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Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	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
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

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.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The data necessary to reproduce figures and results in this study are publicly archived in the repository figshare and were made available to editors and reviewers at the time of submission: https://figshare.com/s/99f6b8973bfd0bb234e8. We place no restrictions on data availability.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☐ Life sciences ☐ Behavioural & social sciences ☒ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	We tested the effect of elevated CO2 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 CO2 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 CO2, 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 CO2 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 CO2) 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 CO2 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 CO2 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

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

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.