Ocean acidification does not impair the behaviour of coral reef fishes

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Summary

The partial pressure of CO_2 in the oceans has increased rapidly over the past century, driving ocean acidification (OA) and sparking concern for the stability of marine ecosystems[1-3]. Coral reef fishes are predicted to be especially susceptible to end-of-century OA, based on several high-profile papers reporting profound behavioural and sensory impairments (e.g., complete attraction to the chemical cues of predators under OA)[4,5]. In contrast, here we comprehensively and transparently show that end-of-century OA has negligible impacts on critical behaviours of coral reef fishes (i.e., avoidance of predator chemical cues, activity levels, and behavioural lateralisation). Using data simulations, we show that the large effect sizes and small within-group variance reported in several previous studies are highly improbable. Together, our findings indicate that reported effects of OA on coral reef fish behaviour are not reproducible, suggesting that behavioural perturbations will not be a major consequence for coral reef fishes in high CO_2 oceans.

Background

The partial pressure of CO_2 in the oceans has increased from average pre-industrial levels of ~280 µatm to current-day levels of ~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], sparking concern 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 pCO_2 levels exceeding 15-times the end-of-century forecasts (i.e., 15,000 µatm)[6]. Therefore, physiologists have historically considered fishes to be robust to near-future levels

of CO_2[7,8]. Intriguingly, many highly-publicised studies report detrimental effects of elevated CO_2 on fish sensory systems and behaviours[4,9], with coral reef fishes appearing to be the most sensitive despite experiencing large daily and seasonal CO_2 fluctuations in nature (e.g., 100 - 1,300 µatm)[7,10]. Indeed, all the sensory systems and associated behaviours of coral reef fishes studied to date appear to be altered or impaired by CO_2 levels of ~1,000 µatm[7,11]. Reported effects across a range of life stages include impairments in olfaction, hearing, vision, learning, behavioural lateralisation, increased activity levels, boldness, anxiety and susceptibility to predation[11]. This body of literature has contributed to dire predictions for fish populations and marine ecosystems facing ocean acidification[12,13].

While the reported effects of ocean acidification on fish sensory systems and behaviour are striking, there are considerable disparities between studies and species even when methodological approaches are similar[14,15]. This discrepancy is surprising given that many of the most prominent studies documenting detrimental effects of ocean acidification on fish behaviour report exceptionally low variability and large effect sizes[4,5,9,16,17], which should maximise the probability of successful replication[18]. Moreover, the proposed mechanism underlying the sensory impairments (interference with $GABA_A$ neurotransmitter function in the brain[17]) is reported to transcend animal phyla[11].

Amidst the 'reproducibility crisis' affecting 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 in

order 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 upon some of the most prominent studies in this field to understand the diversity in behavioural responses within and across species. Importantly, 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].

Response to predator chemical cues

In fishes, the reversal of chemical cue preferences is one of the most alarming impacts 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 maintained at current-day CO_2 (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 on 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 three consecutive years in 560 individuals from six species of pomacentrid coral reef fishes (*Acanthochromis polyacanthus*, *Chromis atripectoralis*, *Dascyllus aruanus*, *Dischistodus perspicillatus*, *Pomacentrus amboinensis*, *Pomacentrus moluccensis*). Experiments covered a range of temperatures (Extended Data Table 1), CO_2 acclimation protocols were kept consistent with previous studies (4+ days at ~1,000 µatm)[4,5,17], and four of our study species (*A. polyacanthus*, *D. aruanus*, *P. amboinensis*, *P. moluccensis*) have previously been reported to exhibit severe behavioural impairments following exposure to high CO_2[16, 25,26].

All four species of adult and sub-adult wild fishes tested in 2014 (*C. atripectoralis*, *D. aruanus*, *P. amboinensis*, *P. moluccensis*) significantly avoided the predator cue (*C. cyanostigma*) in both control and high CO_2 groups (Fig. 1a-d, Extended Data Table 2, pooled $n = 164$, all $P > 0.21$). The following year (2015), we detected a CO_2 treatment effect for captive-reared *A. polyacanthus* juveniles (Extended Data Table 2, $n = 100$, $P < 0.001$): control fish spent 39 ± 2% (model estimate ± SE) of their time in the predator cue (*C. urodeta*) while fish acclimated to high CO_2 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, 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, 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 we examined exhibited attraction to predator cues when acclimated to high CO_2, 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 (i.e., ~9-min trials, using 2 min of data before and after cue switch[4,5,16,17,25,27]. We then used bootstrapping simulations to compare our data with previous datasets (see Supplementary Information). Based on 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 activity levels[27], prompting suggestions that these changes could underlie the higher mortality rates reported for fish 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 (e.g., distances moved) from coral reef fishes have not used video footage but

have been made using direct manual observations, either on SCUBA or by counting the number of gridlines crossed by fish in aquaria (e.g.,[12,26]).

We filmed 582 individuals from six species across three years and quantified swimming activity in behavioural arenas using automated tracking software. Activity levels were assessed in adults and sub-adults of five species in 2014, with three species showing no detectable effects of CO_2 treatment (*C. atripectoralis, P. amboinensis* and *P. moluccensis*; Fig. 4c-e, Extended Data Table 3, pooled $n = 126$, $P > 0.08$). We found some evidence that activity was affected by high CO_2 in *D. aruanus*, whereby an interaction between CO_2 treatment and standard length suggested that activity was elevated by ~59 - 92% in smaller individuals (< 37 mm SL) under high CO_2 (Fig. 4b, Extended Data Fig. 1a, Extended Data Table 3, $n = 46$, $P = 0.03$). In *A. polyacanthus*, activity levels were increased by ~50% ($P = 0.009$) in fish acclimated to elevated CO_2 after controlling for a strong main effect of standard length (Fig. 4a, Extended Data Fig. 1b, 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 (see 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 ($P = 0.04$) whereby activity declined in the high CO_2 fish (but not controls) with increasing body size (Fig. 4a, Extended Data Fig. 1c, 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, 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 lateralisation

A tendency to favour the left or right side during behavioural activities (i.e., behavioural lateralisation) is thought to be an expression of brain functional asymmetries, with importance for tasks such as schooling and predator avoidance[28]. Elevated CO_2 has been reported to reduce or abolish behavioural lateralisation in fishes[17,25], presumably as a result of brain dysfunction[17]. Population-level lateralisation is present when a group of individuals collectively exhibits a side-bias (mean number of turns to one side significantly more than 50%), whereas individual-level lateralisation 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 lateralisation are independent of each other, but 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 on behavioural lateralisation using 175 fish across four species in 2014 (*C. atripectoralis*, *D. aruanus*, *P. amboinensis*, *P. moluccensis*). None of the species exhibited population-level lateralisation under control conditions (Extended Data Fig. 2a-d, Extended Data Table 4), and only *C. atripectoralis* exhibited slight population-level lateralisation under high CO_2 ($P = 0.047$, Extended Data Table 4). Three species (*C. atripectoralis*, *D. aruanus*, *P. moluccensis*)

exhibited no individual-level lateralisation under control conditions, which remained unchanged under high CO_2 (Extended Data Fig. 2a-c, Extended Data Table 4). A treatment effect was detected for individual-level lateralisation in *P. amboinensis*, with the high CO_2 group displaying reduced individual-level lateralisation compared with controls (Extended Data Fig. 2d, 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, Extended Data Table 4). While our sample sizes were comparable to many similar studies (e.g.,[17]), our inconsistent findings for *P. amboinensis* are likely a consequence of low statistical power in a behavioural test that exhibits high inter-individual variability (Extended Data Fig. 2)[18].

We increased statistical power in 2015 when behavioural lateralisation was tested in wild and captive-reared *A. polyacanthus* (n = 120 control, n = 104 high CO_2), a species for which impairments of CO_2 on lateralisation have been reported[25]. In contrast to previously reported results, we found no effect of CO_2 on behavioural lateralisation: *A. polyacanthus* exhibited individual-level lateralisation and no population-level lateralisation, both under control and high CO_2 conditions (Extended Data Fig. 2f, Extended Data Table 5). Based on previous reports that elevated CO_2 impairs visual acuity[26,29], we slightly offset the barrier at one end of the lateralisation arena, creating a shorter path around the barrier to the left. We predicted that fish under high CO_2 would not visually detect the shortcut as strongly as control fish. On the contrary, we found that fish from both treatment groups exhibited a preference for the shorter path (Extended Data Fig. 2g, Extended Data Table 5).

Conclusions and implications

223 We have presented the first multi-species, multi-year, and multi-life stage examination of the
224 profound sensory and behavioural impairments reported for coral reef fishes under end-of-
225 century levels of CO_2, thereby answering an international call for comprehensive replication
226 studies on issues of global significance[21]. Importantly, we took great care to enhance
227 transparency by systematically documenting our experiments and providing raw data and
228 analysis code. In contrast to previous studies on the same and closely related species, we
229 found no consistent detrimental effects of end-of-century CO_2 on avoidance of predator
230 chemical cues, activity levels, or behavioural lateralisation. While CO_2 emissions are an
231 environmental threat[3,30], the catastrophic projections for fish sustainability based on CO_2-
232 induced behavioural impairments[12,13] must be reassessed in light of our findings.
233
234 We went to great lengths to match the species, life stages, location and season of previous
235 studies, yet the discrepancies in findings were striking. This was most apparent for the
236 responses of fish to predator chemical cues, whereby previous studies have reported extreme
237 effect sizes (in which control fish spent < 10% of their time in predator cues compared with >
238 90% of time for fish under high CO_2; Fig. 3a-c) with exceedingly low variability around the
239 group means (Fig. 3d-f). The research community in the field of ocean acidification and coral
240 reef fish behaviour has remained small, and the study systems are often remote and expensive
241 to access, both of which have precluded independent assessments of previous findings. Small
242 sample sizes[18] and other methodological or analytical weaknesses[22] in previous studies could
243 potentially explain the discrepancies between our results and the majority of articles reporting
244 minor impacts (small effect sizes) of CO_2 on fish behaviour. However, we cannot reconcile
245 our findings with those showing extremely large effect sizes and small within-group variance
246 in experiments with large sample sizes (Fig. 3). Inter-individual variation enables the
247 persistence of populations and species and is a fundamental biological phenomenon acted

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

Based on our findings on > 900 wild and captive-reared individuals of six species across three years, we conclude that acclimation to end-of-century levels of CO_2 does not meaningfully alter critical behaviours in coral reef fishes. Reasonably large sample sizes and consistent results across species, locations, life stages and years suggest that the probability of false negatives (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 detailed here (e.g., 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 emphasised that there is strong evidence that increasing atmospheric CO_2 is causing ocean warming which can profoundly affect marine fishes[30].

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Contributions

All authors contributed to the design and execution of behavioural experiments; TDC drafted the manuscript and Supplementary Information with assistance from all authors; TDC and JS managed and prepared the raw data with assistance from co-authors; GDR, DGR and TDC conducted the statistical analyses and created the figures. JS managed the revisions with assistance from all co-authors.

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Figure legends

Fig. 1 Widespread avoidance of predator chemical cues in coral reef damselfishes under present-day and end-of-century levels of CO_2. Percent time (means ± SE) that **a**, *P. amboinensis*, **b**, *C. atripectoralis*, **c**, *D. aruanus*, **d**, *P. moluccensis*, and **e**, *A. polyacanthus* spent in water containing chemical cues of a predator (*C. cyanostigma* for **a-d**, *C. urodeta* for **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 (~410 µatm) in closed grey circles, CO_2 fish (~1,000 µatm) in open blue circles

(*n* of biologically independent animals, i.e., statistically independent samples, are given in figure panels). Data were excluded between 10 and 13 min for predator cue switch. See Extended Data Table 2 for statistics. Fish illustrations by Erin Walsh and Stephanie Rowan.

Fig. 2 Damselfishes avoid predator chemical cues to the same degree when under present-day or end-of-century CO_2 levels irrespective of life stage. Percent time (means±SE) that **a**, **b**, *A. polyacanthus*, **c**, *D. aruanus* and **d**, **e**, *D. perspicillatus* from early life-stages and later life-stages spent on one side of a two-current choice flume during experiments at the Lizard Island Research Station in 2016. Control fish (~520 µatm) in closed grey circles, CO_2 fish (~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*) was introduced to one side of the flume for 20 min and then switched to the other side for a subsequent 20 min. *n* of biologically independent animals, i.e., statistically independent samples, are given in figure panels. See Extended Data Table 2 for statistics. Fish illustrations by Erin Walsh and Stephanie Rowan.

Fig. 3 Bootstrapping data simulations reveal that fish avoid predator chemical cues regardless of whether acclimated to present-day or end-of-century CO_2 (left panels), and the within-group variance in many previous studies is lower than statistically reasonable (right panels). a-c, Frequency outputs from bootstrapping simulations of the mean percent time in predator cue when **a**, $n = 10$, **b**, $n = 20$, or **c**, $n = 60$ 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,

i.e., statistically independent samples). 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 dramatically different from previous reports of major effects of high CO_2 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 (coloured circles and arrows) 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. Coloured circles correspond with references in left panels, while circles in black did not examine effects of high CO_2 and/or predator/alarm cue avoidance and thus do not appear in left panels. References for cited papers are presented in the methods. For additional details, see Supplementary Information.

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. Activity levels (s min^{-1}) following acclimation to control (~450 µatm; closed grey circles) or end-of-century (~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**, *A. polyacanthus* and **b**, *D. aruanus* were collected across multiple years (indicated at the top of each panel), while data for **c**, *C. atripectoralis*, **d**, *P. amboinensis* and **e**, *P. moluccensis* were collected in 2014 and data for **f**,

438 *D. perspicillatus* were collected in 2016 (n along the bottom of the figure panels represent
439 biologically independent animals, i.e., statistically independent samples). Note that there
440 were some statistically significant (two-tailed tests), context-dependent effects of CO_2
441 treatment for *A. polyacanthus* and *D. aruanus*, including interactions with body size (see
442 Extended Data Fig. 1b, c; statistics in Extended Data Table 3).
443

Methods

Experiments were conducted across three years (2014-2016), at two locations in Australia (the Lizard Island Research Station, LIRS, and the Australian Institute of Marine Science, AIMS, in Townsville), and on a total of > 900 individuals from six species across an ontogenetic range. The experimental designs and CO_2-dosing systems (described below) followed best practices for ocean acidification research[31-33]. For all experiments, fish were given at least 4 d to acclimate to the CO_2 treatment before trials commenced. While 4 d is a short acclimation period, this duration was chosen because it has been reported as sufficient to maximise behavioural/sensory impairments in fishes[5,27]. Fish were placed in the two treatment groups 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. 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 since the CO_2 dosing system was visible (both visually and auditory) to any observer physically present for the experiments. However, all activity and predator cue avoidance experiments were video recorded and analysed using automated tracking software. Lateralisation experiments could not be tracked using automated tracking software but were scored in real-time. A more detailed methods description 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 Australian Institute of Marine Science (permit A1924).

Animals and holding conditions

LIRS August 2014

Sub-adult and adult wild fishes (humbug dascyllus, *Dascyllus aruanus*; *n* = 46, Ambon damsel, *Pomacentrus amboinensis*; *n* = 43, lemon damsel, *Pomacentrus moluccensis*; *n* = 49, black-axil chromis, *Chromis atripectoralis*; *n* = 43, and spiny chromis, *Acanthochromis 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), on SCUBA using hand- and/or barrier-nets and spray bottles of clove oil anaesthetic (mixed 1:4 with ethanol). To produce predator chemical cues, predatory bluespotted rock cod (*Cephalopholis cyanostigma*; *n* = 24) were collected using hook and line. All fish 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 damselfish 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.

Following 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±SD) (pH_{total} ~7.72, calculated using the constants of Dickson (1990)[34] and Lueker et al. (2000)[35] in CO2calc [Hansen, USGS, USA]; Extended Data Table 1) 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 (as in Jutfelt et al. (2013)[36]). While 24 h may seem like 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 pCO_2[6,37]. In addition, some previous studies have reported that fish were simply transferred to end-of-

century pCO_2 treatments rather than using a gradual change[27,38,39], or sometimes not reported how fish were transferred to high pCO_2[4,5,17]. The other half of the tanks remained at ambient CO_2 levels of 406 ± 21 µatm (pH_{total} ~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, Finland) connected to an aspiration pump (Vaisala, Finland) and a submerged gas-permeable PFTE probe (Qubit Systems, Kingston, Canada) following Green & Jutfelt (2014)[40]. The CO_2 meter was factory calibrated by Vaisala (Vantaa, Finland) prior to experiments. Water samples were taken at ten different points throughout the experiment for subsequent measurements of total alkalinity (Extended Data Table 1) (60 ml samples of water with 30 µl of mercury chloride to poison any microorganisms). Fish were fed to satiation 1 - 2 times per day with a commercial pellet food, but food was withheld for ~12 h prior to experiments. Tanks were cleaned every 3 - 4 days. Individual fish were re-used for each of the three response variables we measured (activity, behavioural lateralisation, 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 post-hatching, 0.019 ± 0.015 g [mean ± SD] initial wet weight, 9.1 ± 2.3 mm initial standard length [SL]) were obtained from the Reef HQ Aquarium in Townsville, Australia (total n = 1494). Additionally, groups of wild *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) 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 (~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 lateralisation, whereas wild fish were only used in behavioural lateralisation experiments. 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. 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 in Sundin et al. 2017[41]).

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 ± 156 µatm (mean ± SD) (pH_{total} ~7.70; Extended Data Table 1) 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 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} ~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®, Lincoln, Nebraska, USA). 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; 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 nauplii, but food was withheld for 12 - 18 h prior to experiments. Tanks were cleaned weekly. Individual fish were used once; i.e., for one of the three response variables we measured (activity, behavioural lateralisation, or predator chemical cue avoidance). All fish used at AIMS in 2015 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[15]).

LIRS January 2016

Wild fishes were collected from around Lizard Island, as detailed above for LIRS 2014. Adult predatory bluespotted rock cod (*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 (subadult and adult humbug dascyllus [*D. aruanus*; $n = 96$]; juvenile, subadult and adult spiny chromis [*A. polyacanthus*; $n = 112$]; subadult and adult white damsel [*Dischistodus perspicillatus*; $n = 50$]). Note that *A. polyacanthus* does not have a pelagic larval phase (see Supplementary Information). Additionally, larval white damsels (*D. perspicillatus* $n = 72$) were caught near the end of their pelagic phase using established light trapping techniques[42]. 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

565 each; 3 L min^{-1} flow-through) and fed sardine pieces and freshly killed adult damselfish every
566 2 - 3 days. All tanks were provided with pieces of PVC piping to act as shelter for the fish.

567

568 Following 1 - 2 days in captivity, the CO_2 of half of the tanks (n = 11 damselfish tanks, n = 2
569 *C. cyanostigma tanks*) was gradually increased to 1,089 ± 326 µatm (mean ± SD) over 24 h
570 using a CO_2 dosing system as above (for LIRS 2014), while the other half of the tanks
571 remained at ambient CO_2 levels of 521 ± 93 µatm (Extended Data Table 1). Levels of CO_2 in
572 each tank were checked twice daily using the handheld Vaisala as detailed above (for LIRS
573 2014). Fish were fed to satiation 1 - 2 times per day with a commercial fish flake-saltwater
574 slurry (TetraMin Tropical Flakes, Tetra, Blacksburg, VA), but food was withheld for ~12 h
575 prior to experiments. Tanks were cleaned every 3 - 4 days. Individual fish were used once;
576 the two measured response variables were obtained from a single, continuous behavioural
577 trial (activity followed by predator chemical cue avoidance). At the end of the experiments,
578 fish were released at the approximate site of capture.

579

580 **Response to predator chemical cues**

581 **LIRS 2014**

582 Four species were examined for their responses to predator chemical cues (*P. amboinensis*
583 (standard length [SL] range 23 - 53 mm), *C. atripectoralis* (SL 15 - 43 mm), *D. aruanus* (SL
584 16 - 63 mm), and *P. moluccensis* (SL 19 - 34 mm), sample sizes are given in Fig. 1, Extended
585 Data Table 2), using a two-current choice flume. The setup for the two-channel choice flume
586 followed established protocols[43] (for details, see Supplementary Information). The fish in the
587 high CO_2 group had been acclimated to the CO_2 treatment for 5 - 16 days prior to

588 commencement of experiments, while control fish had been held for 4 - 16 days. The choice
589 flume was a custom-built, larger version (L × W × H = 580 × 260 × 280 mm; water depth 80
590 mm) of a two-channel choice flume used in previous studies[44]. Detailed information on the
591 design and function of two-channel choice flumes is given elsewhere[43] (for details, see
592 Supplementary Information). *C. cyanostigma* was used to create predator chemical cues (see
593 Supplementary Information for details). All trials in the choice flume were recorded to a
594 computer using a webcam (Logitech HD Pro C920) positioned 45 cm above the choice arena.
595 At the beginning of a trial, a paper note detailing the treatment history of the individual fish
596 was placed in view of the camera before the fish was placed into the centre of the choice
597 arena within a bottomless mesh cylinder (70 mm diameter) for 1.5 - 2 min. This step was
598 included to ensure that the fish had the opportunity to receive sensory input from both sides
599 of the choice flume – one side flowing with unmanipulated water and the other side flowing
600 with water containing the predator cue. After the settling period, the mesh cylinder was
601 carefully lifted and the fish was allowed to select its position within the flume. After a further
602 8 min, the configuration of flow through each side of the flume was switched using a series of
603 valves such that water containing the predator cue now flowed through the opposite side of
604 the flume. The valves were positioned near the secondary header tanks and could be adjusted
605 without visually or physically disturbing the fish. The fish was given a further 8 min to select
606 its position in the flume with the new flow configuration before being removed and returned
607 to its holding tank. The video files were analysed using tracking software (ViewPoint,
608 Zebralab, Lyon, France) to automatically quantify time spent in the flow of water (side of the
609 flume) containing the predator cue.

610

611 **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 ~45 cm above) recording at 10 frames per second with a resolution of 1,280 × 720 pixels. To match the smaller size of the fish (cf. LIRS 2014), 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, ca. 135 mL min^{-1} $channel^{-1}$). We initially tested flumes built to the exact specifications of those used in previous papers (e.g.,[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* [SL 9 - 11 mm] from Reef HQ public 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; 18 minutes total duration), including the presentation of an explanatory note in front of the camera prior to 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 [SL]) and later life-stages (15.0 - 51.0 mm SL, sample sizes listed in Fig. 2, Extended Data Table 2). Five two-channel choice flumes were used in parallel (610 × 200 mm, 290 × 93 mm, 235 × 45 mm, for details see

636 Supplementary Information). All trials in the choice flumes were recorded to a computer
637 using webcams (Logitech HD Pro C920, FireWire camera, Dragonfly 2, Point Gray,
638 Richmond, BC, Canada; Microsoft LifeCam HD 5000 webcam) positioned 45 - 130 cm
639 above the choice arenas (depending on camera type and flume size). Trials were executed in a
640 similar manner as at LIRS in 2014. At the commencement of a trial, a paper note detailing the
641 treatment history of each fish was placed in view of the relevant camera before the fish was
642 placed into the centre of the choice arena (no mesh cylinder was used) of the flume. Unlike in
643 the predator chemical cue trials described for LIRS 2014 and AIMS 2015, the fish were given
644 40 min to settle in the flumes with unmanipulated water running down both sides (i.e., no
645 predator cue) before the cue was added to one side for 20 min, before switching the predator
646 cue to the other side for the final 20 min. *C. cyanostigma* was used to create predator
647 chemical cues (see Supplementary Information for details). The video files were analysed
648 using tracking software (ViewPoint, Zebralab, Lyon, France) for subsequent analyses of
649 activity levels (defined as seconds per minute spent swimming > 0.5 SL s^{-1}) and time spent in
650 the side of the flume containing the predator cue. An example of a full day of flume trials is
651 presented here: https://youtu.be/iH0w7Wqztjo.

652

653 **Activity levels**

654 **LIRS 2014**

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

for the fish to use as shelter. A video camera (Panasonic HC-V130, Osaka, Japan) 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 minutes. Sample sizes for 2014 swimming activity trials are given in Extended Data Table 3 and in Fig. 4.

AIMS 2015

The two choice flumes described above for use at AIMS in 2015 were also used for separate assessments of captive-reared spiny chromis (*A. polyacanthus*) activity levels for the two acclimation treatments (*n* = 28 fish from control; *n* = 38 fish from high CO2; mean ± SD fish standard length =11.7 ± 1.6 mm; Extended Data Table 3) in unmanipulated acclimation water (i.e., 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 ~45 cm overhead).

LIRS 2016

Activity trials were conducted in the choice flumes described above for LIRS 2016, whereby activity levels were monitored for the first 40 min of the experimental trials prior to releasing any chemical stimulus into either side of the flume. Five flumes were used in parallel and the flume dimensions and water velocities are given above. Additional large adult *A. polyacanthus* (9 control, 9 high CO_2) and *D. aruanus* (6 control, 7 high CO_2) were tested in

white opaque tanks (43 × 32.5 cm, water depth 10 cm). Sample sizes are given in Extended Data Table 3 and in Fig. 4.

Behavioural lateralisation

LIRS 2014

A double-ended opaque plastic T-maze (39 × 29 × 20 cm, L × W × H) was constructed to perform detour tests to examine behavioural lateralisation 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 described previously[45,46]. 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. Lateralisation 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 lateralisation tests were video-recorded (using an Olympus Tough TG1 or a Panasonic Lumix DMC-FT4 camera).

AIMS 2015

A double-ended T-maze (31 × 11 × 13 cm, L × W × H) similar to that described above was constructed to perform detour tests in juvenile *A. polyacanthus*. Wild-caught fish (10 - 33 mm standard length [SL]; control $n = 54$; high CO_2 $n = 42$) as well as captive-reared fish from

Reef HQ Aquarium (8 - 33 mm SL; control $n = 66$; high CO_2 $n = 62$) were used. The lateralisation 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 where the path around the barrier was shorter if the fish turned left rather than right (rationale and further detail given in the Supplementary Information).

Statistics

General

Time spent in predator cue, and activity levels, were quantified for each minute of the fish's behavioural trial using tracking software, which meant many repeat observations for each individual. However, three limitations prevented us from analysing the data over time. Firstly, the effect of time was non-linear. Secondly, the data were temporally autocorrelated. Lastly, the data were bimodal around the minimum and maximum values (see Extended Data Fig. 3 for an example), not conforming to any distribution readily available for use in GAMMs (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 autocorrelated repeated measurements, allowing us to use general linear models. See Supplementary Material for additional details.

Response to predator chemical cues

General linear models (LMs) were used to test for the effects of CO_2 treatment (present day vs. end-of-century) and fish size (standard length [SL] in mm) on the percent time that fish spent on the side of the flume containing the predator cue. Among the six species, there were different sample sizes, size ranges, and years (= 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 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 (but see Nakagawa 2004[47]). Therefore, in our interpretations, while we refer to effects with $P < 0.05$ as "significant", we emphasise 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[48].

Bootstrapping

Most previous studies have used more rapid assessments of cue preferences than in the present study, whereby 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)[4,5,16,17,25,27,49,]. 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[4,5,16,17,25,27,49].

Note that Gould et al. (2015)[50], which is also included in Fig. 3, is included for comparative purposes. The extremely high variance in Welch et al. (2014)[25] (Fig. 3, panel f) 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 Simonsohn (2013)[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 lateralisation

Data collected from each location and year were analysed separately due to the differences in time of year, species used, and exposure duration. Testing for lateralisation is not straightforward because it involves multiple binomial experiments with structure; see Roche et al. (2019)[52] for a description of issues with the statistical approaches used by previous studies to assess lateralisation. A test for detecting lateralisation at the population level requires examining the mean lateralisation score across all individuals in the sample since

775 population-level lateralisation is present when a group of individuals collectively exhibits a
776 side-bias. In contrast, a test for detecting individual-level lateralisation requires examining
777 the sample variance since individual-level lateralisation is present when more individuals
778 exhibit a side-bias than expected by chance (irrespective of whether it is to the left or to the
779 right). For explanations and examples of these two concepts, see Bisazza et al. (1997)[46],
780 Domenici et al. (2012)[53] and Roche et al. (2013)[54]. We tested population-level lateralisation
781 with a generalized linear random-effects model (GLMM with glmer function in R) that sets
782 the intercept equal to the grand mean of the data[52]. We tested individual-level lateralisation
783 with a chi-square test comparing the observed variance (numerator) to the expected variance
784 (denominator) assuming a normal approximation to the binomial distribution[52].This is
785 analogous to testing for overdispersion (i.e., are there more observations in the tail ends of the
786 distribution than expected by chance). See Supplementary Information for further details.

787

788

789 **Data availability**

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

794

795

796 **Code availability**

Scripts for statistical analyses are available in the repository figshare:
https://doi.org/10.6084/m9.figshare.7871522

Extended Data Fig. 1 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 given in Extended Data Table 3 (see "*D. aruanus* (2014)", "*A. polyacanthus (2014)*", and "*A. polyacanthus (2015)*"). For panel a, n = 23 per treatment, for b, n = 8 per treatment; c, $n_{control}$=28, n_{CO2}=38. Sample sizes represent biologically independent animals.

Extended Data Fig. 2 Widespread resilience of behavioural lateralisation in coral reef damselfishes when faced with end-of-century levels of CO_2. Number of right turns (out of 10) under control (closed grey bars) and high CO_2 (open blue bars) conditions for **a**, *P. moluccensis* ($n_{control}$ = 29, n_{CO2} = 20), **b**, *C. atripectoralis* ($n_{control}$ = 26, n_{CO2} = 17), **c**, *D. aruanus* ($n_{control}$ = 19, n_{CO2} = 21), **d**, *P. amboinensis* ($n_{control}$ = 21, n_{CO2} = 22), **e**, *P. amboinensis* retested ($n_{control}$ = 15, n_{CO2} = 15), **f**, *A. polyacanthus* facing a centred barrier at one end of the T-maze ($n_{control}$ = 120, n_{CO2} = 104), and **g**, *A. polyacanthus* facing an offset barrier at the other end of the T-maze (same sample sizes) (sample sizes represent biologically independent animals). **a**-**e** are from the Lizard Island Research Station in 2014, while **f**-**g** are from the Australian Institute of Marine Science in 2015. Dashed lines represent the mean number of right turns for each treatment group. A tick on the panel (coloured

822 according to treatment) indicates significant individual-level lateralisation, while an asterisk
823 at the top of the panel indicates significant population-level lateralisation. See Extended Data
824 Tables 4 and 5 for statistics.
825
826
827 **Extended Data Fig. 3 Histogram of % time in predator cue data for fish used in choice**
828 **flume trials at Lizard Island Research Station in 2016.** Each data point included in this
829 summary represents analysis of one minute of behavioural data for a fish; the plot contains
830 many repeated measurements for each fish.
831
832
833 **Extended Data Fig. 4 Histograms of representative data (4-min means) from Welch et**
834 **al. (2014)[18] (solid bars) showing the disproportionate number of fish that were reported**
835 **to spend 0% of time in conspecific chemical alarm cue when a, acclimated to control**
836 **water, or 100% of time in the cue when b, acclimated to water with elevated CO_2.** The
837 representative treatment groups displayed from Welch et al. (2014)[25] are juvenile *A.*
838 *polyacanthus* in control water from parents acclimated to high CO_2 water (panel **a**, $n = 62$),
839 and juvenile *A. polyacanthus* in high CO_2 water from parents acclimated to high CO_2 water
840 (panel **b**, $n = 62$). Also presented are data (4-min means) from the present study (six species,
841 open bars; $n = 247$ control, $n = 239$ high CO_2) showing peak frequencies around 50% of time
842 in predator cue for both **a**, control and **b**, high CO_2 fish. Sample sizes represent biologically
843 independent animals. Mean values for each of the datasets are indicated with vertical lines,
844 and arrows are directed at modal values in each of the datasets.
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847

848 **Extended Data Table 1 Water chemistry data for the two sites (the Lizard Island**
849 **Research Station and the Australian Institute of Marine Science) during the three years**
850 **(2014 - 2016) of the study.** Partial pressure of carbon dioxide (pCO_2) was measured every 1 -
851 4 days, temperature was logged using iButton data-loggers (one sample per 30 min), and
852 alkalinity was measured on 10 occasions at LIRS in 2014 and on two occasions at AIMS in
853 2015. Data are presented as mean ± SD. Sample sizes are given in the table. Some of the data
854 for AIMS 2015 and LIRS 2016 have previously been presented in Sundin et al. (2017)[41] and
855 (2019)[15] and Clark et al. (2017)[56], respectively.

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858 **Extended Data Table 2 Parameters (± standard error) and their statistical significance**
859 **for general linear models predicting an individual fish's mean percentage of time spent**
860 **on the side of the choice flume containing the predator chemical cue for six species of**
861 **coral reef fishes tested in this study.** We used backwards model selection using *F*-tests to
862 compare AIC of models with and without each predictor variable using the 'drop1' function
863 in R. Only the parameter estimates for final (best) models are given, although we always kept
864 the main effect of acclimation treatment (i.e., the effect of acclimation to elevated CO_2) in
865 place because it was the key variable of interest. Note that for the white damsel (*D.*
866 *perspicillatus)* model, the baseline factor level for size class was "early-stage juveniles" (< 15
867 mm standard length). Sample sizes are given in the table and represent biologically
868 independent animals. Statistical significance is indicated in bold ($\alpha = 0.05$).

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Extended Data Table 3 Parameters and their statistical significance for general linear models predicting 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 AIC of models with and without each predictor variable using the 'drop1' function in R. Only the parameter estimates for 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 are given in the table and represent biologically independent animals. Statistical significance is indicated in bold ($\alpha = 0.05$).

Extended Data Table 4 Individual- and population-level lateralisation for four species of coral reef fishes (*P. amboinensis*, *C. atripectoralis*, *D. aruanus*, *P. moluccensis*) tested in a detour test (LIRS 2014) under control (~400 µatm CO_2) and high CO_2 (~1,000 µatm) conditions. The sample size (*n*) and mean number of right turns ($\bar{X}$) out of a total of 10 turns is indicated for each species and treatment group (sample sizes represent biologically independent animals). A chi-square statistic (ind χ^2) and *P*-value (ind *P*) are presented for tests of individual-level lateralisation; $P < 0.05$ indicates lateralisation. A *z*-value (pop *z*) and *P*-value (pop *P*) are presented for tests of population-level lateralisation; $P < 0.05$ indicates lateralisation. "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 ($\alpha = 0.05$).

Extended Data Table 5 Individual- and population-level lateralisation for wild (*n* = 96) and captive-reared (*n* = 128) *A. polyacanthus* (mean ± SD standard length 20 ± 7 mm) tested in a detour test (AIMS 2015) under control (~400 µatm CO_2; *n* = 54 wild, *n* = 66 captive-reared) and high CO_2 (~1,000 µatm; *n* = 42 wild, *n* = 62 captive-reared) conditions. "offset end" indicates the end of the lateralisation arena where the barrier was offset by 5 mm to create a situation where 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 ($\bar{X}$) out of a total of 10 turns (per arena end) is indicated for each treatment group (sample sizes represent biologically independent animals). A chi-square statistic (ind χ^2) and *P*-value (ind *P*) are presented for tests on individual-level lateralisation; $P < 0.05$ indicates lateralisation. A *z*-value (pop *z*) and *P*-value (pop *P*) are presented for tests of population-level lateralisation; $P < 0.05$ indicates lateralisation. Statistical significance is indicated in bold ($\alpha = 0.05$).

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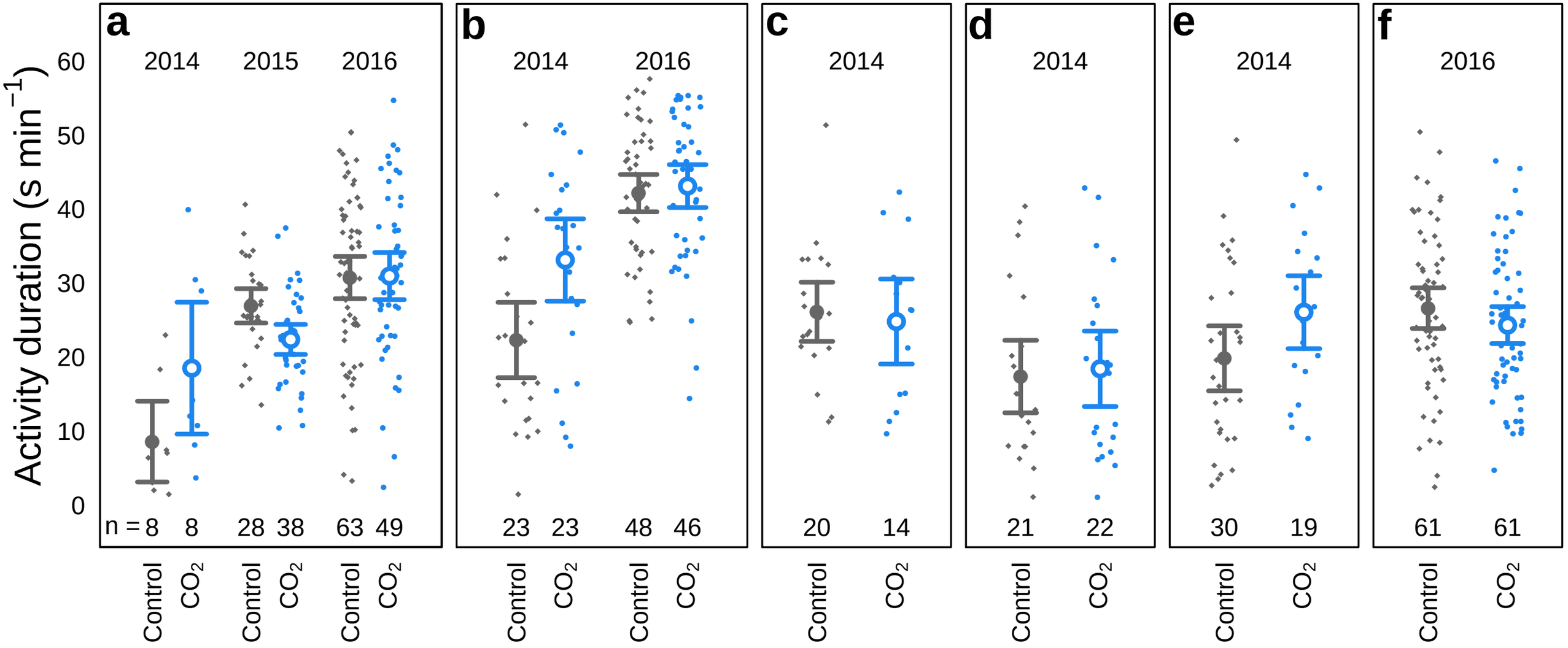
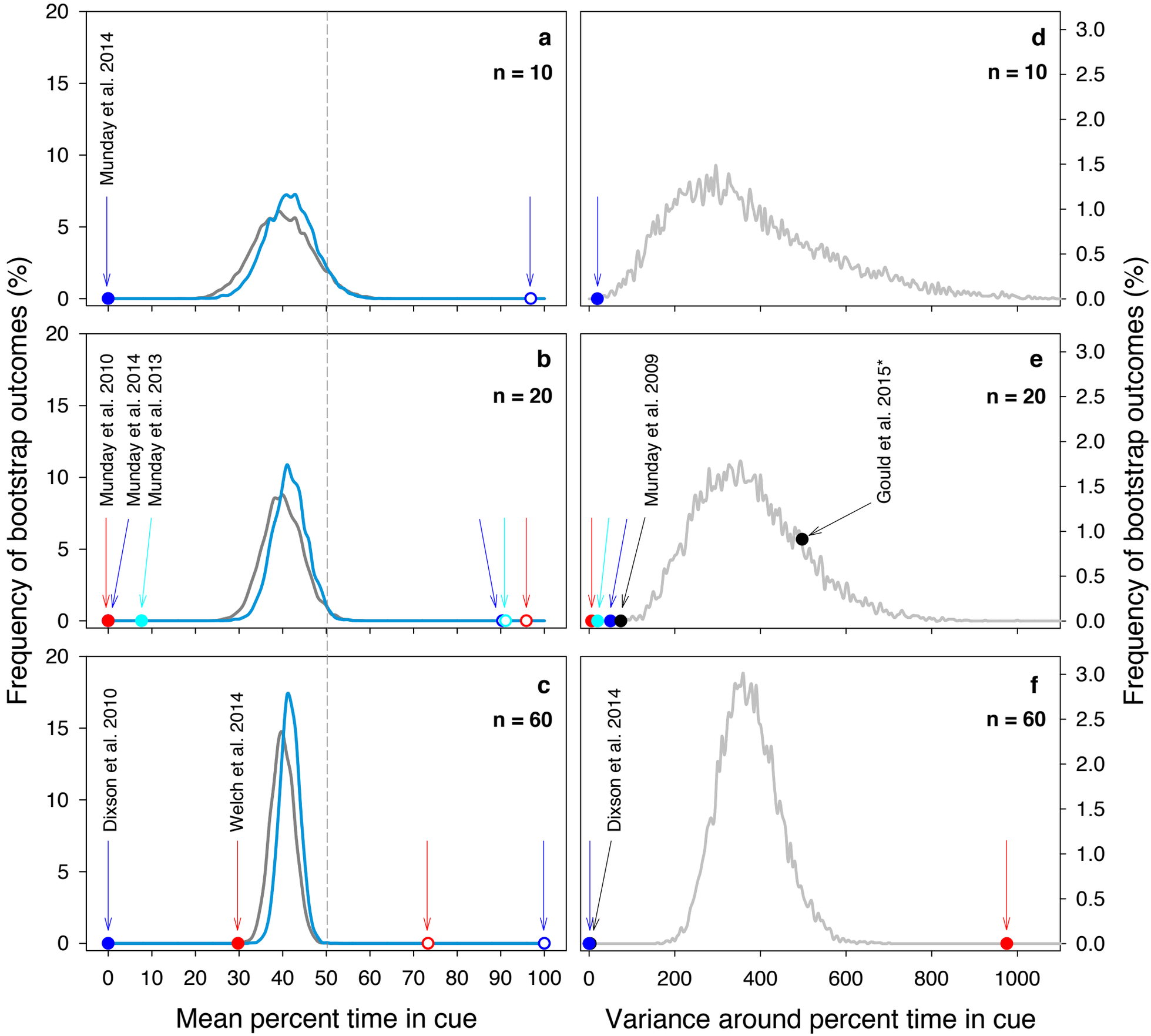
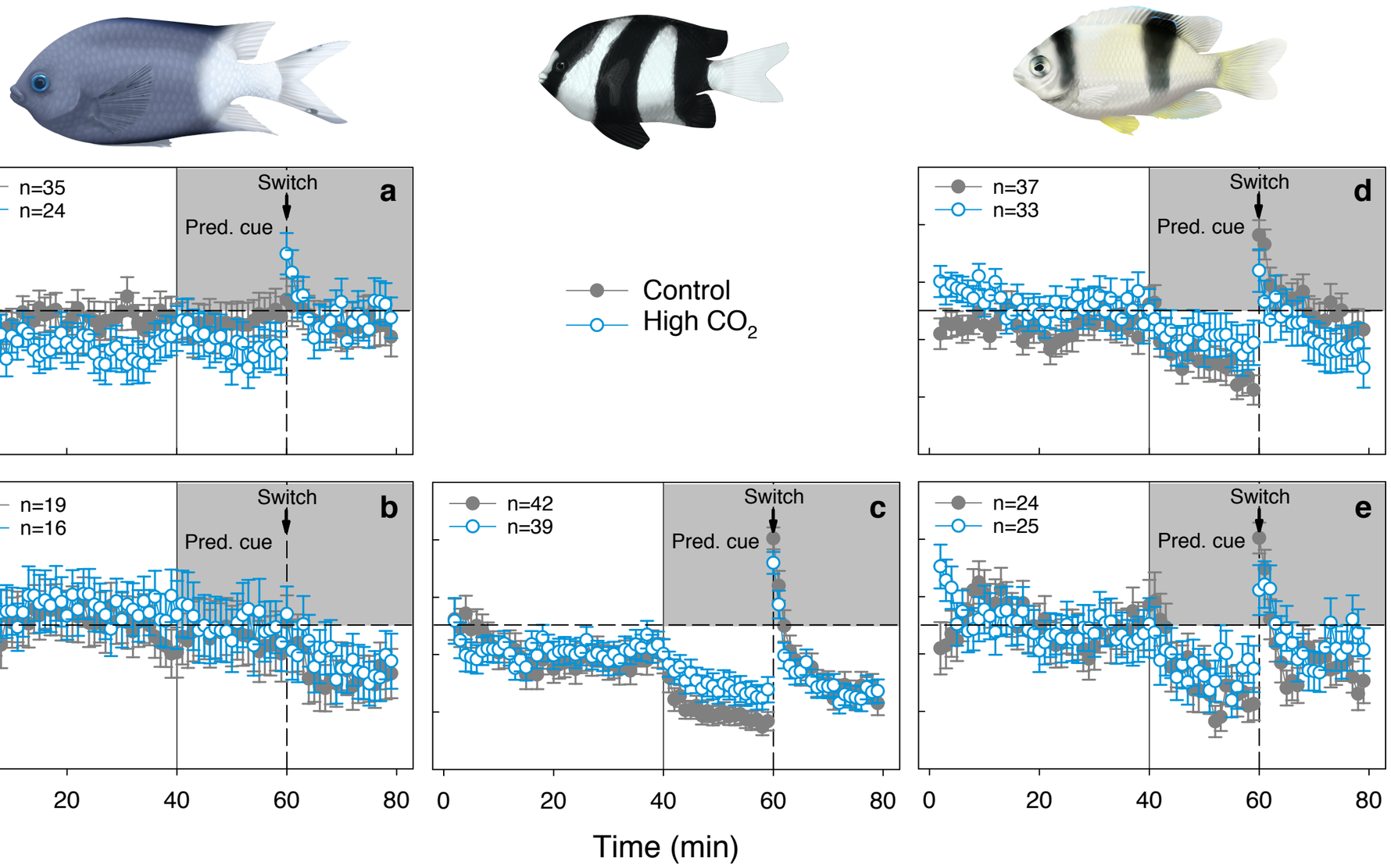
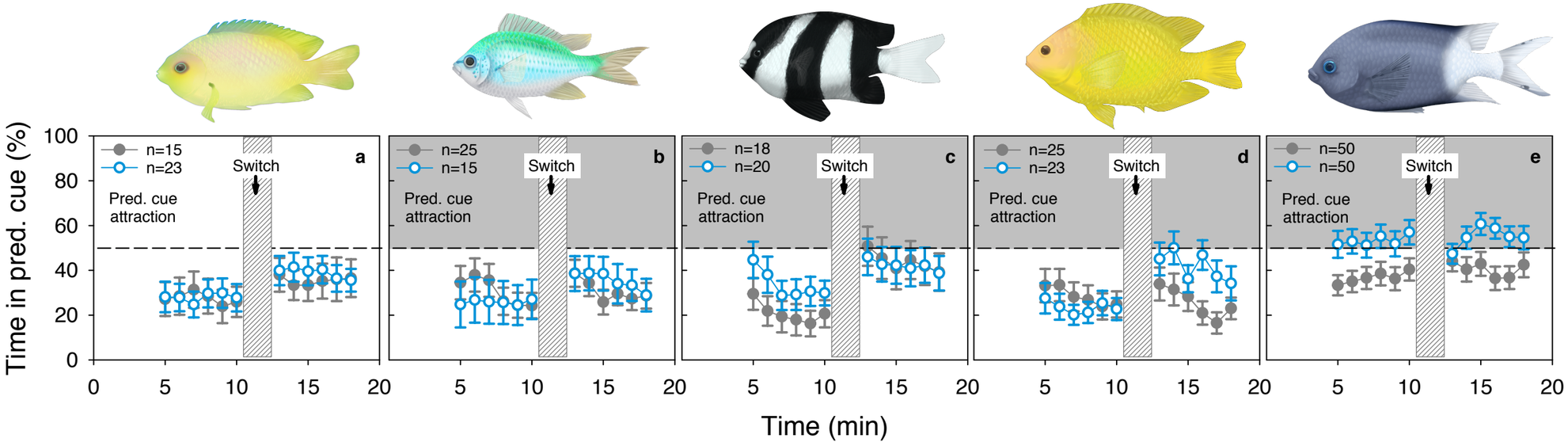

a
b
c
d
e
f
2014
2015
2016
Activity duration (s min^{-1})
0
10
20
30
40
50
60
n = 8 8 28 38 63 49
23 23 48 46
20 14
21 22
30 19
61 61
Control
CO_2


a
n = 10
Munday et al. 2014
b
n = 20
Munday et al. 2010
Munday et al. 2014
Munday et al. 2013
c
n = 60
Dixson et al. 2010
Welch et al. 2014
d
n = 10
e
n = 20
Munday et al. 2009
Gould et al. 2015*
f
n = 60
Dixson et al. 2014
Frequency of bootstrap outcomes (%)
Frequency of bootstrap outcomes (%)
Mean percent time in cue
Variance around percent time in cue


Early life stages
Mid & late life stages
Time on one side (%)
Time (min)
Control
High CO2
a
b
c
d
e
n=35
n=24
n=19
n=16
n=42
n=39
n=37
n=33
n=24
n=25
Switch
Pred. cue
0
20
40
60
80
100


Time in pred. cue (%)
100
80
60
40
20
0
n=15
n=23
Switch
a
Pred. cue
attraction
n=25
n=15
Switch
b
Pred. cue
attraction
n=18
n=20
Switch
c
Pred. cue
attraction
n=25
n=23
Switch
d
Pred. cue
attraction
n=50
n=50
Switch
e
Pred. cue
attraction
0
5
10
15
20
Time (min)