**0.04**
A. polyacanthus (2016), *n* = 112, model R^2 = 0.34			
Model intercept	38.24 ± 1.53	24.98	<.001
Acclimation (CO_2)	0.34 ± 1.77	0.19	0.85
SL (mm)	-0.32 ± 0.04	-7.64	**<.001**
P. amboinensis (2014), *n* = 43, model R^2 = -0.02			
Model intercept	17.34 ± 2.58	6.71	<.001
Acclimation (CO_2)	1.05 ± 3.61	0.29	0.77
D. perspicillatus (2016), *n* = 122, model R^2 = 0.21			
Model intercept	35.47 ± 1.96	18.10	<.001
Acclimation (CO_2)	-1.93 ± 1.69	-1.15	0.25
SL (mm)	-0.64 ± 0.11	-5.71	**<.001**
P. moluccensis (2014), *n* = 49, model R^2 = 0.05			
Model intercept	19.80 ± 2.15	9.21	<.001
Acclimation (CO_2)	6.44 ± 3.45	1.81	0.08
C. atripectoralis (2014), *n* = 34, model R^2 = -0.03			
Model intercept	26.10 ± 2.22	11.77	<.001
Acclimation (CO_2)	-1.32 ± 3.46	-0.38	0.71

Parameters and their statistical significance for general linear models that predict the individual mean activity levels (swimming (s min⁻¹)) for six species of coral reef fishes tested in this study. We used backwards model selection using *F*-tests to compare the AIC of models with and without each predictor variable using the 'drop1' function in R. Only the parameter estimates for the final (best) models are given, although we always kept the main effect of acclimation treatment in place because it was the key variable of interest. Sample sizes represent biologically independent animals. Statistical significance is indicated in bold (*α* = 0.05).

Extended Data Table 4 | Individual- and population-level lateralization of coral reef fishes

Treatment	Species	n	ind χ^2	ind P	$\overline{X}$	pop z	pop P
Control	*P. amboinensis*	21	36.38	**0.014**	5.38	0.83	0.404
	C. atripectoralis	26	37.18	0.055	5.04	0.11	0.912
	D. aruanus	19	9.52	0.947	5.11	0.29	0.772
	P. moluccensis	29	26.89	0.524	5.52	1.76	0.079
	P. amboinensis (retest)	15	22.56	0.068	4.20	-1.59	0.112
High CO_2	*P. amboinensis*	22	7.51	0.997	5.31	0.943	0.346
	C. atripectoralis	17	14.82	0.538	5.76	1.99	**0.047**
	D. aruanus	21	10.78	0.952	4.95	-0.138	0.890
	P. moluccensis	20	18.08	0.517	5.20	0.566	0.572
	P. amboinensis (retest)	15	16.96	0.258	5.20	0.463	0.644

Individual- and population-level lateralization was tested for four species of coral reef fishes (*P. amboinensis, C. atripectoralis, D. aruanus* and *P. moluccensis*) in a detour test (LIRS 2014) under control (around 400 µatm CO_2) and high CO_2 (about 1,000 µatm) conditions. The sample size (n) and mean number of right turns ($\overline{X}$) out of a total of 10 turns is indicated for each species and treatment group (sample sizes represent biologically independent animals). χ^2 statistics (ind χ^2) and P values (ind P) are presented for tests of individual-level lateralization. z values (pop z) and P values (pop P) are presented for tests of population-level lateralization. $P < 0.05$ indicates lateralization. 'retest' indicates a subset of individuals that underwent a second trial in an effort to validate the findings from their first trial. Statistical significance is indicated in bold ($a = 0.05$).

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Extended Data Table 5 | Individual- and population-level lateralization of wild and captive-reared *A. polyacanthus*

Treatment	Species	n	ind χ^2	ind P	$\overline{X}$	pop z	pop P
Control	*A. polyacanthus* (cantered end)	120	219.18	**6.28E-08**	5.18	0.974	0.33
	A. polyacanthus (offset end)	120	170.79	**0.001**	3.59	-7.76	**8.24E-15**
High CO$_2$	*A. polyacanthus* (cantered end)	104	130.58	**0.034**	4.73	-1.56	0.119
	A. polyacanthus (offset end)	104	152.33	**0.001**	4.14	-4.51	**6.56E-06**

The individual- and population-level lateralization of wild ($n = 96$) and captive-reared ($n = 128$) *A. polyacanthus* (mean ± s.d. standard length, 20 ± 7 mm) was tested in a detour test (AIMS 2015) under control (around 400 µatm CO$_2$; $n = 54$ wild, $n = 66$ captive-reared) and high CO$_2$ (about 1,000 µatm; $n = 42$ wild, $n = 62$ captive-reared) conditions. 'offset end' indicates the end of the lateralization arena where the barrier was offset by 5 mm to create a situation in which the path around the barrier was shorter if the fish turned left rather than right. The sample size (n) and mean number of right turns ($\overline{X}$) out of a total of 10 turns (per arena end) is indicated for each treatment group (sample sizes represent biologically independent animals). χ^2 statistics (ind χ^2) and P values (ind P) are presented for tests of individual-level lateralization. z values (pop z) and P values (pop P) are presented for tests of population-level lateralization. $P < 0.05$ indicates lateralization. Statistical significance is indicated in bold ($\alpha = 0.05$).

nature research

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☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
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Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	To extract fish behavioural data from videos we recorded of fish in behavioural arenas, we used the commercial software ViewPoint (version 3, 20, 5, 83) (details given in the Supplementary Information).
Data analysis	For data analyses, we used a combination of Microsoft Excel (for organizing and summarizing outputs from the ViewPoint video tracking software) and R (versions 3.5.0 and 3.5.2, for doing statistical analyses and making most of the figures). Within R, we specifically used glmer which is a function of the analysis package lme4 (version 1.1-20). For data exploration we used mgcv (version 1.8-26). Further details are given in the statistical analyses section of the Methods and Supplementary Information.

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Ecological, evolutionary & environmental sciences study design

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Study description	We tested the effect of elevated CO_2 on predator cue avoidance, swimming activity and behavioural lateralisation. Experiments were conducted across three years (August-September 2014, April-July 2015, January 2016), at two locations, and on a total of >900 individuals from six species. Predator cue avoidance and activity trials were conducted at the Lizard Island Research Station (LIRS) in 2014 and 2016, and at the Australian Institute of Marine Science (AIMS) in Townsville in 2015. Behavioural lateralisation trials were conducted at LIRS in 2014 and at AIMS in 2015. We used General Linear Models to test for the effect of elevated CO_2 on predator cue avoidance. We built separate models for each species × year combination due to the many differences between years. Fish size was included as a fixed effect. Swimming activity was analysed using the same general linear modelling procedures as for predator cue avoidance. Population-level lateralisation was analysed with a generalized linear random-effects model, and individual-level lateralisation was analysed with a chi-square test comparing the observed variance (numerator) to the expected variance (denominator) assuming a normal approximation to the binomial distribution. All statistical procedures are described in detail in the Methods section and in the Supplementary Information.
Research sample	We used six species of coral reef fish (humbug dascyllus Dascyllus aruanus, ambon damsel Pomacentrus amboinensis, lemon damsel Pomacentrus moluccensis, black-axil chromis Chromis atripectoralis, spiny chromis Acanthochromis polyacanthus, white damsel Dischistodus perspicillatus) and two species of predators (bluespotted rock cod Cephalopholis cyanostigma and flagtail grouper Cephalopholis urodeta). Species were selected based on previously used coral reef fishes (for direct replication) and two species not previously used in ocean acidification research (for conceptual replication). Fish were collected from the wild at Lizard Island, obtained from a public aquarium (Reef HQ Aquarium, Townsville) and obtained from the northern Great Barrier Reef (by Cairns Marine Pty Ltd). Fish were juveniles, sub-adults and adults. No fish were sexed. Fish were exposed to CO_2, no additional manipulations were applied.
Sampling strategy	Sample sizes were based on previous studies and fish availability. Previous studies have typically used 10 fish or less per treatment group, which formed the lowest sample size used in this study. When possible, we used a higher sample size (e.g., n = 50) to increase statistical power but without depleting any wild fish populations.
Data collection	For predator cue avoidance and activity, trials were video recorded and analysed using tracking software. Data from lateralisation trials was collected manually in real-time (video recorded at LIRS in 2014, data collected by JS, for AIMS 2015, data was collected by GDR, NS and AY).
Timing and spatial scale	Timing: For LIRS 2014, data was collected in August and September (Aug 30-Sept 10, data was collected every day). For AIMS 2015, predator cue avoidance trials were conducted on May 21, 22, 27, and 27; activity trials were conducted on May 24, 26, 29, 30, 31, and June 1 and 2-9; behavioural lateralization trials were done on April 15-17, April 23, 27, 28, and May 17, 19, 23, and 24. Temporal gaps in data collection at AIMS was related to logistical factors, including days off, and availability of experimental equipment and personnel. For LIRS 2016, data was collected in January (16-31, data collection every day except for Jan 24 which was a day off). Rationale for data collection was to perform the maximum number of experiments per day after the fish had been exposed to CO_2 for an adequate amount of time. Spatial scale: For LIRS 2014 and 2016, the experiments were performed at the Lizard Island Research Station, Great Barrier Reef, Australia. The fish were collected from around Lizard Island at the northern end of the Great Barrier Reef, Australia (14°40' S; 145° 28' E). For AIMS 2015, the experiments were performed at the Australian Institute of Marine Science, Townsville, Australia. Fish were obtained from the Reef HQ Aquarium in Townsville. Additionally, groups of wild fish were collected at Davies Reef (18.8238° S, 147.6429° E) Great Barrier Reef, Australia (in April 2015) and transported to AIMS.
Data exclusions	Data where excluded in instances where the automated tracking software we used failed, causing erroneous tracks of animal movement.
Reproducibility	Activity and predator cue avoidance was replicated across three years. Lateralisation was replicated across two years.
Randomization	Fish were placed in the two treatment groups (control and elevated CO_2) at random. Other aspects of water chemistry (i.e., the water supply used and the temperature it was kept at), lighting, and feeding were kept constant among replicate tanks across the two acclimation treatments.
Blinding	Complete blinding was not possible since the CO_2 dosing system was visible (both visually and auditory) to any observer physically present for the experiments, making it obvious which replicate tanks contained the elevated CO_2 treatment. However, all activity and predator cue avoidance experiments were video recorded and analysed using automated tracking software rather than being manually scored by a human observer.

Did the study involve field work?   ☐ Yes   ☒ No

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Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals

The study did not involve laboratory animals

Wild animals

Lizard Island Research Station 2014
Sub-adult and adult wild fishes were collected in August 2014 from around Lizard Island at the northern end of the Great Barrier Reef, Australia (14°40' S; 145° 28' E), on SCUBA using hand- and/or barrier-nets and spray bottles of clove oil anaesthetic (mixed 1:4 with ethanol). Five species of damselfishes were collected: humbug dascyllus (Dascyllus aruanus), ambon damsel (Pomacentrus amboinensis), lemon damsel (Pomacentrus moluccensis), black-axil chromis (Chromis atripectoralis), and spiny chromis (Acanthochromis polyacanthus). Predatory bluespotted rock cod (Cephalopholis cyanostigma) were collected using hook and line. Fish were not sexed. All fish were transported in aerated seawater to LIRS. At the end of the experiments, fish were released at the site of capture.

Australian Institute of Marine Science 2015
A. polyacanthus juveniles (~10-15 days post-hatching) were corralled into clear containers by SCUBA divers from four distinct schools (four breeding pairs) at depths of 8-10 m at Davies Reef (18.8238° S, 147.6429° E), Lizard Island, in April 2015. Fish were transported in aerated seawater to AIMS. Fish were not sexed. All fish were euthanized with an overdose of tricaine methanesulfonate (MS-222, ca. 500 mg L-1) at the end of the experiments, or at intermittent times through experiments when they were sacrificed to take precise length and weight measurements for another study (Sundin et al., 2019).

Lizard Island Research Station 2016
Wild fishes were collected in January 2016 from around Lizard Island, as detailed above for LIRS 2014. Adult predatory bluespotted rock cod (C. cyanostigma) were caught using hook-and-line, and three damselfish species were caught using clove oil spray and hand- or barrier-nets (subadult and adult humbug dascyllus [D. aruanus]; juvenile, subadult and adult spiny chromis [A. polyacanthus]; subadult and adult white damsel [Dischistodus perspicillatus]). Additionally, larval white damsels were caught near the end of their pelagic phase using established light trapping techniques. Fish were not sexed. At the end of the experiments, fish were released at the site of capture.

Field-collected samples

Lizard Island Research Station 2014
Damselfishes were placed in tanks with flow-through seawater (35 PSU) at ambient temperature (23±1oC, actual range), divided in approximately even numbers between eight identical tanks (25 L each; 3 L min-1 flow-through). Fish were fed to satiation 1-2 times per day with a commercial pellet food. C. cyanostigma were divided in even numbers between two identical tanks (200 L each; 12 L min-1 flow-through) and fed pieces of sardine (Sardinops sagax) every 2-3 d. Tanks were cleaned every 3-4 days.

Australian Institute of Marine Science 2015
Juvenile spiny chromis (A. polyacanthus) were obtained from the Reef HQ Aquarium in Townsville, Australia in May. The fish were housed in 25 L tanks with seawater recirculating (~3.5 L min-1), connected to one of four independent 200 L sumps, which themselves were continuously flushed with fresh seawater at 4-7 L min-1. Fish were fed ad libitum 1-2 times per day using commercial aquaculture pellets crushed to a powder and/or Artemia nauplii. Four wild predatory fish (flagtail grouper; Cephalopholis urodeta) were freighted to AIMS and split evenly between two tanks after being caught from the northern Great Barrier Reef by Cairns Marine Pty Ltd. C. urodeta were fed freshly killed juvenile A. polyacanthus every 1-2 days. Fish were exposed to natural water temperatures for the region (quantified using thermal data-loggers sampling every 30 min; iButton, Maxim Integrated, San Jose, CA, USA). Temperature declined seasonally from 26.1±0.2°C during the first week of acclimation (May 2015) to 24.8±0.5°C during the final week of experiments (June 2015; Table S1). Salinity was regulated through the AIMS SeaSim aquarium facility and remained at 35.8±0.15 PSU. Tanks were cleaned weekly.

Lizard Island Research Station 2016
Fishes were placed in tanks with flow-through seawater at ambient temperature (29.5±1oC, actual range). The damselfishes were divided in approximately even numbers between 22 identical tanks (one species per tank, 7-8 tanks per species; 10-25 L each and 1-3 L min-1 flow-through, depending on fish size). Fish were fed to satiation 1-2 times per day with a commercial fish flake-saltwater slurry (TetraMin Tropical Flakes, Tetra, Blacksburg, VA). C. cyanostigma were divided in even numbers between

four identical tanks (60 L each; 3 L min-1 flow-through) and fed sardine pieces and freshly killed adult damselfish every 2-3 d. All tanks were provided with pieces of PVC piping to act as shelter for the fish. Tanks were cleaned every 3-4 days.

Ethics oversight

All experiments were conducted in compliance with all relevant ethical regulations under approval from the James Cook University Animal Ethics Committee in association with the Australian Institute of Marine Science (permit A1924).

Note that full information on the approval of the study protocol must also be provided in the manuscript.