No effect of short-term exposure to ocean acidification on individual-level variation in behaviour and susceptibility to predation in a Great Barrier Reef damselfish

Dominique G. Roche[1,2], Josefin Sundin[3,4], Ben Speers-Roesch[5], Shinichi Nakagawa[6,7], Timothy D. Clark[8], Sandra A. Binning[2,9]

[1] *Fish Ecology and Conservation Physiology Laboratory, Department of Biology, Carleton University, 1125 Colonel By Dr., Ottawa, ON, K1S5B6, Canada.*

[2] *Institut de Biologie, Éco-Éthologie, Université de Neuchâtel, Rue Emilie-Argand 11, 2000, Neuchâtel, Switzerland.*

[3] *Department of Neuroscience, Uppsala University, Uppsala, Sweden.*

[4] *Department of Aquatic Resources, Swedish University of Agricultural Sciences, Drottningholm, Sweden.*

[5] *Department of Biological Sciences, University of New Brunswick, Saint John, New Brunswick, Canada.*

[6] *School of Biological, Earth and Environmental Sciences, University of New South Wales, Australia.*

7 Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada.

[8] *School of Life and Environmental Sciences, Deakin University, Geelong, VIC, Australia 3216.*

[9] *Département de sciences biologiques, Université de Montréal, Montréal, Québec, Canada.*

Corresponding author:

dominique.roche@mail.mcgill.ca; sandra.ann.binning@umontreal.ca

Abstract

1) Ocean acidification, caused by rising carbon dioxide (CO_2) in the atmosphere, has been reported to negatively impact a wide variety of behaviours in fishes, including activity, exploration, and predator avoidance.
2) These effects have been documented at the population level, but many animal species naturally show large and repeatable individual-level differences in behaviour. How environmental stressors, such as ocean acidification, affect behavioural variation at the individual level remains largely unknown but is vital in understanding adaptation given natural selection operates on variation at the individual rather than population level.
3) Using a statistical approach allowing variation in means and variation in variance to be modeled within a single framework, we quantified individual-level differences across five behaviours in the coral reef Ambon damselfish *Pomacentrus amboinensis* (emergence time, activity level, time spent sheltering, thigmotaxis, novel object inspection). We measured behaviour in a novel environment assay, twice before (CO_2 ~450 μatm) and twice following short-term exposure to predicted end-of-century ocean acidification conditions (~1,100 μatm).
4) Following behavioural assays, we tested individual survival in a live predation experiment. We used predatory rock cod, *Cephalopholis microprion,* acclimated to the same CO_2 treatments as Ambon damsel and examined predictors of survival probability.
5) All behaviours in damselfish were moderately and significantly repeatable, with no marked differences in repeatability estimates between the ambient CO_2 and elevated CO_2 treatment groups. Short-term exposure to end-of-century ocean acidification conditions had no effect

on any of the five behaviours measured, both in terms of group means and residual (within-individual) variance.

6) The probability of survival in the predation trials was similar for damselfish in the ambient and elevated CO_2 treatment groups. Smaller damselfish as well as those that spent a greater amount of time inspecting a novel object (i.e., bolder individuals) had a lower probability of survival regardless of their CO_2 treatment.

7) Our results challenge assumptions about the impacts of ocean acidification on coral reef fish behaviour and susceptibility to predation, both at the population and individual level. They also provide support for a trade-off between boldness and predation risk in fish.

Keywords

Behavioural traits, coral reef fish, open field test, personality, predator-prey interaction, novel object test, repeatability, survival analysis

Résumé

1) L'acidification des océans, causée par l'augmentation du dioxyde de carbone (CO_2) atmosphérique, a été associée à des effets négatifs sur le comportement des poissons, notamment l'activité, l'exploration et l'évitement des prédateurs.

2) Ces effets ont principalement été étudiés à l'échelle des populations, alors que de nombreuses espèces présentent d'importantes différences interindividuelles répétables dans leur comportement. Comment les facteurs de stress environnementaux influencent

cette variation individuelle demeure largement inconnue, bien qu'elle soit essentielle à notre compréhension de l'adaptation.

3) À l'aide d'une approche statistique permettant de modéliser simultanément les changements de moyenne et de variance, nous avons quantifié les différences interindividuelles dans cinq comportements chez le poisson-demoiselle (*Pomacentrus amboinensis*) : le temps d'émergence, le niveau d'activité, le temps passé à l'abri, la thigmotaxie et l'inspection d'un nouvel objet. Le comportement a été mesuré deux fois avant (CO_2 ~450 µatm) et deux fois après une exposition à court terme aux conditions d'acidification prévues pour la fin du siècle (~1 100 µatm).
4) Suite aux tests comportementaux, nous avons évalué la survie individuelle lors d'expériences de prédation avec le mérou *Cephalopholis microprion*, acclimaté aux mêmes conditions de CO_2 que les poissons-demoiselles, afin d'identifier les facteurs prédictifs de la survie.
5) Les cinq comportements présentaient une répétabilité modérée et significative, sans différence marquée entre les traitements de CO_2 ambiant et élevé. L'exposition à court terme aux conditions d'acidification prévues pour la fin du siècle n'a eu aucun effet sur les comportements étudiés, tant sur les moyennes des groupes que sur la variance résiduelle intra-individuelle.
6) La probabilité de survie lors des expériences de prédation était similaire entre les deux traitements de CO_2. Les individus de plus petite taille et ceux consacrant davantage de temps à l'inspection d'un nouvel objet (c'est-à-dire plus téméraire), présentaient toutefois une probabilité de survie plus faible, indépendamment du traitement de CO_2.

7) Nos résultats remettent en question certaines hypothèses concernant les effets de l'acidification des océans sur le comportement et la vulnérabilité à la prédation des poissons de récifs coralliens, tant à l'échelle populationnelle qu'individuelle. Ils appuient également l'existence d'un compromis entre les comportements téméraires et le risque de prédation chez les poissons.

Mots-clés

Analyse de survie, interaction prédateur-proie, poissons de récifs coralliens, répétabilité, traits comportementaux, test en champ ouvert, personnalité, test de nouvel objet

Introduction

The partial pressure of carbon dioxide (CO_2) in the oceans has increased by ~45% over the past century, driving a process known as ocean acidification (OA) (Lüthi *et al.* 2008; Hönisch *et al.* 2012). This environmental change shows no signs of stopping: forecasts predict that oceanic CO_2 levels at the beginning of the next century will reach those not seen in the last 30 million years (Lüthi *et al.* 2008). Among the range of detrimental impacts reported for animals under ocean acidification, altered species interactions — including between predators and their prey — have received considerable attention (Draper *et al.* 2019). For example, lab-based experiments have suggested that end-of-century levels of CO_2 cause coral reef fishes to become highly attracted to the chemical cues of their predators (Dixson *et al.* 2010; Munday *et al.* 2010), implying increased predation risk under these scenarios (but see Clark et al. 2020, which questions the repeatability of this finding). Indeed, damselfish exposed to end-of-century CO_2 and then returned to their natural coral reefs were reported to disappear faster than control individuals (Munday *et al.* 2010; Munday *et al.* 2012). The exact fate of these fish was not recorded, but suggestions for their disappearance included predator attraction, increased activity/boldness, and poorer homing/sheltering ability, all leading to increased predation (Munday *et al.* 2010; Briffa *et al.* 2012; Devine *et al.* 2012; Devine *et al.* 2013). However, a limited number of studies have directly tested the hypothesis that ocean acidification leads to decreased prey survival during a predator interaction. Interestingly, the few laboratory studies on predator-prey interactions between coral reef fishes following short-term (4 - 7 days) exposure to end-of-century levels of CO_2 suggest that the performance of both predators and prey may be altered (e.g. Allan *et al.* 2013; Allan *et al.* 2017). Conversely, and contrary to early findings (e.g. Dixson *et al.* 2010; Munday *et al.* 2010), there is increasing evidence that ocean acidification may have only negligible effects on coral reef

121 fish behaviour (Clark *et al.* 2020; Clements *et al.* 2022; Sundin 2023). This uncertainty makes it
122 difficult to predict the impact of future ocean acidification on predator-prey relationships on coral
123 reefs.

124 Many experiments exploring fish behaviour following exposure to ocean acidification
125 report extremely low inter-individual variability in behavioural responses (e.g. Dixson *et al.* 2010;
126 Munday *et al.* 2010; but see Clements *et al.* 2022). This low variability is particularly alarming
127 given that the resilience of a species to environmental perturbations depends on variation in
128 individual responses (Tuomainen *et al.* 2011). Phenotypic variation is an inherent biological trait
129 that forms the basis for natural selection and enables an adaptive response to stressors across
130 generations.

131 Individual behaviours that are repeatable across time and contexts are often referred to as
132 animal "personality" (Wolf *et al.* 2012; Carter *et al.* 2013; Roche *et al.* 2016), and interest in
133 understanding the implications of these individual-level differences on trait evolution has grown
134 over the last 20 years. Behavioural differences influence both the ways in which individuals
135 interact with their environment as well as the outcome of biotic interactions such as predation,
136 competition, parasitism, cooperation and mate-choice (Wolf *et al.* 2012; Roche *et al.* 2016; Dubois
137 *et al.* 2022; Gradito *et al.* 2024). For example, individuals that are consistently 'bold' may expend
138 more energy on activity, increasing their susceptibility to predation but also providing greater
139 foraging opportunities compared with 'shy' individuals that remain in hiding for longer durations.
140 Thus, in order to understand how a species might respond to environmental stressors, including
141 ocean acidification, variability in individual responses should be explicitly considered rather than
142 overlooked.

143 Focusing on individual-level variation as opposed to population mean responses to
144 environmental perturbations has major conservation implications by allowing us to understand the
145 traits responsible for conferring resilience to stressful conditions (Browman 2016; Killen *et al.*
146 2016). As such, behavioural ecologists are increasingly adopting a reaction norm approach to the
147 study of behavioural variation in order to examine how behaviour and phenotypic plasticity are
148 correlated and/or under selection in a given environment (Dingemanse *et al.* 2010; Dingemanse *et*
149 *al.* 2013; Roche *et al.* 2016; Fig. 1). A reaction norm is the range of phenotypes (behaviours,
150 morphologies, or physiological states) that an individual can express across time or a range of
151 environmental conditions. Hypothetically, a population-level increase in a behaviour (e.g., activity
152 level) due to elevated CO_2 exposure can occur from all individuals increasing their activity score
153 (Fig. 1 A1), or only some individuals exhibiting a marked increase in activity (Fig. 1 A2, A3). In
154 this example, individuals that do not show a change in activity after elevated CO_2 exposure may
155 be considered tolerant. Identifying tolerant individuals and their associated behaviours is a critical
156 step in predicting how species will respond and adapt to future ocean acidification conditions.

157 To address several of the knowledge gaps highlighted above, we studied individual-level
158 behavioural differences and reaction norms in a coral reef damselfish, the Ambon damselfish,
159 *Pomacentrus amboinensis*, after short-term exposure to current day, ambient CO_2 conditions and
160 elevated CO_2 conditions approximating end-of-century levels of ocean acidification. We also
161 performed a live predation experiment with the same individuals and the predatory dot head rock
162 cod (*Cephalopholis microprion,* hereafter rock cod) to explicitly test the effect of ocean
163 acidification on fitness while considering the influence of individual behavioural differences on
164 the outcome of predator-prey interactions. The Ambon damselfish is common and abundant on
165 many shallow reefs in the Indo-Pacific. It is planktivorous, diurnal, and commonly preyed upon

166 by mesopredators including rock cod (Vail & McCormick 2011). *C. microprion* and *P.*
167 *amboinensis* have been used as predator and prey, respectively, in previous predation studies
168 (Holmes & McCormick 2010; Vail & McCormick 2011).

169 Our study is the first to explicitly test how individual-level behavioural variation affects
170 resilience and susceptibility to ocean acidification while incorporating a direct measure of the
171 fitness consequences of any CO_2-induced behavioural changes. The approaches used here enable
172 us to address key outstanding questions in this field of research: (1) Are individual-level
173 behavioural differences in Ambon damselfish repeatable?; (2) How does ocean acidification affect
174 population- and individual-level differences in behaviour?; (3) Do effects of ocean acidification
175 on behaviour lead to higher predation rates?; and (4) Do behavioural traits predict susceptibility to
176 predation? We hypothesized that the repeatability of the behaviours we examined would depend
177 on CO_2 treatment and that short-term exposure to elevated CO_2 would induce behavioural
178 differences compared with individuals in the ambient CO_2 treatment at both the population and
179 individual levels. We also hypothesized that behavioural traits and short-term CO_2 exposure would
180 impact survival in a predation trial with bolder fish acclimated to elevated CO_2 being the most
181 likely to be eaten quickly.

182

183 **Materials and methods**

184 ***Fish collection and husbandry***

185 All experiments were conducted between January 12th and February 1st, 2016. Post-
186 settlement juvenile *P. amboinensis* were collected by SCUBA divers on January 12-13 from
187 lagoon reefs at Lizard Island on the northern Great Barrier Reef, Australia (14°40’ S; 145° 28’ E)
188 using hand nets and a barrier net (10 mm stretch monofilament). Fish were transported within 90

189 minutes of capture to the aquarium facilities at the Lizard Island Research Station (LIRS) in 20 L
190 buckets containing seawater aerated with battery-operated pumps. At LIRS, the fish were placed
191 in 30 L holding tanks receiving seawater at ~ 750 ml min^{-1} from one of two flow-through 32 L
192 header tanks (receiving seawater pumped directly from the reef) diffused with ambient air. At 24-
193 48 h post-capture, fish were weighed (Mettler Toledo PL602-S, d = 0.01 g) and measured for
194 standard length in a water-filled plastic bag to the nearest 0.1 mm with digital callipers. Seventy-
195 four *P. amboinensis* (33.1 ± 2.5 mm; 1.43 ± 0.35 g; mean ± SD) were then randomly placed
196 individually in 1 L flow-through plastic aquaria (17 × 12 × 7 cm, L × W × H), determined using
197 the randomisation tool in Excel 2013 (numbers were randomly generated between 1 and 74 without
198 replacement). These aquaria were supplied with seawater pumped directly from the reef to four
199 header tanks (32 L), and from there diffused to the 74 aquaria (i.e., 18-19 holding aquaria per
200 header tank). The header tanks received seawater at ~ 750 ml min^{-1}, and each 1 L holding aquaria
201 received seawater at ~40 ml min^{-1}. Each aquarium contained a 4 cm long white PVC pipe for
202 shelter. The outer sides of each plastic aquarium were covered in black tape to prevent visual
203 interaction and disturbance. Fish were fed daily with 0.5 ml of a commercial fish flake-saltwater
204 slurry (TetraMin Tropical Flakes, Tetra, Blacksburg, VA). All animals fed readily by day three
205 post-capture. Fish were habituated in their aquaria for three days prior to the start of the
206 experiment. Seawater temperature in the aquaria was 29.5 ± 1˚C. Behavioural assessments for
207 Experiment 1 (below) were performed on each individual twice under ambient CO_2 conditions,
208 then half the fish were acclimated to elevated CO_2 for 4-6 days and half were maintained at ambient
209 CO_2 conditions for the same length of time before behavioural assessments were performed
210 another two times on all individuals. Predation experiments for Experiment 2 (below) were
211 conducted 1-4 days following the last (4^{th}) behavioural trial.

Experiment 1: behavioural trials before and after CO_2 exposure

We examined behavioural differences among *P. amboinensis* using a novel environment assay. This assay is a modified version of the open-field test in which an individual is introduced into an unfamiliar environment, where the environment also included a novel object (Carter *et al.* 2013; Roche *et al.* 2016). Coral reef fishes regularly encounter novel situations and organisms (e.g., unfamiliar predators, substrates, competitors) and must rapidly evaluate potential risks and rewards. Consequently, responses to novel stimuli are ecologically meaningful proxies for risk assessment and exploratory behaviour in reef fishes. Novel environment assays are particularly relevant for Ambon damselfish, which experience predation pressure from a range of mesopredators with different predatory strategies (i.e., ambush, pursuit) as well as inter- and intraspecific competition for refuges in the coral matrix. Behavioural assays using novel environments and objects have been used to assess traits such as boldness and neophobia in coral reef fishes including damselfishes (e.g. White *et al.* 2013; Bowers *et al.* 2024). Each assay lasted 25 min and was repeated twice (two days between trials) on all fish at ambient CO_2 (458 ± 17.9 µatm; mean ± standard deviation) and then two more times (two days between trials) after half the fish were acclimated to elevated CO_2 and the other half were maintained at ambient CO_2 (see CO_2 exposure below and Fig. S1). All trials were conducted between 9:00-16:00, and the time of day was randomized across replicates to control for diurnal patterns in activity. Each trial was filmed (top view) with a digital camera (GoPro Hero 3+ black; GoPro, San Mateo USA). The stock lens of the camera was replaced with a commercially available lens to avoid image distortion (4.14 mm f/3.0 86° HFOV 5MP GP41430; Peau Productions Inc., San Diego, USA). We presented a note with the fish ID and trial number at the start of each video (Clark *et al.* 2016). Fish were transferred

from their individual aquaria to the experimental arena using their water-filled shelter (the experimenter blocked both ends of the PVC shelter with the palm of their hands). Fish either entered their shelter when the experimenter approached their aquaria or were carefully placed into the shelter by the experimenter. This procedure minimized air exposure and reduced handling stress prior to introduction into the experimental arena. In instances where a fish was accidentally air exposed, we noted the handling time to account for differences in handling stress prior to the start of the experiment.

The four experimental arenas (38 × 28 × 30 cm, L × W × H) had white, opaque sides and were devoid of structure other than a novel object affixed in the centre. Four different novel objects were used (one object was used per arena at any given trial), each measuring approximately 2 cm in diameter and between 2 and 8 cm in height. The objects differed in shape, height and colour to enhance the novelty of the stimuli across trials. The object was changed when the assay was repeated, and the order of objects was randomized between fish and trials such that individual fish were presented with each object only once. The experimenter positioned the shelter and the fish at a fixed, pre-determined location in the arena and exited the room for the duration of the trial to minimize disturbance. The fish was then free to exit its shelter and explore its surroundings over the 25-minute duration of the trial. Water height (seawater pumped directly from the reef) was maintained at 7 cm and the arena was emptied and rinsed with water between each trial. At the end of each trial, the fish was returned to its individual aquarium with its shelter.

To avoid observer bias, we extracted behavioural data from the videos using the automated tracking software ViewPoint (ZebraLab, Lyon, France). For each trial, we recorded five behavioural measurements: 1) emergence time (the time at which the fish's entire body first exited the shelter in seconds); 2) activity level (the distance covered during the trial [25 min] after

emergence in mm per minute), 3) sheltering (the time spent in the vicinity of the shelter [within 3 cm; approximately 1 body length; BL] in seconds); 4) thigmotaxis (the time spent within 3 cm [approximately 1 BL] of the arena walls in seconds); and 5) novel object inspection (time spent within 5 cm i.e. <2 BL, of the novel object) in seconds.

CO_2 exposure

Once all individuals had repeated the behavioural assay twice under ambient CO_2, half of the fish (n = 37) were maintained in ambient water at present-day pCO_2 (ambient; 458 ± 17.9 µatm; mean ± SD; min: 450 µatm, max = 490 µatm based on 55 measurements) and the other half (n = 37) were exposed to end-of-century pCO_2 levels (elevated; 1104 ± 175 µatm; mean ± SD, min: 660 µatm, max = 1490 µatm based on 55 measurements) for 4-6 days. This exposure time was chosen to be consistent with studies reporting altered behaviour in coral reef fishes and reports that longer exposure durations (10 or 25 days) do not alter the response any further (e.g. Munday *et al.* 2010; Allan *et al.* 2013; Munday *et al.* 2013). Control aquaria received seawater from one of two flow-through 32 L header tanks diffused with ambient air and filled at ~ 750 ml min^{-1} from seawater pumped from the reef (i.e., 18-19 holding aquaria per control header tank, flow through control seawater in each 1 L control holding aquaria: ~40 ml min^{-1}). CO_2-treatment aquaria received water from one of two additional aerated header tanks (32 L, flow-through filled at ~ 750 ml min^{-1} , i.e., 18-19 holding aquaria per CO_2 header tank, flow through CO_2 seawater in each 1 L CO_2 holding aquaria: ~40 ml min^{-1}) in which the pCO_2 level was gradually increased over 24 h using pH stat computers (Aqua Medic GmbH, Bissendorf, Germany) connected to solenoid valves regulating administration of 100% CO_2. We monitored pCO_2 in the header tanks and holding tanks multiple times daily using a handheld CO_2 meter (Vaisala GMT 222, Finland) connected to an aspiration

pump (Vaisala GM 70, Finland) and a submerged gas-permeable PFTE probe (Qubit Systems, Kingston, Canada) following established protocols (Hari *et al.* 2008; Jutfelt *et al.* 2013). The Vaisala CO_2 meter was factory calibrated prior to experiments. The experimental design and CO_2-dosing system thus followed best practices for ocean acidification research (Reibesell *et al.* 2011; Cornwall *et al.* 2015).

Experiment 2: predation trials

We collected 22 rock cod (*C. microprion*) between January 12th and 17th 2016 from lagoon reefs nearby LIRS using hook-and-line. They were transported to the aquarium facility in 20 L aerated buckets within two hours of capture and housed in large (~300 l) flow-through aquaria. We selected 14 individuals that began feeding within 12 h of capture and moved them to individual 32 L white opaque plastic aquaria (38 × 28 × 30 cm, L × W × H). Each aquarium contained a 12.5 cm long PVC pipe (55 mm diameter) providing shelter for the rock cod at one end of the tank. At the other end, we positioned six pieces of half PVC pipe (55 mm diameter) glued together to create a three-dimensional refuge for *P. amboinensis*, which was inaccessible to *C. microprion*. This allowed us to standardize the refuge size and location across all aquaria. The predator shelter and prey refuge were affixed to the bottom of the tank with silicone. This design intentionally lowered the probability of prey escaping the predator compared to the natural habitat because predation is a rare event that we wanted to observe in our experiment. To habituate rock cod in these aquaria, we fed them pieces of cut, defrosted pilchard during four days prior to the experiments. During this time, seven rock cod were kept in present-day control water (508 ± 48.4 µatm; mean ± SD) and seven were exposed to end-of-century pCO_2 levels (1229 ± 252 µatm; mean ± SD) with seawater temperature at approximately 29.5 ± 1˚C. CO_2 dosing and measurements followed the

304 procedures as detailed in the '*CO_2 exposure*' section above. Two header tanks were used for the
305 control fish and four for the CO_2 fish.

306 Predator trials commenced when Experiment 1 had been completed (January 28 – February
307 1). Twenty minutes before the onset of a predation trial, we introduced an opaque partition in the
308 middle of an aquarium containing a rock cod to restrict *C. microprion* to the area of the tank
309 containing its shelter. One *P. amboinensis* from Experiment 1 was introduced to the other half of
310 the tank, using its PVC shelter as in Experiment 1, and allowed a 20-minute exploration period.
311 We then lifted the partition, and allowed the predator and prey to interact for 60 min. We filmed
312 interactions with the same cameras as in Experiment 1 mounted above the aquarium (GoPro Hero
313 3+ black fitted with a commercial lens). Prey that were not consumed within 60 min were returned
314 to their holding aquarium. Control and CO_2-treated *P. amboinensis* were paired with a predator
315 receiving the same experimental treatment using stratified randomization so that each predator
316 received approximately the same number of prey, which spanned a range of behavioural scores.
317 Each predator was tested between 5 and 6 times with different prey individuals. Each predator was
318 tested only once in a given day and all predators were fed one piece of pilchard daily, in the
319 evening.

320

321 ***Statistical analysis***

322 *Experiment 1: behavioural trials before and after CO_2 exposure*

323 We checked the distributions of each of the five behavioural measurements (Fig. S2) and
324 determined appropriate transformations to comply with the normality assumption of model
325 residuals. We used the R package *lme4* (Bates *et al.* 2015) to fit five general Linear Mixed-effects
326 Models (LMM), one for each behavioural measurement. We specified the following fixed effects

in each model: treatment (binary variable: ambient vs. elevated CO_2); trial number (ordinal variable from 1 to 4 with trials 3 and 4 corresponding to elevated CO_2 exposure for the treatment group); the interaction between treatment and trial number; and the standard length of fish (continuous variable). The random effects for these models included trial number as random slopes and fish identity as random intercepts. Activity level and thigmotaxis (time spent close to the wall) were square-root-transformed; latency to approach a novel object was log-transformed after adding 0.5; time to emergence from the shelter was log-transformed; time spent in shelter was not transformed (Fig. S2).

We used the *brms* package (Bürkner 2017) to fit double-hierarchical general linear mixed-effects model (DHGLM), one for each of the five behavioural measurements. A DHGLMs comprises two parts, allowing variation in means and variation in variances to be modelled within a single framework (Cleasby *et al.* 2015; O'Dea *et al.* 2022). The mean component of the DGHLMs was identical to the LMMs above, and the variation component included the fixed effects treatment, trial, and their interaction, as well as the random effect fish identity. The DGHLM allowed us to investigate the effect of CO_2 treatment on both mean and residual variance (or within-individual variance), and to compute an estimate of population-wide variation in predictability (CV_P, the coefficient of variation in predictability) (Cleasby *et al.* 2015). Predictability refers to the level of variability in an individual's behaviour in a given environment (i.e., within-individual variation in behaviour), in contrast to repeatability, which is a measure of the consistency in how individuals differ in their average phenotypes (i.e., between-individual variation in behaviour; see below). CV_P represents standardized variation in within-individual variance among individuals and can serve as a standardized effect size for comparisons across traits, groups, or studies.

We used the *rptR* package (Stoffel *et al.* 2017) with 10,000 bootstrapping iterations to calculate three estimates of repeatability (*R*) for each of the five behavioural measurements: one for all fish, irrespective of treatment group (N=73); one for fish in the ambient CO_2 treatment group (N=37); and one for fish in the elevated CO_2 treatment group (N=36). In total, we computed 15 *R* estimates. *R* ranges between 0 and 1 and is the proportion of the total phenotypic variance attributable to differences between individuals; it provides a standardized measure of the consistency of phenotypes across time or contexts (Nakagawa *et al.* 2010; Roche *et al.* 2016). We note that *R* estimates can be obtained from DGHLMs, but these models could not provide separate estimates for the ambient and elevated CO_2 treatment groups in our study (see Cleasby *et al.* 2015). The *rptR* package uses mixed models from the *lme4* package to obtain the repeatability of behavioural measurements; we obtained 'adjusted' *R* estimates by fitting trial number as a fixed effect to control for time-related changes in behaviour (Mitchell *et al.* 2019). We tested whether *R* differed between the ambient and elevated CO_2 treatment groups for each behavioural measurement by calculating contrasts between the two groups (five contrasts in total). When the 95% confidence interval (CI) for a given contrast excluded zero, we considered the two *R* estimates to be statistically different.

Experiment 2: predation trials

We examined differences in the survival of elevated CO_2-exposed (N=34) and ambient CO_2 (N=34) Ambon damsel in predation trials with a Cox Proportional Hazards model, using the *survival* package (Therneau 2020). Five Ambon damsel used in *Experiment 1* were excluded from this analysis due to issues during the predation trials (i.e., camera malfunction, manipulation error). Seven explanatory variables were included in the model: CO_2 treatment, prey body size, and the

mean of each of the five behavioural measurements recorded four times per Ambon damsel in *Experiment 1*. Predator identity was specified as a cluster (i.e., random) variable to account for repeated measurements. We examined the assumption of proportional hazards using cox.zph() in the package *survival* and *ggcoxdiagnostics()* in the package *survminer* (Therneau 2020).

We examined differences in the number of predatory attacks resulting in capture for fish in the elevated CO_2 (N=30) and ambient CO_2 (N=28) groups with a Generalized Linear Mixed-effects Model (GLMM), specifying a Poisson error distribution. Fixed and random effects in the model were the same as in the survival analysis. Marginal ($R^2_{GLMM(m)}$) and conditional ($R^2_{GLMM(c)}$) R-squared values were computed with *r.squaredGLMM()* in the package *MuMIn* (Barton 2016). We examined model assumptions with a qqplot of residuals and other diagnostic plots of residuals using *simulateResiduals()* in the package *DHARMa* (Hartig 2018).

The assumptions of both models were met although the was an indication overdispersion and slight quantile deviations for the GLMM (Dispersion test P=0.040). All analyses were done in R 4.2.2 (R Core Team 2023). We used the packages *ggplot2* (Wickham 2016), *tidybayes* (Kay 2024), *emmeans* (Lenth 2025), and *survminer* (Therneau 2020) for data visualization.

Results

Experiment 1: behavioural trials before and after CO_2 exposure

Short-term exposure to projected end-of-century CO_2-induced acidification had no effect on any of the five behaviours measured in *P. amboinensis*, both in terms of group means (i.e., population-level) (Fig. 2, Table 1) and residual (i.e., within-individual) variance (Fig. 3, Table 1). Thigmotaxis decreased, and time spent near the novel object increased with trial number for fish in both the ambient CO_2 and elevated CO_2 groups, indicating habituation to the experimental setup and/or

procedure in both treatment groups (Fig. 2, Table 1). No such population-level trends were observed for emergence time, activity level, or time spent sheltering (Fig. 2, Table 1). Intra-individual variability decreased with trial number for all five behaviours for fish in both the ambient CO_2 and elevated CO_2 groups (Fig. 3), indicating some degree of habituation to the setup/manipulation.

All behaviours were moderately and significantly repeatable, with no marked differences in repeatability estimates between CO_2 groups (Table 2). There was substantial variability in predictability (i.e., within-individual variance) for all five behavioural measurements for both groups (CV_p; see Table 1).

Experiment 2: predation trials

The probability of survival in a predation trial was similar for Ambon damsel in the elevated CO_2 and ambient groups (Fig. 4), with fish in both treatments having a similar hazard of death (ambient CO_2 treatment hazard ratio relative to elevated CO_2 treatment group = 0.88, 95% CI = 0.43–1.78, P=0.719; Table 3). While body size, emergence time, activity level, time sheltering and thigmotaxis were unrelated to survival probability (Table 3), Ambon damsel that spent a greater amount of time inspecting a novel object had a lower probability of survival (hazard ratio = 2.66, 95% CI = 1.36–5.20, P=0.004; Table 3).

Rock cod often failed to capture their prey upon striking: only 6 of 58 Ambon damsel that were ultimately eaten were captured on the first strike, with several evading their predator more than 10 times (Fig. S4). Ten Ambon damsel were not captured during the 60-min trial (Fig. 4). The number of predatory attacks leading to capture was similar for fish in the elevated CO_2 (mean ± SD: 5.96 ± 3.18) and ambient CO_2 groups (mean ± SD: 5.39 ± 3.86) (GLMM estimate ± SE: -

418 0.040 ± 0.182, *P*=0.827; Table S1, Fig. S4). Predators required more attacks to capture large
419 Ambon damsel (estimate ± SE: 0.147 ± 0.067, *P*=0.027) as well as Ambon damsel that spent more
420 time near a shelter (estimate ± SE: 0.490 ± 0.243, *P*=0.044) and in the vicinity of the novel object
421 during the repeated behavioural trials (estimate ± SE: 0.386 ± 0.137, *P*=0.005) (Table S1; marginal
422 R^2_{GLMM}= 0.19; conditional R^2_{GLMM}= 0.35).
423
424 **Discussion**
425 Studies on the impacts of end-of-century ocean acidification on coral reef fish behaviour are
426 largely based on comparisons between treatment means rather than individual-level effects.
427 Although it has been argued that some individuals are potentially more tolerant to elevated CO_2
428 exposure than others (Tresguerres *et al.* 2017; Esbaugh 2018), the extent to which individual
429 behaviour affects responses to ocean acidification has never been tested.

430 We investigated the repeatability, individual- and population-level differences in five
431 commonly measured behaviours as well as survival outcomes during a predation trial in the context
432 of ocean acidification. All individuals habituated to the experimental set-up as evidenced by
433 decreased intra-individual variability over the course of the four behaviourial trials (Fig. 3).
434 Habituation to experimental arenas is common when repeatedly measuring behaviour, especially
435 in laboratory settings when the timing between assays is short (Martin *et al.* 2008; Blumstein
436 2016). Nevertheless, we found that all five behaviours measured were significantly repeatable in
437 both treatments, indicating that our assays measured consistent behavioural differences in Ambon
438 damsels. Our repeatability estimates (0.278 – 0.497; Table 2) are relatively high considering that
439 when measured in the laboratory and on ectotherms, estimates tend to be lower than when
440 measured in the field and/or on endotherms (Bell *et al.* 2009). Contrary to widespread assumptions,

441 this behavioural consistency was not affected by CO_2 treatment: both control and elevated CO_2
442 groups had similar estimates of repeatability in all behaviours, and population reaction-norms
443 followed similar trends in both groups even after CO_2 exposure (scenario B1 in Fig. 1). This
444 finding suggests that individual behavioural traits are not differentially impacted by short-term
445 CO_2 exposure, providing evidence refuting the suggestion that some individual phenotypes are
446 more tolerant or susceptible to elevated CO_2 exposure than others.

447 When population-level differences in behaviours were considered, exposure to projected
448 end-of-century CO_2 had no effect on any of the five behaviours measured in Ambon damselfish.
449 Our results are in line with Clark et al. (2020) suggesting that short-term CO_2 exposure does not
450 meaningfully alter coral reef fish behaviour. This result contrasts with earlier literature showing
451 large effects of short-term CO_2 exposure on many aspects of fish behaviour (reviewed in Briffa *et*
452 *al.* 2012; Clements *et al.* 2015; Clements *et al.* 2022; Sundin 2023; Clark *et al.* 2024). A lack of a
453 CO_2 treatment effect is perhaps not surprising since fishes have a well-developed acid-base
454 regulatory system, which allows them to maintain tissue pH under acidified conditions (Ishimatsu
455 *et al.* 2005). In fact, more than a century of research has shown that fish physiology is generally
456 tolerant to elevated CO_2 levels including those that exceed climate change projections (Clark *et al.*
457 2024). That behavioural responses are similarly resilient is consistent with this idea, and there is
458 growing empirical evidence of a decline effect in results testing for behavioural differences
459 between control individuals and individuals exposed to ocean acidification (Clements *et al.* 2022;
460 Clements *et al.* 2023). The results from our carefully designed study provide robust evidence that
461 fish behaviour is not altered following short-term exposure to elevated CO_2 whether individual or
462 population-level effects are considered. Although ocean acidification may indeed have negative
463 impacts on some marine life, especially calcifying organisms (Kroeker *et al.* 2010; Clark *et al.*

2024), our results highlight the growing agreement around the behavioural resilience of fishes to ocean acidification, as well as the value of transparent and reproducible research practices in achieving consensus around controversial research topics (O'Dea *et al.* 2021; Roche *et al.* 2022).

CO_2 treatment had no effect on the probability of prey fish surviving in a predation trial; Ambon damsel from both treatment groups had a similar hazard of death (Fig. 4). The number of predatory attacks leading to capture was also similar between treatment groups (Fig. S4). The experimental arena was intentionally designed to increase predator-prey encounter rates relative to those experienced on natural reefs so that predation attempts could be observed within our experimental period. Consequently, the assay was intended to provide a standardized comparison of relative predator evasion between experimental treatments rather than to estimate absolute predation risk in nature. Although escape opportunities for the damselfish in our arena were reduced compared with what they might have access to in the field, individuals frequently evaded repeated attacks and approximately 15% survived the entire trial (Fig. 4; S4), indicating that substantial variation in escape performance remained despite the constrained space. These results are consistent with several other studies suggesting that CO_2 exposure has limited effects on predator-prey interactions in coral reef fishes (Allan *et al.* 2017; Ferrari *et al.* 2017; McCormick *et al.* 2018), refuting early assertions, based on indirect evidence, that ocean acidification dramatically increases prey susceptibility to predation (e.g. Munday *et al.* 2010; Devine *et al.* 2013). Indeed, Allan et al. (2013) found that when both the predator and prey had been exposed to elevated CO_2 (as in our study), the capture rate was similar to that of the control group (where both predators and prey were exposed to present-day CO_2). Similarly, a study exploring the effect of elevated CO_2 on predation in freshwater fishes found no effect of CO_2 treatment on predator success or time to predation (Midway *et al.* 2017). Our results further highlight the importance of

behavioural tests on interacting animals when assessing the impact of anthropogenic stressors such as ocean acidification on complex ecological interactions such as predation.

Although elevated CO_2 had no impact on survival outcomes during predation trials in our study, we did observe small differences in the survival of Ambon damsel based on individual traits. We found that predators required more attacks to capture large Ambon damsel, which may be due to minor differences in mouth gape requirements of the predators and/or higher absolute burst swimming speeds in larger versus smaller Ambon damsel conspecifics (Taylor *et al.* 1985). While emergence time, activity level, time spent sheltering, and thigmotaxis behaviours were not related to individual survival, Ambon damsels that spent a greater amount of time inspecting the novel object had a lower probability of survival. This trend was independent of CO_2 treatment. Our results are consistent with the notion that bolder individuals face higher predation risk than shy individuals (Hulthén *et al.* 2017). However, as four out of five behaviours tested had no relationship with survival, the role of individual behavioural traits in predicting predation risk should be interpreted with caution. Furthermore, although we predicted higher mortality in bolder fish from the elevated CO_2 treatment group compared to the control group, the effect of behaviour on survival outcome was independent of CO_2 exposure. This result runs counter to those of several studies that have linked elevated CO_2 to increased boldness in fishes (e.g. Ferrari *et al.* 2011; Munday *et al.* 2014; Ferrari *et al.* 2017; Mitchell *et al.* 2022). Again, our results align with more recent studies demonstrating negligible effects of ocean acidification on fish behaviour (Clark *et al.* 2020; Clements *et al.* 2022; Clements *et al.* 2023; Clark *et al.* 2024) and do not support the notion of differences in individual tolerance or susceptibility to ocean acidification within a fish population.

We used elevated CO_2 exposure durations of 4-6 days in our experiments, which is consistent with studies that remain the most highly cited in the field (see Clements *et al.* 2022), and comparable with recent studies testing OA-induced behavioural alterations (e.g. 5-7 days in Hamilton *et al.* 2023; 10 days in Durant *et al*. 2023). In the limited number of studies that have examined the topic, longer exposure durations have not been reported to cause more pronounced behaviour differences between treatment groups: Munday *et al.* (2010) concluded for larval/settlement stages of clownfish and damselfish that behavioural impairments were maximized at 4 days, and Munday *et al.* (2013) did not report any change in the behavioural impairments of juvenile coral trout (*Plectropomus leopardus*) after 28 days of elevated CO_2 exposure compared with the impairments already existing after 4 days suggesting that behavioural impairments do not increase proportionally with exposure time. Clark *et al.* (2024) analysed existing data for the effects of CO_2 on chemical cue detection and found no effect of fish age or duration of CO_2 exposure on the outcomes (see fig. 5.2, table 5.2 in Clark *et al.* 2024). Thus, it is unlikely that the lack of behavioural differences we observed is solely due to the short duration of CO_2 exposure used.

Conclusion

Ocean acidification presents a growing threat to marine ecosystems globally because of anthropogenic activities. Concerns for how this change in water chemistry will impact aquatic organisms is warranted and an important area of continued research. Yet, there are limited resources to understand the complexities of ocean acidification impacts on marine life and thus researchers should focus their time and effort on species, lifestages, interactions and physiological processes that are most likely to suffer consequences as a result of ocean acidification in order to

532 inform decision making and conservation strategies (Buxton *et al.* 2021). Based on our results, as
533 well as the growing body of evidence from different species and study systems, we suggest that
534 fish behaviour is not likely to be meaningfully impacted by near-future ocean acidification levels.
535 Thus, future research should prioritize studying how other aspects of elevated atmospheric CO_2
536 impact marine fishes, including the rapid rise in the temperature of the global oceans.

537

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546

547 **Conflicts of Interest**

548 None to declare.

549

550 **Author contributions**

551 Dominique G. Roche, Ben Speers-Roesch, Josefin Sundin, Timothy D. Clark and Sandra A.
552 Binning conceived the ideas and designed methodology; Dominique G. Roche, Ben Speers-Roesch
553 and Sandra A. Binning collected the data; Timothy D. Clark processed the data; Dominique G.
554 Roche and Shinichi Nakagawa analysed the data; Dominique G. Roche and Sandra A. Binning led

555 the writing of the manuscript with substantial input from Josefin Sundin and Timothy D. Clark.
556 All authors contributed critically to the drafts and gave final approval for publication. Our study
557 brings together authors from a number of different countries, including two scientists based in
558 Australia where the study was carried out.
559

560 **Data and code availability statement**

561 The data and analytical code to reproduce the results of this study were shared with the editors and
562 reviewers on submission and are publicly available on the Open Science Framework:
563 https://doi.org/10.17605/OSF.IO/CZVSF.
564

565 **Ethics statement**

566 Animals were collected and cared for under Marine Parks Permit no. G13/35909.1 issued by the
567 Australian Government's Great Barrier Reef Marine Park Authority. All experiments were
568 approved by the Animal Experimentation Ethics Committee at James Cook University (permit no.:
569 A1924) according to the Australian Code of Practice for the Care and Use of Animals for Scientific
570 Purposes, 7th edition 2007. AI was not used in any part of the study, data analysis, or manuscript
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572

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759

TABLES

Table 1. Estimates and 95% confidence intervals (CI) for predictors included in five double hierarchical linear mixed-effects models (DHGLMs) assessing how end-of-century CO_2-induced aquatic acidification affects five behavioural measurements in the Ambon damselfish (*Pomacentrus amboinensis*): activity level (Activity), emergence time (Emergence), time spent inspecting a novel object (Novel), time sheltering (Sheltering), time close to the arena walls (Thigmotaxis). Predictors 1-4 relate to the mean component of the DHGLMs and predictors 5-7 relate to the dispersion component (i.e., residual variances for individuals). Estimates in bold have a 95% credible interval non-overlapping zero.

Predictor	**Activity (95% CI)**	**Emergence (95% CI)**	**Novel (95% CI)**	**Sheltering (95% CI)**	**Thigmotaxis (95% CI)**
Intercept	57.74 (53.40, 61.90)	2.45 (1.74, 3.18)	0.70 (0.39, 1.02)	32.17 (27.69, 36.69)	3.55 (3.17, 3.92)
sigma_Intercept	2.37 (2.10, 2.63)	0.73 (0.50, 0.95)	-0.44 (-0.66, -0.22)	2.41 (2.19, 2.62)	0.23 0.01, 0.43)

1. groupcontrol	1.05	0.09	0.27	-1.37	-0.48
	(-4.65, 7.00)	-0.92, 1.08)	(-0.17, 0.73)	(-7.76, 4.90)	(-1.01, 0.04)
2. trial	-0.96	-0.13	**0.51**	2.53	**-0.77**
	(-6.60, 4.83)	(-0.80, 0.56)	(0.18, 0.83)	(-3.08, 8.17)	(-1.31, -0.21)
3. SL	0.24	0.6	0.19	-5.09	0.16
	(-5.60, 6.13)	(-0.40, 1.61)	(-0.24, 0.63)	(-11.48, 1.46)	(-0.36, 0.66)
4. groupcontrol:trial	3.42	**-1.64**	0.24	-4.5	0.30
	(-4.51, 11.30)	(-2.54, -0.70)	(-0.21, 0.69)	(-12.29, 3.09)	(-0.48, 1.07)
5. sigma_groupcontrol	0.01	-0.13	0.06	-0.06	-0.12
	(-0.34, 0.37)	(-0.43, 0.19)	(-0.24, 0.36)	(-0.35, 0.22)	(-0.39, 0.15)
6. sigma_trial	**-0.56**	-0.2	-0.24	-0.22	-0.24
	(-1.01, -0.11)	(-0.58, 0.15)	(-0.69, 0.23)	(-0.66, 0.26)	(-0.64, 0.16)
7. sigma_groupcontrol:trial	0.15 (-0.48, 0.80)	0.27 (-0.25, 0.82)	0.13 (-0.5, 0.78)	-0.34 (-1.01, 0.28)	-0.23 (-0.78, 0.31)

768

Table 2. Estimates of repeatability (*R*) and variation in predictability (CVp) for five behavioural traits measured experimentally four times in the Ambon damselfish, *Pomacentrus amboinensis*. Behaviour was assessed in all fish under ambient CO_2 conditions twice, and thereafter twice more after having exposed half of the fish to elevated CO_2. The difference in *R* between the ambient CO_2 group (control) and elevated CO_2 treatment group for each behaviour is presented as a contrast (Contrast). 95% confidence intervals are indicated in parentheses. The behaviours measured were activity level (Activity), emergence time (Emergence), time spent inspecting a novel object (Novel), time sheltering (Sheltering), and time close to the arena walls (Thigmotaxis).

Behaviour	**Overall *R* (95% CI)**	**Control *R* (95% CI)**	**Treatment *R* (95% CI)**	**Contrast (95% CI)**	**CVp (95% CI)**
Activity	0.278 (0.149, 0.402)	0.255 (0.068, 0.427)	0.303 (0.113, 0.472)	0.046 (-0.211, 0.302)	0.805 (0.625, 0.997)
Emergence	0.298 (0.169, 0.424)	0.317 (0.124, 0.488)	0.303 (0.118, 0.478)	-0.015 (-0.269, 0.247)	0.754 (0.533, 0.967)
Novel object	0.497 (0.363, 0.605)	0.568 (0.390, 0.703)	0.405 (0.210, 0.571)	-0.162 (-0.403, 0.082)	0.636 (0.324, 0.856)

Shelter	0.381 (0.247, 0.502)	0.427 (0.230, 0.588)	0.340 (0.147, 0.509)	-0.085 (-0.335, 0.174)	0.545 (0.202, 0.775)
Thigmotaxis	0.245 (0.118, 0.368)	0.243 (0.066, 0.413)	0.225 (0.043, 0.398)	-0.020 (-0.271, 0.229)	0.568 (0.175, 0.805)

777

778 **Table 3.** Hazard ratios (HR) for fixed-effects predictors in a Cox Proportional Hazards model
779 examining differences in survival probability for CO_2-exposed (N=34) and control (N=34) Ambon
780 damsel (*Pomacentrus amboinensis*) (prey). A hazard ratio lower than 1 indicates reduced hazard
781 of death whereas a hazard ratio greater than 1 indicates an increased hazard of death. 95%
782 confidence intervals, Z scores, and p-values are indicated. The predator (*Cephalopholis*
783 *microprion*) was exposed to the same conditions of water chemistry as the prey.
784

Predictor	**HR**	**95% CI**	**Z**	***P***
Prey standard length	1.11	0.87, 1.42	0.833	0.405
Treatment_control	0.88	0.43, 1.78	-0.360	0.719
Emergence time	1.11	0.84, 1.47	0.746	0.456
Activity level	1.36	0.87, 2.14	1.349	0.177
Time sheltering	2.99	0.90, 9.96	1.785	0.074
Thigmotaxis	1.60	0.69, 3.71	1.084	0.278
Novel object	2.66	1.36, 5.20	2.871	0.004

785

FIGURES

Figure 1. Examples of hypothetical behavioural reaction norms from exposure to high CO_2. On average, individuals might exhibit either (A) an increase or (B) no change in a given behavioural trait (e.g. activity, anxiety). Different combinations of individual-level responses can underpin population-level patterns, where individuals might or might not respond similarly to high CO_2 exposure. A population-level increase in behaviour can result from (A1) all individuals increasing their behaviour or (A2-A3) some individuals exhibiting a sharp increase in behaviour and others, no change in behaviour. A lack of population-level change in behaviour can result from: (B1) the behaviour of all individuals remaining unchanged; (B2) differences in the behaviour of individuals accentuating; or (B3) no consistent changes in behaviour across individuals.

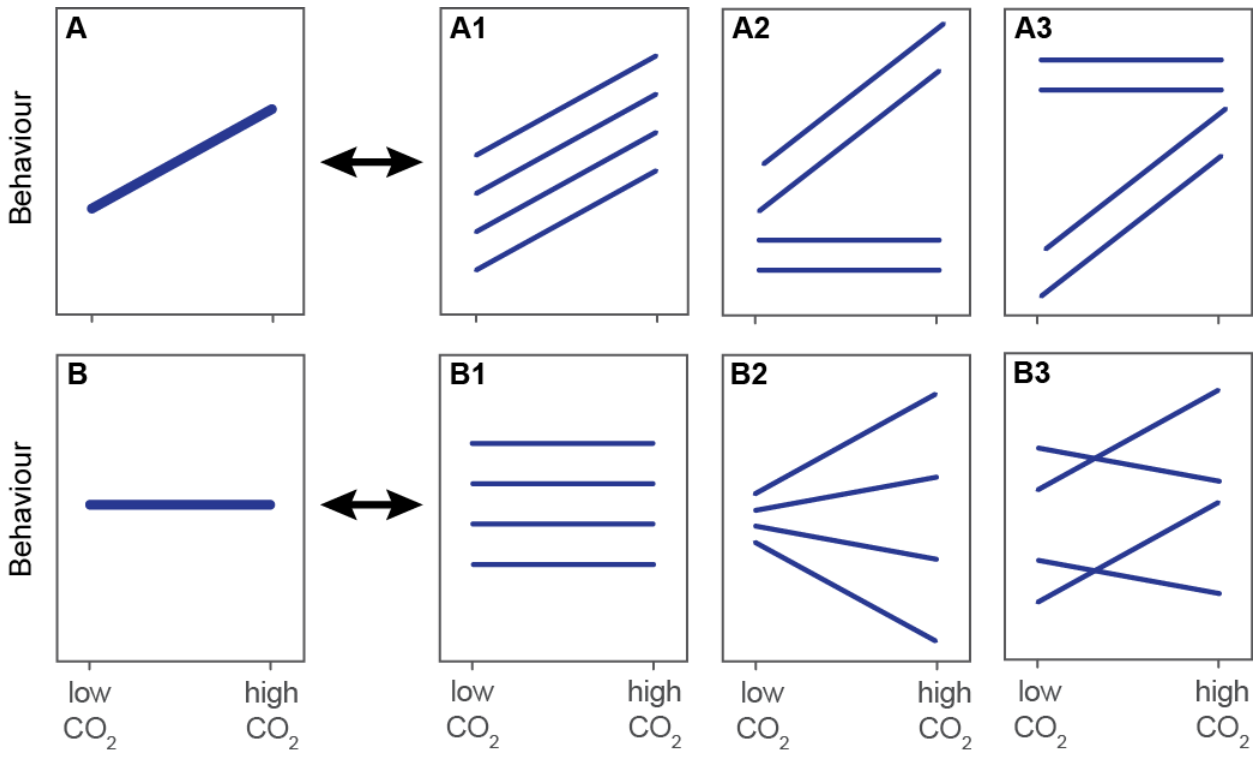


Figure 2. Reaction norm plots of 5 behavioural measurements recorded across 4 trials for Ambon damselfish (*P. amboinensis*) exposed to present-day levels of dissolved CO_2 (Ambient CO_2; n=36) and projected end-of-century CO_2-induced aquatic acidification (Elevated CO_2, n=37): (A) time to emergence from the shelter, (B) activity, (C) time sheltering, (D) thigmotaxis and (E) inspection of a novel object. Fish in the control group were exposed to present-day CO_2 levels in all 4 trials, whereas fish in the CO_2 group were exposed to present-day CO_2 levels in trials 1-2 and projected end-of-century CO_2 levels in trials 3-4. Data points and coloured lines represent individual fish; the black line is the population-level (i.e., mean) response with the 95% CI credible interval indicated by dotted lines. Non-transformed data are shown in Figure S3.

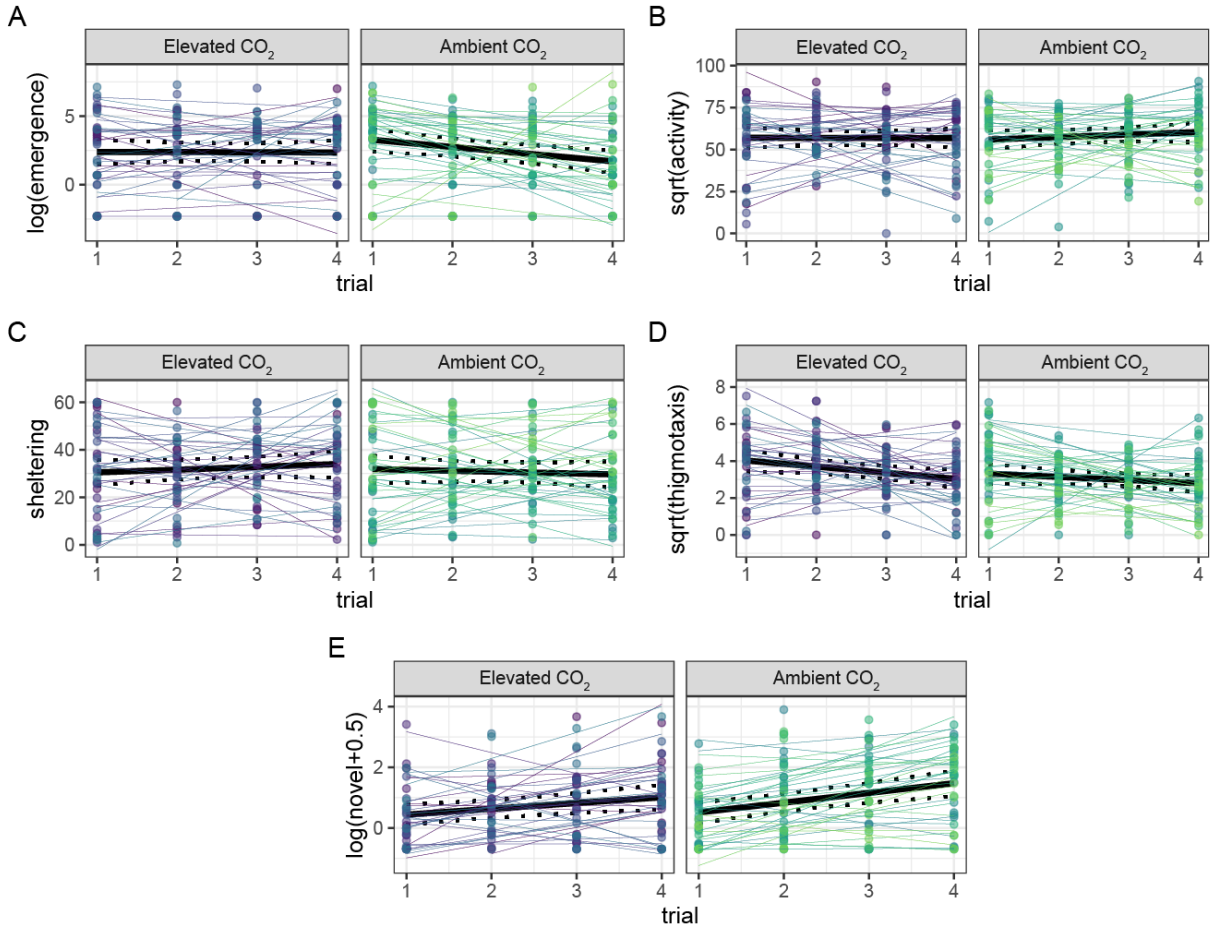


Figure 3. Intra-individual variation (residual variance for individual fish) in 5 behavioural measurements across 4 trials in Ambon damselfish (*P. amboinensis*) exposed to present-day levels of dissolved CO_2 (Ambient CO_2, n=36) and projected end-of-century CO2-induced aquatic acidification (Elevated CO_2, n=37): (A) time to emergence from the shelter, (B) activity, (C) time sheltering, (D) thigmotaxis, and (E) inspection of a novel object. Fish in the control group were exposed to present-day CO_2 levels in all 4 trials, whereas fish in the CO_2 group were exposed to present-day CO_2 levels in trials 1 and 2, and projected end-of-century CO_2 levels in trials 3 and 4.

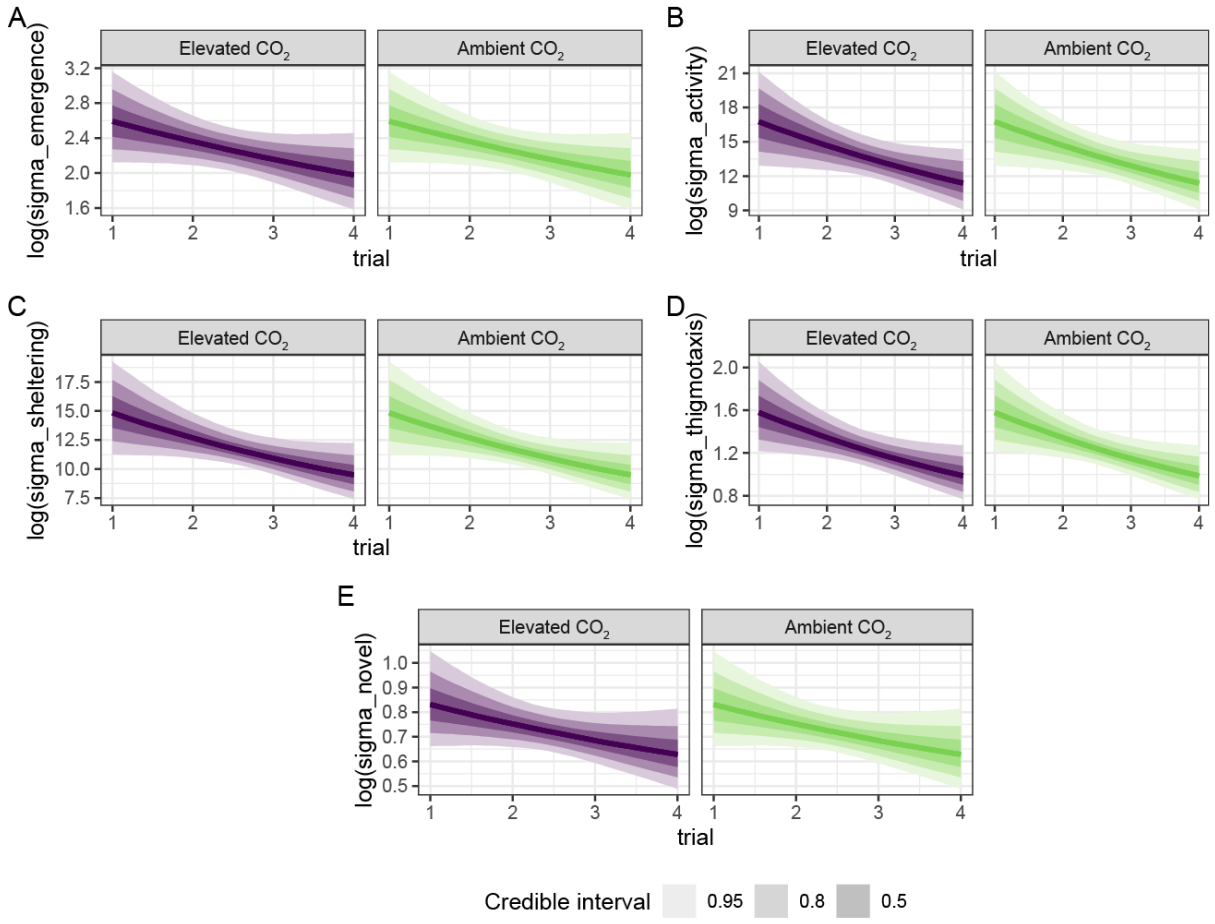
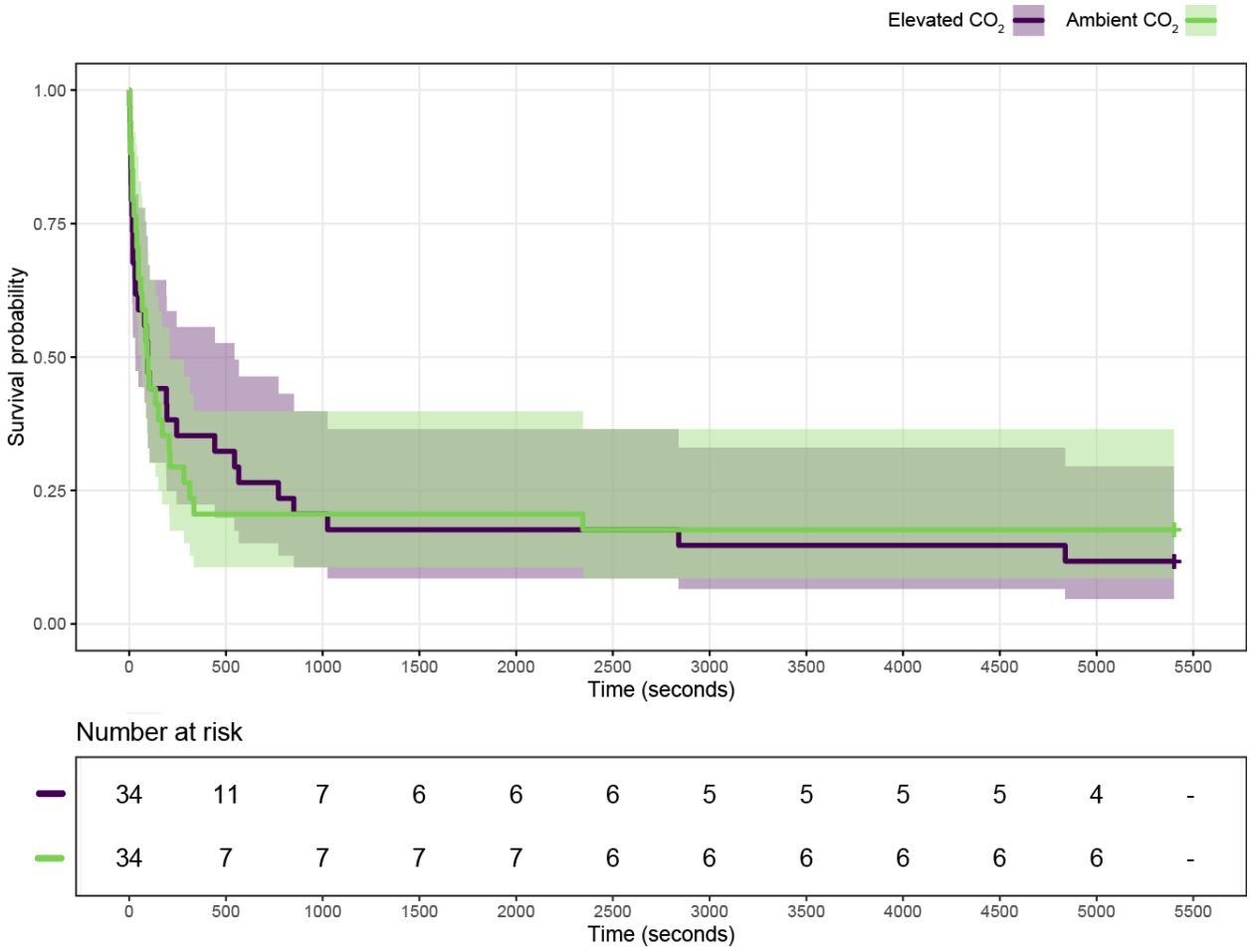


Figure 4. Kaplan Meier survival curves for Ambon damselfish (*P. amboinensis*) exposed to present-day levels of dissolved CO_2 (Ambient CO_2, n=34) and projected end-of-century CO_2-induced aquatic acidification (Elevated CO_2, n=34). Fish were subjected to a 90 min predation trial involving a predator, the rock cod *Cephalopholis microprion*, exposed to the same conditions of water chemistry.



SUPPLEMENTARY MATERIAL

No effect of short-term exposure to ocean acidification on individual-level variation in behaviour and susceptibility to predation in a Great Barrier Reef damselfish

Table S1. Summary table with estimates, standard errors (SE), Z and *P* values for fixed effects included in a Poisson generalized linear mixed-effects model examining differences in number of predatory attacks that resulted in the capture of Ambon damsel (*Pomacentrus amboinensis*) in ambient CO_2 (N=28) and elevated CO_2 conditions (N=30). The sample size excludes Ambon damsel that were not captured. Predators (*Cephalopholis microprion*) were exposed to the same conditions of water chemistry as their prey. Predator identity was included as a random effect.

Explanatory variable	**Estimate**	**SE**	**Z**	***P***
Prey standard length	0.147	0.067	2.210	0.027
Mean emergence time	0.017	0.075	0.223	0.823
Mean time active	0.168	0.108	1.555	0.120
Mean time in/near shelter	0.490	0.243	2.012	0.044
Mean time displaying thigmotaxis	0.260	0.146	1.785	0.074
Mean time near novel object	0.386	0.137	2.815	0.005
Treatment_control	-0.040	0.182	-0.219	0.827

Figure S1. Graphical representation of the experimental timeline used to examine the effect of exposure to end-of-century CO_2 levels on personality and susceptibility to predation in *Pomacentrus amboinensis*. Fish personality was assessed by repeatedly measuring behaviour in the same fish in a novel environment assay on specific days (orange arrows) during 4 days exposure to present day, ambient, CO_2 (control, n=74) followed the next day by treatment with either present day CO_2 or elevated CO_2 for 6 days (n=37 each). At the end of the CO_2 treatments, each fish was exposed to a predator-prey trial (using *Cephalopholis microprion* as predator) under the same CO_2 treatment.

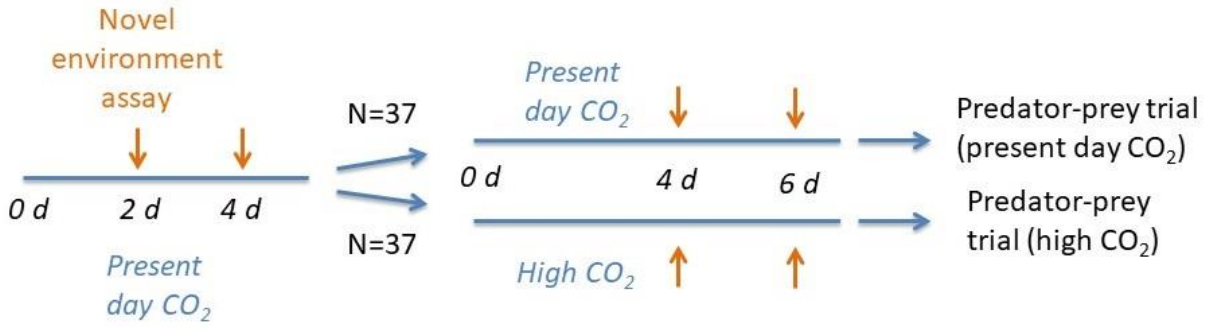


Figure S2. The distribution of model residuals (m_) after the following transformations: time to emergence from the shelter (emergT) was log-transformed; activity levels (activity) and thigmotaxis (thigmotaxis) were square-root-transformed; time spent sheltering (shelter) was not transformed; and time near a novel (novel) object was log-transformed after adding 0.5.

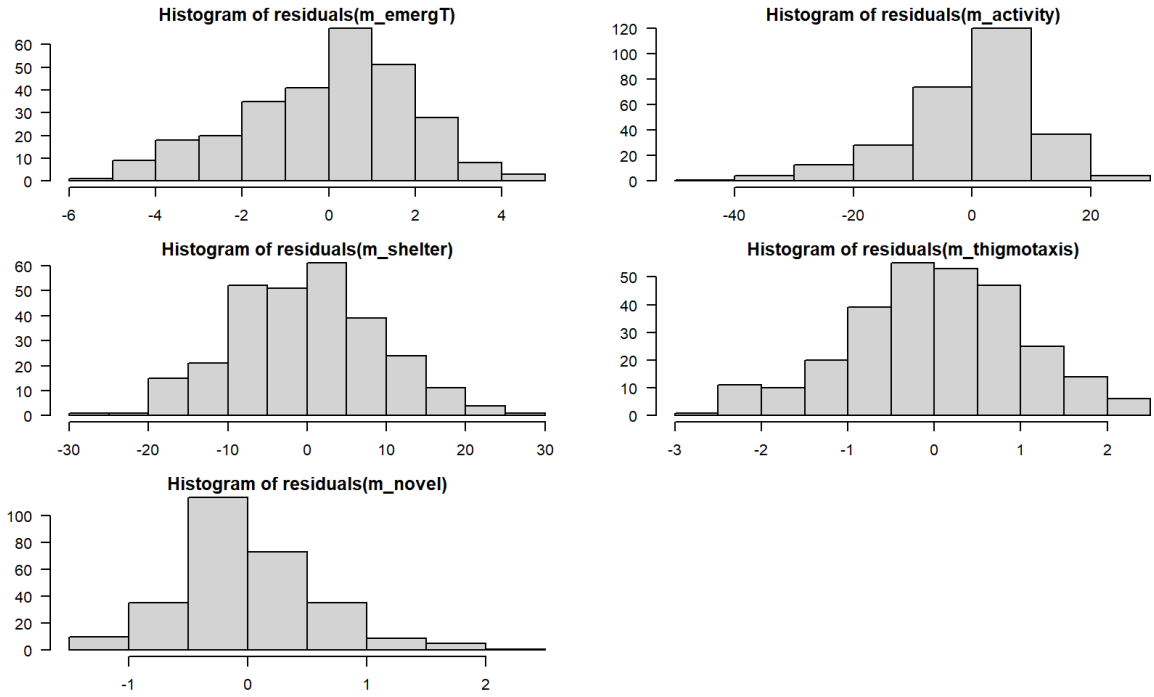


Figure S3. Reaction norm plots of non-transformed data for 5 behavioural measurements recorded across 4 trials for Ambon damselfish (*P. amboinensis*) exposed to present-day levels of dissolved CO_2 (Ambient CO_2; n=36) and projected end-of-century CO_2-induced aquatic acidification (Elevated CO_2, n=37): (A) emergence, (B) activity, (C) time sheltering, (D) thigmotaxis and (E) inspection of a novel object. Fish in the control group were exposed to present-day CO_2 levels in all 4 trials, whereas fish in the CO_2 group were exposed to present-day CO_2 levels in trials 1-2 and projected end-of-century CO_2 levels in trials 3-4. Data points and coloured lines represent individual fish; the black line is the population-level (i.e., mean) response with the 95% CI credible interval indicated by dotted lines.

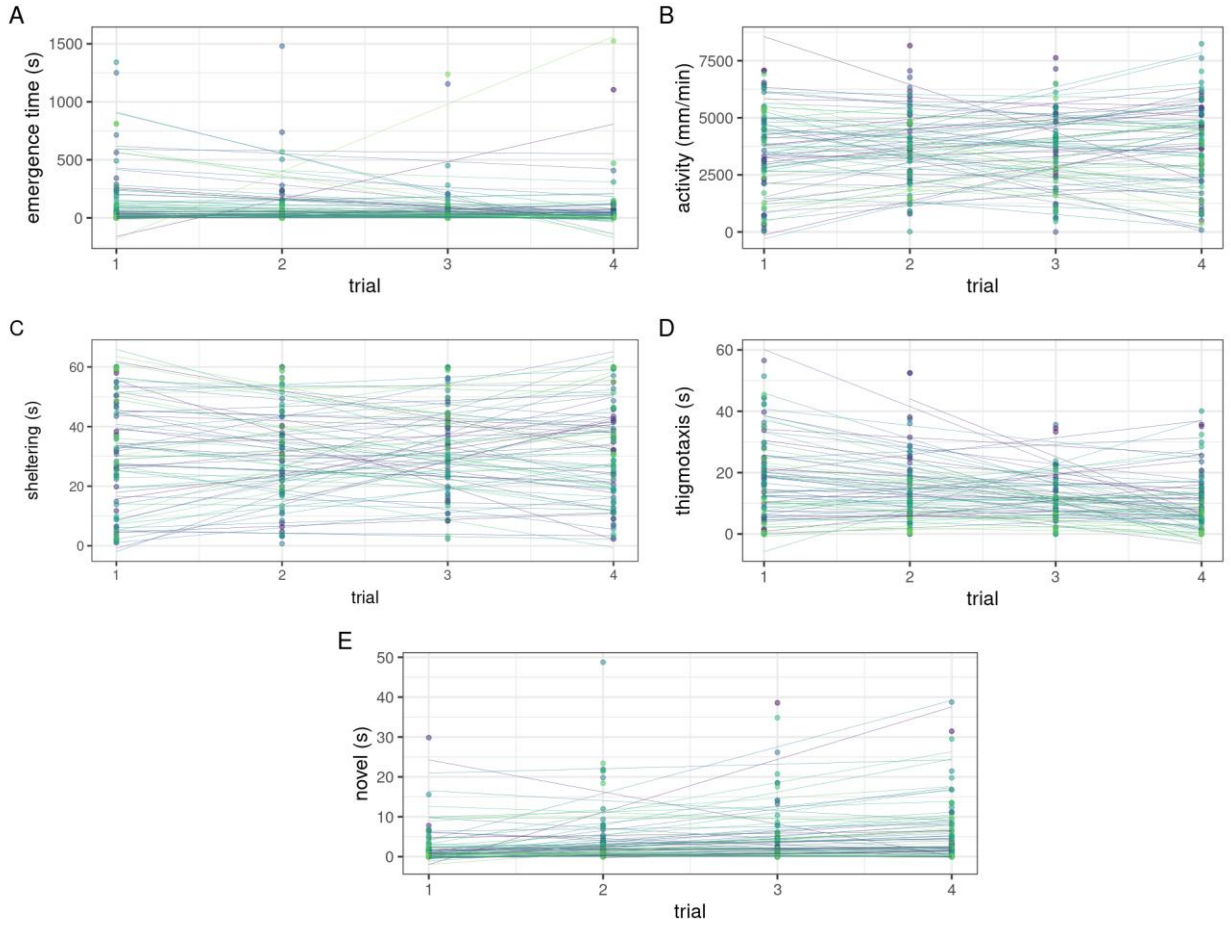


Figure S4. The number of predatory attacks by dot-head rock cod (*Cephalopholis microprion*) resulting in the capture of Ambon damsel (*Pomacentrus amboinensis*) in ambient CO_2 (N=28) and elevated CO_2 (N=30) treatment groups. Ambon damsels that were not captured are not shown.