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1 **Living in a high CO₂ world: a global meta-analysis shows multiple trait-mediated responses**
2 **of fish to ocean acidification.**

3
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20
21 **Running headline:** Fishes response to ocean acidification

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23 **Type of manuscript:** Review

25 **ABSTRACT**

- 26 1. Understanding how marine organisms will be affected by global change is of primary
27 importance to ensure ecosystem functioning and human well-being through nature's
28 contribution to people. This study meets the call for addressing how life-history traits
29 mediate effects of ocean acidification on fish.
- 30 2. We built a database of overall and trait-mediated responses of teleost fish to future CO₂
31 levels by searching the scientific literature. Using a meta-analytical approach, we
32 investigated the effects of projected CO₂ for 2050-2070 and 2100 on fish eco-physiology
33 and behaviour from 327 experiments on 44 species, stemming from polar to tropical
34 regions.
- 35 3. With anticipated CO₂ emission scenarios, multiple CO₂-dose dependent effects on
36 calcification, resting metabolic rate, yolk, behavioural performances along with increased
37 predation risk and decreased foraging will occur, particularly for larvae. Importantly, no
38 traits confer fish tolerance to elevated CO₂.
- 39 4. Far-reaching ecological consequences on fish population replenishment and community
40 structure are likely to occur.
- 41 5. Fish are a key resource for livelihoods in coastal communities and a key component for
42 ecosystem stability. Given the multiple CO₂-dose dependent effects evidenced here,
43 especially for larvae, we stress the need to expand the number and duration of ocean
44 acidification studies to multi-generational, multiple stressor (e.g. warming, hypoxia,
45 fishing) and species interactions experiments to elucidate complex ecosystem-level
46 changes and how these changes alter ecosystem services supply.

47
48 **Keywords:** Behaviour, Calcification, Development, Fish-traits, Growth, Heterogeneity,
49 Metabolism, Survival, Teleosts.

50

51 INTRODUCTION

52 By absorbing >25% of the carbon dioxide (CO₂) and >90% of the heat accumulating in the
53 atmosphere, the ocean plays a key role in regulating the Earth's climate (Gattuso et al., 2015).
54 However, this occurs at a cost, as changes in the ocean's carbonate system resulting from
55 increasing anthropogenic CO₂ emissions – a process termed ocean acidification (OA) – have been
56 progressing since the industrial revolution at a historically unprecedented rate (Doney et al., 2009;
57 Hönisch et al., 2012). OA is predicted to rise even more rapidly during this century, as average
58 ocean *P*CO₂ (partial pressure CO₂) could reach ~1000 μatm (Meinshausen et al., 2011) leading to
59 0.3 units reduction in pH (Bopp et al., 2013; Stocker et al., 2013; Pörtner et al., 2014; McNeil &
60 Sasse, 2016), well beyond the threshold expected to be detrimental to many marine organisms
61 (Kroeker et al., 2013). This may have far-reaching consequences for marine ecosystems, including
62 biodiversity loss and changes to marine community structure (Nagelkerken & Connell, 2015).

63 International organizations warn that global economy could risk losing as much as \$1 trillion
64 USD annually if countries do not take urgent steps to slow down anthropogenic CO₂ emissions by
65 the end of the century (CBD, 2014). Reflecting mounting concerns, research attempts to predict
66 biological impacts of OA have gone through a striking increase in publishing rate (with more than
67 500 articles published in 2013), which is unparalleled by any other scientific field (Riebesell &
68 Gattuso, 2015).

69 Biological consequences of OA are fundamentally linked to the ecology and physiology of
70 marine species (Pörtner et al., 2014; Heuer & Grosell, 2014). Existing quantitative syntheses have
71 found that effects vary among life stages, species or broader taxonomic groups (Hendriks et al.,
72 2010; Kroeker et al., 2010; Harvey et al., 2013; Kroeker et al., 2013; Wittmann & Pörtner, 2013).
73 Fish were initially thought to be resilient to OA as they are able to defend their internal pH in a
74 high CO₂ environment through active ion transport (Claiborne et al., 2002; Brauner & Baker 2009;

75 Melzner et al., 2009). Nevertheless, increased acid-base regulation activity may have energetic
76 consequences that might affect physiology and development, especially in early life stages
77 (Ishimatsu et al., 2008). More recently, the compensatory responses that occur during acid-base
78 regulation were found to influence fish calcification, behaviour and ion transport (Nilsson et al.,
79 2012; Heuer & Grosell, 2014; 2016). Calcification in fish occurs in the inner ear for formation of
80 otoliths, carbonated structures playing a role in sound detection, acceleration and orientation, and
81 in the intestinal lumen, where calcium carbonate is precipitated to excrete excess calcium ions
82 (Heuer & Grosell, 2014). Fish potentially contribute with up to 45% of global calcium carbonate
83 budget (Wilson et al., 2009), therefore playing an important role for ocean chemistry and carbon
84 sequestration. Most notably, changes in the concentrations of acid-base relevant ions at higher CO₂
85 levels appear to impair the function of neurotransmitter receptors causing a range of behavioural
86 changes (Nilsson et al., 2012; Hamilton et al., 2014; Heuer & Grosell, 2014). In fish, documented
87 behavioural effects of OA involve impaired learning ability, sensory functions or decision-making,
88 along with disrupted anti-predator responses, increased boldness, and altered homing behaviour
89 (see Briffa et al., 2012; Heuer & Grosell, 2014; Clements & Hunt, 2015, for reviews). Several
90 studies also documented that exposure to elevated CO₂ alters fish lateralization, the preference for
91 left or right side during behavioural activity that confers benefits in terms of spatial orientation,
92 reactivity, cognition and group coordination (Domenici et al., 2012; 2014 Jutfelt et al., 2013).
93 Behavioural and sensory functions changes may affect key ecological processes such as predator-
94 prey interactions, dispersal, settlement and habitat choice, with cascading implications at the
95 community level (Nagelkerken & Munday, 2016).

96 To date, the large majority of studies documenting effects of high levels of *PCO*₂ on fish
97 have been performed in laboratory conditions, showing variable and sometime contradictory
98 results or in some instances underestimating the potential ability of fish to acclimate and adapt to
99 predicted OA in the long term (Wittmann & Pörtner 2013). Indeed, species sensitivity to OA may

100 depend on some life history traits that could confer tolerance to the expected changes in CO₂
101 levels. For instance, species living in pelagic environments, where CO₂ concentrations are stable
102 over time could be more sensitive to OA than coastal benthic species that usually experience high
103 fluctuations of CO₂ concentrations due to the alternation of respiration and photosynthesis
104 processes by reef organisms (Munday et al. 2008; Hoffman et al. 2011).

105 To date, existing quantitative generalisations of OA consequences for fish under different
106 CO₂ projections have been controversial, with uncertainty associated with the small number of
107 experiments conducted (Harvey *et al.* 2013; Kroeker *et al.* 2013; Wittmann & Pörtner 2013). A
108 more comprehensive picture of fish responses across different species characteristics and altered
109 CO₂ concentrations is now possible, as research on the topic has progressed in the few last years.
110 Indeed, this represents an opportunity to test the robustness of the patterns found in previous
111 studies and to highlight more general insights.

112 Here, we used a mixed-effects meta-analytical approach to investigate the effects of
113 projected CO₂ for 2050-2070 and 2100 under intermediate or high emission scenarios (RCP 6 and
114 8.5, respectively; Meinshausen *et al.* 2011; Pörtner *et al.* 2014), on several response variables
115 extracted from 75 studies (327 contrasts) on 44 species, from polar to tropical regions. We
116 classified and analysed potential effects of OA according to two broad types of fish responses: (1)
117 the eco-physiological responses (mortality, reproduction, metabolism, growth, calcification and
118 yolk dimension); and (2) the behavioural responses (i.e. effects on fish behaviour and activity
119 levels). To assess potential differences in responses, we also analysed such response variables both
120 in short and longer term CO₂ exposure experiments, as well as under chronic exposure in natural
121 CO₂ seeps.

122 In addition to this, since fish responses to OA can vary among ontogenetic stages and
123 species traits, we also examined the effects of elevated CO₂ on the above response typologies
124 across different life-stages, physiological types (i.e. stenohaline or euryhaline), climatic zones, and

125 habitats (see Appendix S1 for details). Specific hypotheses associated to these different fish
126 characteristics were the following. 1) Due to their inefficient system for ionic regulation, we
127 expected fish early life stages (i.e. embryos, larvae and juveniles) to be more affected by elevated
128 CO₂ levels than the adult stage. 2) Stenohaline species should be more sensitive than euryhaline
129 fish, as these latter experience highly fluctuating *PCO*₂ environments during their life cycle. 3)
130 The eco-physiology and behaviour of tropical and polar species is more disrupted under OA
131 conditions than that of temperate and sub-tropical ones, which evolved in a relatively more
132 variable pH/CO₂ environment. 4) Similarly, being adapted to more stable CO₂ levels in offshore
133 waters, pelagic species should be more sensitive than benthic and benthic-pelagic ones.

134 Several studies elucidated a clear relation between fish brain function, behaviour and
135 ecological processes under expected OA levels (Nilsson *et al.* 2012; Chivers *et al.* 2014; Lai *et al.*
136 2015). Documented fish behavioural changes in fish are a consequence of (upstream)
137 neurosensory disruptions (Heuer & Grosell 2014; 2016), and may lead to altered (downstream)
138 ecological processes (recruitment, predation or competition; Nagelkerken & Munday 2016).
139 Therefore, we also assessed to what extent fish behavioural responses were related to
140 neurosensory (cognition, audition, olfaction, vision, and a combination among olfaction and
141 vision) and ecological (foraging, predation risk and habitat choice) categories under elevated CO₂
142 levels (see Appendix S1 for details).

143

144

145 **MATERIAL AND METHODS**

146 *Data collection and selection criteria*

147 We searched for studies testing the effects of altered *PCO*₂ levels on marine teleost fish
148 published online or printed through to January 2017 using Google Scholar and ISI Web of Science
149 with the combination of the following three keywords: ocean acidification, fish and CO₂. We also

150 used the reference lists of the retrieved papers. In addition, we searched for OA papers in the news
151 stream managed by the Ocean Acidification International Coordination Centre (OA-ICC;
152 <http://news-oceanacidification-icc.org>). Papers not reporting mean responses, error estimates and
153 sample sizes in at least one control CO₂ condition and one elevated CO₂ treatment were discarded
154 (in few instances, corresponding authors were contacted to obtain missing information).

155 As seawater carbonate chemistry varies regionally and between ecosystems (Hofmann *et al.*
156 2011), control CO₂ levels used in the retrieved experiments spanned from 300 to 800 $\mu\text{atm } P\text{CO}_2$.
157 Therefore, we considered for each study a $P\text{CO}_2$ range ($\Delta P\text{CO}_2$) – expressed as the absolute
158 difference between the $P\text{CO}_2$ values in the elevated and in the control CO₂ condition – that was
159 assigned to one of the following OA projections under intermediate or high emission scenarios
160 (RCP 6 and 8.5, respectively; Meinshausen *et al.* 2011): (1) moderate $P\text{CO}_2$ expected by 2050-
161 2070 following the RCP 8.5 (business-as-usual) scenario or expected by 2100 following the RCP
162 6 scenario (~500-700 μatm ; $\Delta P\text{CO}_2 \leq 350 \mu\text{atm}$); (2) high $P\text{CO}_2$ expected by 2100 following the
163 RCP 8.5 scenario or beyond 2100 following the RCP 6 scenario (~1000 μatm ; $\Delta P\text{CO}_2$ between
164 350 and 700 μatm) (Meinshausen *et al.* 2011). We have not included in the analyses studies using
165 $P\text{CO}_2$ levels beyond the predictions for 2100 under the business as usual scenario (i.e. $P\text{CO}_2 >$
166 1300 μatm), but a few studies (n=3) where control CO₂ conditions were above-average values as
167 they reflected the conditions of specific ecosystems where acidification is predicted to be heavier
168 (Hoffmann *et al.* 2011). Studies manipulating carbonate chemistry using acid addition were not
169 considered as they do not mimic the expected changes in HCO₃⁻ concentrations and dissolved
170 CO₂.

171 We kept multi-species experiments only when data were independently collected. In the case
172 of time series, we used only the final data point, except for mortality data, where the first time
173 point of a time series was considered in order to maintain a conservative estimate and avoid bias of
174 rearing fish in aquaria (e.g. Murray *et al.* 2014). When a single experiment reported several

175 metrics related to the same response typology (e.g., length and weight to estimate growth) we
176 randomly chose one metric to avoid autocorrelation. For studies designed to assess interactive
177 effects of multiple stressors (for instance food availability, oxygen, salinity, and temperature along
178 with PCO_2) we included only responses to altered PCO_2 and considered control levels for the
179 other factors. We included data from trans-generational acclimation experiments when parents
180 were reared under control CO_2 conditions. The data on survival were transformed to mortality
181 estimates ($= 1 - \text{survival}$). For the calcification, we included only studies dealing with otolith
182 formation (e.g. otolith area). To assess OA effects on fish metabolism, we analysed the resting
183 metabolic rate (RMR, i.e. oxygen consumption at resting) and its proxies, and the metabolic scope
184 (i.e. aerobic scope - MS, i.e. the difference between maximum and resting metabolic rates)
185 separately, as the directionality of each response under OA may be different. For instance, an
186 increase of RMR may be related to an increase in the energy spent on acid base regulation and
187 ventilation (Perry & Gilmour 2006), with this being potentially detrimental for the species
188 performance as an increase in cost of acid-base regulation can shunt away the energy from growth
189 (Ishimatsu *et al.* 2008) by decreasing MS. By contrast, a reduction in MS results in less energy
190 being available for life-history processes, such as growth, foraging activity and reproduction
191 (Pörtner & Farrell 2008) and can be due to a reduction in maximum metabolic rate even if RMR is
192 not affected.

193 When assessing the fish behavioural response types to OA, we kept distinct those
194 experiments showing behavioural changes (generally reported as reductions of behavioural
195 performances, i.e. negative effects) from those experiments exhibiting changes in activity or
196 boldness (which in turn often recorded increased effects, i.e. positive effects; but see Hamilton *et*
197 *al.* 2014) and split these into behaviour and activity, respectively. Importantly, the definitions of
198 positive or negative effects (see also effect size calculation) apply to the directionality (above or
199 below the null effect) of each response variable separately and may not reflect the actual fish

200 performance outcome. As an example, in some experiments, fish may have exhibited either a
201 behavioural (negative) change (i.e. a decreasing time percentage spent in the home site cue;
202 Devine *et al.* 2012) or an increased (positive) activity (i.e. venturing at an increasing distance from
203 a shelter; Munday *et al.* 2013) under elevated CO₂, although both types of response could
204 theoretically lead to an increased mortality (hence decreased performance).

205 When available, both metadata and/or raw data were obtained from the Pangaea data
206 repository (<http://www.pangaea.de>). When not available, data were retrieved directly from the text
207 or tables or with Data Thief III (B. Tummers, DataThief III 2006; <http://datathief.org>) to get data
208 from figures.

209 For each type of response we assigned metrics to the following response variables: (1)
210 mortality, growth, calcification, reproduction, metabolism [resting metabolic rate (RMR) and
211 metabolic scope (MS)] and yolk (for the eco-physiological response types); (2) behaviour and
212 activity (for the behavioural response types) (see Appendix S1).

213 We assessed how the following categories of life history traits could mediate the response of
214 fish to OA: (1) life stage (egg-embryo, larvae, juvenile, or adult), (2) physiology (stenohaline or
215 euryhaline), (3) climatic zone (polar, temperate, sub-tropical and tropical), and (4) adult habitat
216 (benthic, benthopelagic and pelagic (see Appendix S1). In addition, behavioural contrasts were
217 also categorised according to aspects of (5) the neurosensory system (i.e. cognition, audition,
218 olfaction, vision and olfaction+vision when visual stimuli and olfaction cues were not kept
219 separate in a given experiment) and (6) the ecological processes involved such as foraging (i.e.,
220 feeding activity and prey detection), predation risk and habitat choice (settlement and homing
221 ability) (see Appendix S1). The hatchling stage, instead of the egg-embryo stage, was considered
222 for categorising the behavioural, the neuro-sensory and the ecological contrasts. The habitat of
223 eggs (benthic or pelagic) was also considered as a category when assessing OA effects on yolk .
224 The assignment of these different categories was made using Fishbase (www.fishbase.org) and

225 information available in each paper considered. Moreover, to evaluate the effects of different time
 226 of exposure to OA experimental conditions, we compared data from short-term experiments (≤ 4
 227 weeks), from long-term experiments (> 4 weeks) and from experiments conducted along natural
 228 CO_2 vents where fish with limited mobility are chronically exposed to elevated CO_2
 229 concentrations (i.e. chronic exposure experiments) following Nagelkerken & Connell (2015).

230 Details on the studies we used in the meta-analysis along with response types, response
 231 variables, categories, category levels, metrics and effect sizes are reported in the supplementary
 232 Appendix S1.

233

234 *Data analysis*

235 We used a weighted, random-effects meta-analysis to quantify the response of fish to OA
 236 and assess how the studied categories can mediate this response. For each combination of response
 237 type i , response variable j , category k , category level m and study l , the effect size of the fish
 238 response to elevated $p\text{CO}_2$ levels was calculated as log-ratios (Osenberg *et al.* 1997; Hedges *et al.*
 239 1999):

$$240 \quad E_{ijklm} = \ln \left(\frac{\bar{X}_{I_{ijklm}}}{\bar{X}_{C_{ijklm}}} \right)$$

241 where $\bar{X}_{I_{ijklm}}$ and $\bar{X}_{C_{ijklm}}$ are the mean values of response variable j , in response type i , in
 242 category k , and category level m from study in the impacted (elevated $p\text{CO}_2$) and control $p\text{CO}_2$
 243 conditions, respectively.

244 The variance $v_{E_{ijklm}}$ associated with the effect size E_{ijklm} was calculated as follows:

$$245 \quad v_{E_{ijklm}} = \frac{\sigma^2_{I_{ijklm}}}{n_{I_{ijklm}} \times \bar{X}_{I_{ijklm}}^2} + \frac{\sigma^2_{C_{ijklm}}}{n_{C_{ijklm}} \times \bar{X}_{C_{ijklm}}^2}$$

246

247 where $\sigma_{I_{ijklm}}^2$ and $\sigma_{C_{ijklm}}^2$ are the variances associated with the means $\bar{X}_{I_{ijklm}}$ and $\bar{X}_{C_{ijklm}}$,
248 respectively, where $n_{I_{ijklm}}$ and $n_{C_{ijklm}}$ are the number of replicates in the impacted (elevated
249 PCO_2) and control PCO_2 conditions, and where i, j, k, m and l are defined as above. All effect
250 sizes were weighted, accounting for both the within- and among-study variance components.
251 Models were fitted and heterogeneity tests were run to assess how many fish traits (i.e., life stage,
252 physiology, climatic zone, habitat, neurosensory system, ecological processes) could mediate the
253 response of fish to OA. Models fitting and heterogeneity tests were done using the *metaphor*
254 package (Viechtbauer 2010) in R (R Core Team 2016).

255

256

257 **RESULTS**

258 *Eco-physiological responses*

259 Overall we found that rising CO_2 levels will significantly affect fish calcification (+5%),
260 RMR (+7%) and yolk (-5%), while mortality, metabolic scope, reproduction and growth seemed to
261 be unaffected (Fig. 1a, 2a, 3a; see Table S1, S2). Examining the effects of different CO_2
262 projections under the two RCP scenarios, we found that only high PCO_2 levels significantly
263 increased calcification and RMR, by 6% and 8%, respectively, and decreased yolk area by 5%
264 (Fig. 1a, 2a; 3a; see Table S1, S2).

265 However, we documented multiple adverse responses to ocean acidification when different
266 fish traits were considered in our meta-analysis.

267 Trait-mediated effects of rising pCO_2 levels on fish mortality are reported in Fig. 1b (see
268 Table S3, Fig. S1 for full results). Increased mortality was recorded for larvae exposed to high
269 PCO_2 levels, whereas embryos, juveniles and adults were unaffected at both PCO_2 level ranges
270 considered (Fig. 1b; see Table S3, Fig. S1). Effects of OA on mortality of stenohaline species were
271 not evident, whereas euryhaline species exhibited higher mortality at high PCO_2 (+29%; Fig. 1b;

272 see Table S3, Fig. S1). When considering climatic zones, mortality increased only for sub-tropical
273 species exposed to high PCO_2 (+38%), and no effects were evident for polar, tropical and
274 temperate species exposed at moderate and high PCO_2 levels (Fig. 1b; see Table S3, Fig. S1).
275 Mortality of pelagic fish increased at high PCO_2 levels, whereas benthic and benthopelagic
276 species did not show significant effects at the two of PCO_2 level ranges considered (Fig. 1b; see
277 Table S3, Fig. S1).

278 Growth of larvae significantly decreased by 4% at high PCO_2 , and no effect was observed at
279 moderate PCO_2 (Fig. 1b; see Table S3, Fig. S2). This neutrality in growth response was also
280 apparent on embryos and juveniles at both PCO_2 levels and on adults at high PCO_2 levels (Fig. 1b;
281 see Table S3, Fig. S2). We did not find any OA effects on fish from different climatic zone, except
282 for subtropical species that showed decreased growth (-14%) at high PCO_2 levels (Fig. 1b; see
283 Table S3, Fig. S2). Growth of pelagic species significantly decreased by 9% at high PCO_2
284 conditions, whereas benthic and benthopelagic fish were neutrally affected by altered CO_2 (Fig.
285 1b; see Table S3, Fig. S2).

286 Calcification (otolith formation) responses to OA differed between fish life stages,
287 physiologies, climatic zones, and habitats (Fig. 1b; see Table S3). Exposure to high PCO_2 levels
288 significantly increased calcification of fish larvae (+6%), and of sub-tropical (+7%), temperate
289 (+6%) and benthic (+6%) species (Fig. 1b; see Table S3, Fig. S3). In addition to this, the
290 calcification of benthopelagic species was also affected but only one contrast was available for this
291 analysis.

292 Fish reproduction and metabolic scope (MS) were unaffected at both PCO_2 levels, no matter
293 the different fish traits considered (Fig. 2; see Table S3, Fig. S4, S6). Resting metabolic rate
294 (RMR) increased in adult (+13%) and stenohaline (+8%) fish exposed to high PCO_2 levels.
295 Interestingly, polar species exposed to high CO_2 concentrations showed a RMR 33% higher than
296 control conditions. Trait-mediated responses were also evident among fish habitats, as benthic fish

297 increased their RMR at high PCO_2 levels, whereas benthopelagic and pelagic species resulted
298 unaffected under elevated CO_2 conditions (Fig. 2b; see Table S3, Fig. S5).

299 We found that OA may cause an overall significant decrease in yolk size (Fig. 3a; see Table
300 S1). When considering the effects of the different ranges of CO_2 , only high pCO_2 levels had a
301 negative effect on yolk size. This result was evident in embryos (yolk size decreased by 7% at
302 high pCO_2 levels) but not in larvae (Fig. 3b; see Table S3, Fig. S7 for full results). Stenohaline and
303 benthic fish showed a negative effect on yolk size at high PCO_2 levels (-7% and -8%,
304 respectively). When we compared data on fish species from different climatic zones, temperate
305 species showed decreased yolk size only at moderate PCO_2 levels (but only one contrast was
306 available for the analysis), whereas yolk of tropical species resulted to be affected under high
307 PCO_2 levels. Offspring of benthic fish species showed decreased yolk size (-6%) after exposure to
308 high CO_2 concentrations (Fig. 3b; see Table S3, Fig. S7).

309

310 *Behavioural responses*

311 Overall, OA conditions significantly and strongly affected behaviour (-54 %) and activity
312 (+108 %) of fish (Fig. 4a, Table S1) and such responses did vary among PCO_2 levels (Fig. 4a, see
313 Table S2). Moderate CO_2 concentrations decreased by 45% the behavioural performances of fish
314 and increased their activity levels by 150% on average (Fig. 4a, see Table S2). At high PCO_2
315 levels fish displayed significant reductions of their behavioural performances (-60%), whereas no
316 significant effects were evident on their activity (Fig. 4a, see Table S2). Behaviour of larvae was
317 negatively affected at high PCO_2 levels, whereas juveniles showed significant reductions in
318 behavioural performances at both PCO_2 levels considered (Fig. 4b; see Table S4, Fig. S8).
319 Behavioural disruptions were also evident for tropical species exposed at moderate and high CO_2
320 concentrations (Fig. 4b; see Table S4, Fig. S8). OA did not seem to affect the behaviour of
321 subtropical and temperate species, but the number of available studies is too low to detect a clear

322 response pattern (Fig. 4b; see Table S4, Fig. S8). No data on OA effects on the behaviour of polar
323 species were available. When considering fish physiology and habitat traits, most of the contrasts
324 revealed OA effects on stenohaline and benthic species, showing severe behavioural disruptions at
325 both moderate and high PCO_2 levels (Fig. 4b; see Table S4, Fig. S8).

326 A significant 1.5-fold increase in juvenile fish activity (boldness) was recorded at moderate
327 but not at high pCO_2 levels, whilst neutral effects were recorded for larval and adult stages at the
328 two ranges of PCO_2 levels (Fig. 4b; see Table S4, Fig. S9). Fish activity increased only in
329 stenohaline and benthic species exposed to moderate PCO_2 levels, whereas data on euryhaline,
330 benthopelagic and pelagic species resulted understudied or not available (Fig. 4b; see Table S4,
331 Fig. S9).

332 Significant alterations of fish behaviour mostly resulted from impaired olfaction, with on
333 average 40% and 70% decreases at moderate and high PCO_2 levels, respectively (Fig. 5a; see
334 Table S4). Fish behaviour was unaffected in experiments involving cognition and vision (Fig. 5a;
335 see Table S4). Fish simultaneously exposed to visual and olfactory stimuli showed a decreased
336 behavioural performance at moderate PCO_2 (only 2 contrasts included in the analyses) and no
337 effects at high PCO_2 levels (Fig. 5a; see Table S4).

338 The observed fish behavioural impairments resulted in higher predation risk at both
339 moderate (by 55% on average) and high (by 77%) PCO_2 levels. Feeding activity and detection of
340 prey (i.e., the foraging activity of fishes) also decreased by 59% at high PCO_2 levels, but were
341 unaffected at lower CO_2 concentrations (Fig. 5b; see Table S4). No effects on habitat choice were
342 found for any of the PCO_2 levels considered.

343

344 *Experimental CO_2 exposure time*

345 Our analysis showed that fish sensitivity to OA scenarios differed according to experimental
346 CO_2 exposure time and the PCO_2 ranges considered (Fig. 6; see Table S4). Short-term experiments

347 at moderate PCO_2 levels did not affect any eco-physiological and behavioural responses, whereas
348 short-term exposure to high PCO_2 levels significantly increased fish calcification (+6%) and
349 mortality (+24%), and decreased growth (-3%) and behavioural performances (-60%) (Fig. 6; see
350 Table S4). Long-term exposure at both moderate and high PCO_2 levels significantly increased fish
351 activity/boldness (+232% and +2119%, respectively). High PCO_2 levels increased fish RMR
352 (+20%) and decreased their behavioural performances (-58%). The effects of chronic exposure to
353 elevated CO_2 concentrations were evident at moderate PCO_2 levels for behaviour (-88%) and for
354 activity (+200%), no data are available for high PCO_2 levels .

355

356 **DISCUSSION**

357 New insights are gained from our findings by including the increasing number of studies
358 published in recent years and the responses of fish to different CO_2 projections. This also allowed
359 our analyses to identify a range of trait-based variations in fish sensitivity to OA, with eco-
360 physiological and behavioural responses varying according to CO_2 scenarios, and fish ontogenetic
361 phases and species characteristics. Specifically, we show that, if anthropogenic CO_2 emissions
362 continue to rise both under the RCP 6 and RCP 8.5 scenarios, overall significant effects of
363 elevated PCO_2 levels on fish metabolism, calcification (otolith formation), yolk consumption,
364 behaviour and activity (boldness) are expected to occur in the next few decades. It is now well-
365 established that under elevated CO_2 conditions, any ATP-demanding compensation activities for
366 acid-base balance and enhanced transport of ions may incur elevated energetic costs, potentially
367 leading to subtle fitness consequences for such response variables in marine fish (Sokolova 2013;
368 Heuer & Grosell 2014). Hence, at the same time, we suggest that more subtle consequences of OA
369 on mortality and growth of fish, as well as downstream ecological consequences, might occur.

370 There has been a clear call for addressing the important effects of species traits when making
371 predictions about the impacts of environmental change (e.g. Estrada *et al.* 2016). Our work meets

372 this call and sharpens the focus to fish. Previous meta-analyses did not take into account different
373 fish traits and life stages instead focussing on a small amount of experiments available at that time
374 (Harvey *et al.* 2013; Kroeker *et al.* 2013; Wittmann & Pörtner 2013; Lefevre, 2016). As an
375 example, it has previously been argued that OA studies on fish were biased by the high number of
376 experiments carried out on tropical species (Wittmann & Pörtner 2013), therefore suggesting that
377 responses were predominantly focussed towards species with short life cycles, which are typical of
378 this climate zone (Leis & McCormick 2002). By analyzing the body of literature through to
379 January 2017 and by including data on additional 30 non-tropical species and 115 contrasts, here
380 we were able to provide a clearer picture and show that fish responses to elevated CO₂ levels may
381 be CO₂ dependent and vary according to different fish characteristics and traits.

382

383 *OA effects on marine fish are mediated by their life history traits and characteristics*

384 We documented varying adverse responses when examining specific fish traits. In this
385 regard, we did not find any specific fish characteristics which could potentially confer tolerance to
386 fish under elevated CO₂ concentrations. These are important results as in addition to identifying
387 the direction and quantifying the magnitude of these responses, we were able to show that, if fish
388 traits are not taken into account, consequences of ocean acidification on marine animals might not
389 be detected as in some cases species-specific responses could be antagonistic (Esbaugh, 2017).
390 Our study adds on previous evidence showing that larvae are the most sensitive life stage as they
391 displayed increased mortality and calcification (i.e. otolith formation), and decreased growth at
392 elevated CO₂ conditions (~1000 µatm PCO₂). The observed higher sensitivity to elevated PCO₂
393 levels for early life stages of fish might be linked to their high surface to volume ratio affecting
394 diffusive processes and to their acid-base balance system not being fully developed, with ionic
395 exchanges occurring across the skin of larvae and the yolk of embryos until their gills can develop
396 (Munday *et al.* 2009a; Baumann *et al.* 2012; Hurst *et al.* 2013).

397 Mortality at individual level affects population dynamics (Munday *et al.* 2010), which in
398 turn can extend responses to higher community- and ecosystem-levels (Nagelkerken & Connell
399 2015). Since we only included experiments testing for the direct effects of elevated CO₂ on fish
400 mortality, the increased mortality of larvae we recorded may be a conservative estimate, as more
401 indirect effects can also occur leading to even higher mortality rates. As an example, we found that
402 larval growth was significantly reduced at high CO₂ levels, and smaller larvae may exhibit a lower
403 performance and survival in the wild as a result of their reduced swimming ability and less
404 efficient predator avoidance (e.g. Miller *et al.* 1988), therefore leading to increased mortality
405 through predation.

406 Heavily calcifying organisms like molluscs, echinoderms and corals are considered to be the
407 most threatened marine taxa under OA (Kroeker *et al.* 2013; Wittmann & Pörtner 2013). Fish are
408 not considered calcifying organisms despite producing calcium carbonate in the inner ear for
409 otolith formation or precipitating CaCO₃ in the intestinal lumen to reduce osmotic pressure,
410 release calcium ions excess in the environment and thus facilitate water absorption (Heuer &
411 Grosell 2014). Here we found a significant increase in the size of otoliths in larvae exposed to high
412 PCO₂ levels expected to occur by 2050 (following the business-as-usual RCP8.5 scenario) or by
413 2100 (following the intermediate RCP6.0 scenario). Our results also showed that otolith
414 calcification in both benthic and benthic-pelagic species may increase at high PCO₂ levels. Recent
415 studies shed light on the potential mechanisms involved in increased otolith formation under OA.
416 Specifically, rising dissolved CO₂ concentrations in the ocean may lead to increased HCO₃⁻ levels
417 in the fish plasma (which is used to compensate pH), while plasma PCO₂ levels remain elevated
418 (Esbaugh *et al.*, 2012). It appears that plasma HCO₃⁻ import in the alkaline endolymph and the
419 hydration of CO₂ in the saccular epithelium lead to increased HCO₃⁻ and hence to increased otolith
420 size when fish are exposed to elevated CO₂ levels (Heuer & Grosell, 2014). It is presently
421 unknown whether larger otoliths may lower the ability of sound detection and the survival of fish

422 in the wild, although we know that asymmetry between otoliths can be detrimental (Gagliano et
423 al., 2008).

424 Despite the lack of effects of elevated CO₂ on the metabolism of fish early stages, adults
425 experimentally exposed to high *p*CO₂ levels significantly increased their metabolism at rest.
426 Similar responses were detected on polar fish species. At present, some studies suggest that
427 temperature effects lead to higher costs for pH regulation in cold-adapted eurytherms compared to
428 polar stenotherms (Pörtner, 2004), however existing knowledge on OA effects on the metabolism
429 of cold environment fish species is scant. Although an increase in RMR may be expected to result
430 in a decrease in metabolic scope, our results show that MS is not affected by elevated CO₂. This
431 apparent contradiction could be due to either (1) a compensatory increase in maximum metabolic
432 rate or (2) the fact that the number of studies on MS is much smaller than that for RMR and
433 therefore the analysis for MS is less robust. A recent meta-analysis on warming and acidification
434 effects on metabolism of marine organisms showed no significant effects of altered *p*CO₂ levels on
435 resting metabolic rate and metabolic scope of teleost fish, highlighting high variability in the
436 metabolic responses of fish (Lefevre, 2016). In this regard, our analyses suggest that fish
437 metabolic responses might be trait-mediated as we mostly found elevated CO₂ effects on RMR of
438 benthic, stenohaline and polar species. Earlier studies hypothesised that exposure to high *PCO*₂
439 levels could lead to increased fish resting metabolism due to the costs associated with internal CO₂
440 balance, however mixed responses were found, with some studies confirming this assumption both
441 in tropical (Munday et al., 2009b; Couturier et al., 2013; Ferrari et al., 2015) and non-tropical
442 species (Esbaugh et al., 2016; Flynn et al., 2015), whereas other studies revealed decreased resting
443 metabolic rate after prolonged high-CO₂ exposure (Rummer et al., 2013; Pimentel et al., 2014;
444 Heuer & Grosell, 2016).

445 Fish reproduction – indeed an energetically costly process – was unaffected by altered CO₂
446 levels, no matter which specific fish trait was considered. However, in this case, there were too

447 few experiments to draw a clear picture of the effect of rising CO₂ on fish reproduction. In this
448 regard, work on OA effects on fish reproduction *per se* should be a priority given its importance
449 for population replenishment.

450 Marine habitats with different salinity levels also vary greatly in PCO₂ and thus euryhaline
451 species – e.g. those fish having the ability to effectively osmoregulate across a broad range of
452 salinities – are likely to experience highly variable PCO₂ environments during their life cycle. This
453 suggests these organisms (i.e. marine-brackish fish) can be pre-adapted to OA conditions.
454 Contrary to what we expected, we found a significantly higher mortality of euryhaline fish
455 exposed to high PCO₂ levels. Mortality of stenohaline fish (which cannot tolerate a broad range of
456 salinities) was unaffected by OA but these fish showed increased calcification rates (otolith
457 formation) and metabolic rate at resting when exposed at high PCO₂ levels. To date, the impact of
458 OA on fish osmoregulation at ecologically relevant CO₂ conditions is critically understudied
459 (Heuer & Grosell 2014).

460 Overall, we found a negative effect on yolk consumption (i.e. decreased yolk size) in fish
461 exposed to elevated CO₂. A decreased yolk size was evident after exposure to high PCO₂ levels
462 for early developmental stages of benthic species, but not for pelagic ones. Similar to the trend
463 proposed for stenohaline and euryhaline fish, some authors have hypothesised that embryos and
464 larvae of pelagic spawning fish, which likely experience lower and more stable CO₂ conditions in
465 the open ocean (Hofmann *et al.* 2011), might be more susceptible to CO₂ increases compared to
466 the offspring of fish laying eggs in the benthic environment, which in turn might be better adapted
467 to natural CO₂ fluctuations due to the alternation of photosynthetic and respiration processes by
468 primary producers (Hofmann *et al.* 2011; Munday *et al.* 2011). Contrary to these expectations,
469 when categorising yolk size responses according to the habitat of developing embryos, negative
470 (i.e. decreased yolk size) responses have been noted at high PCO₂ levels only in benthic spawning
471 fish, but not in offspring with pelagic development. It is known that through maternal

472 provisioning, females may adjust eggs characteristics (e.g. offspring yolk size) to environmental
473 conditions (Chambers 1997) and that yolk consumption is affected by elevated CO₂ conditions
474 during the embryonic development (Chambers *et al.* 2014).

475 Our meta-analysis also confirmed that OA could strongly influence the behaviour of tropical
476 fish, particularly that of larvae and juveniles. Indeed, these responses were rather consistent at
477 environmentally relevant *PCO*₂ levels expected by the next few decades. Importantly, the
478 observed behavioural impairments were due to alterations of a range of different neurosensory
479 functions. Olfaction was the most sensitive sensory function affecting fish behaviour even at *PCO*₂
480 levels expected in 2050. At the same time, in many experiments, fish reared in elevated CO₂
481 conditions were bolder, displaying up to 1.5-fold increase in their activity rates (e.g. less time
482 spent in a shelter, or increased distance ventured) and therefore increased vulnerability to
483 predators (e.g. Munday *et al.* 2010).

484 As animals respond to environmental change through behavioural modifications, the
485 downstream consequences of the observed behavioural and activity effects (particularly on larvae
486 and juvenile fish) are expected to lower fish ecological performance (e.g. with respect to predator-
487 prey dynamics, recruitment success, settlement, homing and habitat choice) and therefore to affect
488 the outcomes at community level (Nagelkerken & Munday 2016). Consistently, our quantitative
489 findings indicate that the behavioural disruptions we identified could result in a higher predation
490 risk and a lowered foraging activity, which in turn may lead to increased mortality and decreased
491 growth, therefore decreasing the ability of a species to persist in a given community. Unexpectedly
492 the settlement and homing ability (i.e., habitat choice) of fish was unaffected by rising CO₂ levels.
493 Certain effects, such as an increase in fish activity (boldness) and changes in fish lateralization, do
494 not have an immediate bearing on ecological “fitness”, because in nature they represent a trade off
495 in which for example, bold individuals can find food faster (Mamuneas *et al.*, 2015) but are more
496 exposed to predation (Ward *et al.*, 2004) than shy individuals. Similarly, although a high degree of

497 lateralization has been considered an advantage in terms of multitasking (escaping from predators
498 and schooling; Bisazza & Dadda, 2005; Dadda & Bisazza, 2006; Dadda et al., 2010, Domenici et
499 al., 2012), it was suggested to represent a trade off with the ability to deal equally well with stimuli
500 of threats from all directions (Vallortigara & Rogers, 2005). In these cases, the effect of elevated
501 CO₂ may be detrimental for fish because it alters the balance within the continuum of bold vs. shy
502 and lateralized vs. non-lateralized individuals, with potential ecological consequences at
503 population level (Sih et al., 2012).

504

505 *Ocean acidification effects on fish under different experimental CO₂ exposure duration*

506 To date, a great uncertainty associated with the consequences of different CO₂ projections on
507 teleost fish, relies on whether such organisms will be able to cope with ongoing rapid changes in
508 the carbonate chemistry of the ocean. Our analyses following different CO₂ exposure times from
509 field- and lab-based experiments revealed that moderate *PCO₂* levels have no effects on fish eco-
510 physiology both in short- and long-term experiments, and that fish behavioural performances are
511 disrupted particularly in chronically exposed fish populations. Despite consistent responses on fish
512 calcification and behaviour, we found that high *PCO₂* levels significantly affect mortality and
513 growth of fish only after short- but not after long-term elevated CO₂ exposures. Indeed, our
514 analyses suggests that further longer term and multi-generational experiments are needed to test
515 for a potential adaptation of fish occurring at altered *PCO₂* levels and we identify this issue as a
516 critical gap of knowledge, which is further discussed in the next paragraph.

517

518 *Future directions and conclusions*

519 Acclimation and adaptation may have significant consequences for how marine organisms
520 will respond to future high CO₂ oceans (Sunday et al., 2014) and some previous work argues that
521 adaptation may be too slow for long-lived species (Pörtner et al., 2014). Due to the small number

522 of experiments carried out so far, quantitative generalisations are not possible yet, and our
523 investigation identified a critical need to increase the number of fish studies addressing such
524 processes under OA. To date, experimental findings are not in line with conclusions made using
525 fossil records and observations from paleo-studies which suggested that teleosts are very tolerant
526 to elevated PCO_2 levels when compared to invertebrate taxa (Wittmann & Pörtner, 2013). In this
527 context, a few studies showed mixed potential for transgenerational acclimation (e.g. Allan et al.,
528 2014; Welch et al., 2014) while others, examining OA effects on chronically CO_2 exposed fish
529 populations (e.g. those from volcanic CO_2 seeps), documented changes in embryo metabolism,
530 olfaction, spawning behaviour, and escape response (Munday et al., 2014; Nagelkerken et al.,
531 2015; Cattano et al., 2016; Milazzo et al., 2016). Although much progress has been made in
532 designing experiments to assess how elevated PCO_2 can alter fish ecophysiology and behaviour, to
533 date we are still far from understanding adaptive capacity to OA (Munday et al., 2013).

534 Given that additional environmental stressors will lead to dramatic ocean change in the
535 future – i.e. increasing seawater temperature and more severe hypoxia are predicted to occur
536 concomitantly to rising seawater CO_2 – their effects are unlikely to operate independently and
537 there is a need to understand how the combined consequences of these stressors will affect the
538 fitness of marine fish. Pörtner and Farrell (2008) have hypothesised additive and synergic effects
539 of temperature and elevated CO_2 on individual performance, increasing the energy requirements
540 for homeostasis regulation, and therefore, reducing the amount of energy available for other
541 biological processes. Similarly, by constraining metabolism, predicted warming and hypoxia can
542 be expected to contract the distribution of marine fish (Deutsch et al., 2015). Importantly, there
543 may be genetic correlations between phenotypic variation associated ocean acidification, warming
544 and hypoxia that could potentially limit the rate of adaptation to these stressors when two or more
545 of them occur simultaneously, and have antagonistic effects (Munday et al., 2013). Identifying
546 such constraints will be an important issue for studies on the adaptive potential of fishes to OA.

547 An additional unavoidable limitation of most OA studies on fish and other taxa is their un-
548 representativeness of ecosystem effects since they do not take into account the complex species
549 interactions acting at multiple levels of organisation in the real world. Yet, some attempts have
550 succeeded in testing OA effects on key community ecological interactions (e.g. competition and
551 predation) involving fish in their natural habitat (Nagelkerken & Munday, 2016). In this context,
552 our study might also identify the need to expand the number of OA studies examining fish
553 responses to community shifts and biogenic habitat modifications in naturally high CO₂
554 environments (Munday et al., 2014, Enochs et al., 2015; Nagelkerken et al., 2015; Sunday et al.,
555 2016).

556 In summary, here we document negative effects on many fish eco-physiological and
557 behavioural responses at CO₂ levels expected by the next few decades or by the end of this century
558 depending on the CO₂ emission scenario considered. We suggest that some direct effects –
559 particularly on fish mortality and growth – may be underestimated as we found increased
560 predation risk and decreased foraging, confirm that larvae are the most sensitive life stages
561 (Munday et al., 2009a), and advise that downstream ecological consequences can be very likely.
562 Importantly, we do not find any traits potentially conferring fish tolerance to elevated CO₂, and
563 thus reject previous hypotheses that some specific fish traits (e.g., those species characteristics
564 associated to highly fluctuating PCO₂ environments) may mitigate such responses. Many
565 explanations for documented eco-physiological and behavioural responses of marine fish to
566 elevated CO₂ rely on changes expected to occur in fish brain function (Nilsson *et al.* 2012) and
567 acid-base compensation (Heuer & Grosell 2014). Likely, effects on fish will be heaviest where
568 ocean acidification, warming, and hypoxia regionally coexist, indicating that studies like the one
569 we present focussing on an individual environmental drivers of change maybe conservative.
570 However, some level of uncertainty remains over the long-term persistence of the observed effects
571 on fish, particularly on some eco-physiological response variables. Given the importance of

572 marine fish livelihoods in coastal communities, for ecosystem stability and food webs, it is
573 fundamentally critical to slow down current rates of CO₂ emissions and deviate from the expected
574 scenarios to mitigate OA effects on fish.

575

576 **AUTHORS CONTRIBUTIONS**

577 C.C., J.C. and M.M. conceptualized and designed the paper; C.C. assembled the data; C.C.,
578 J.C. and M.M. analyzed the data. C.C. and M.M. produced figures and drafted the paper; all
579 authors contributed to discussion, writing, and interpretation.

580

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586

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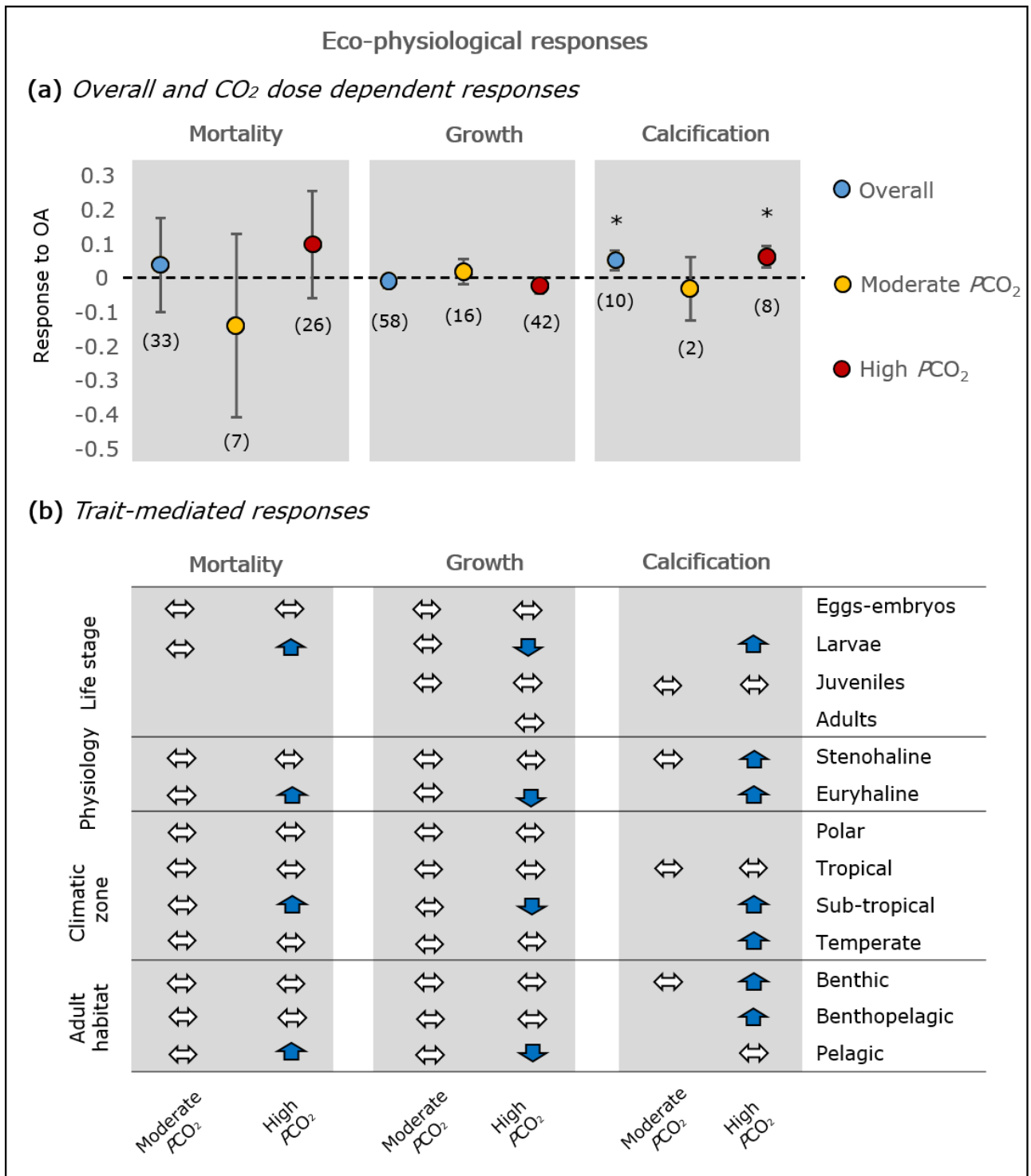
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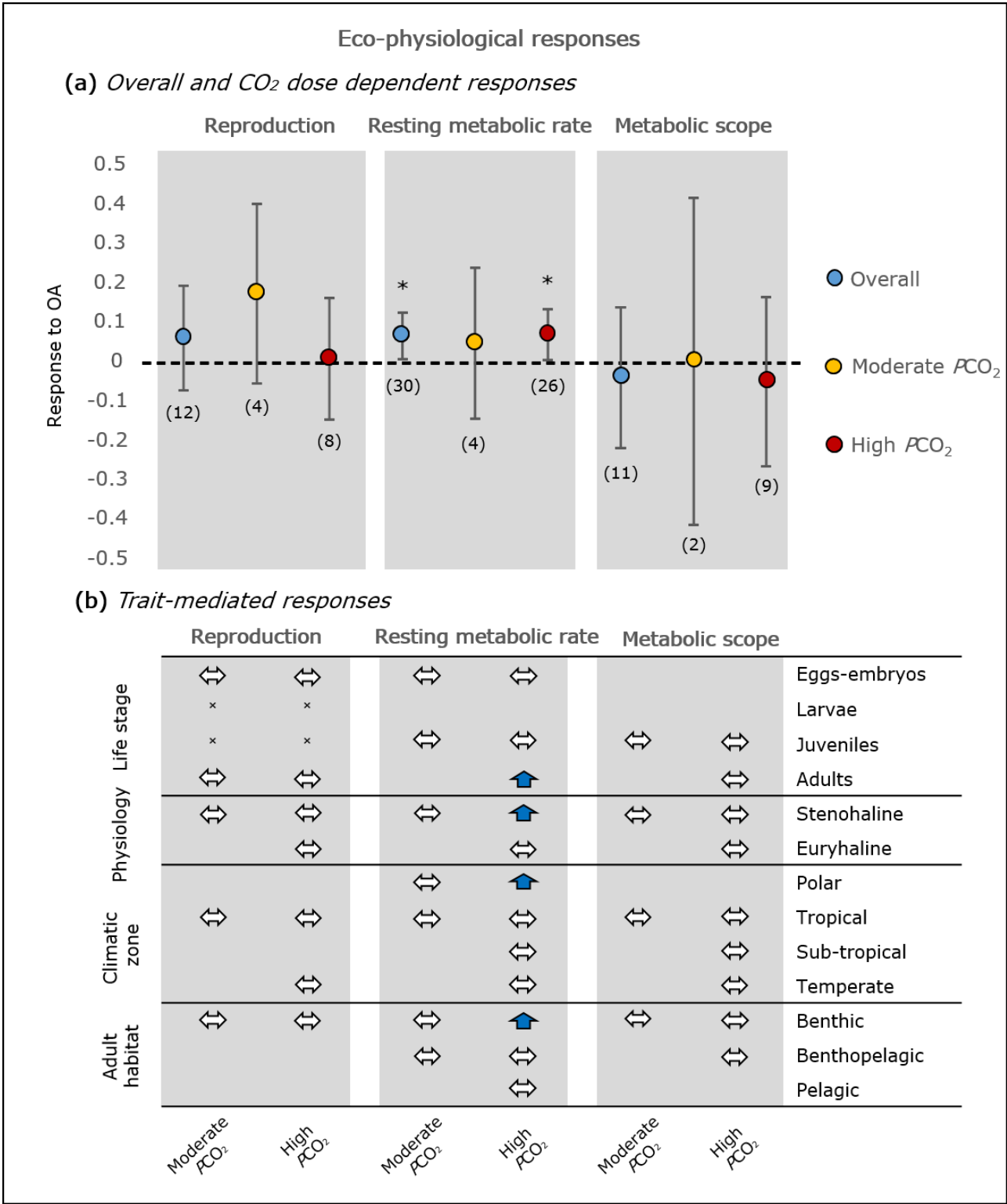
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813 **Figure 1** OA effects on fish eco-physiological responses (mortality, resting metabolic rate and
 814 metabolic scope). **(a)** Overall and CO₂ dose-dependent responses. Effect sizes (±95% CI) are
 815 shown for each response variable, overall and for each CO₂ projection (see Materials and methods
 816 for details). The number of contrasts considered per response is showed in parentheses. Asterisk

817 indicates significant difference from zero. **(b)** Summary of trait-mediated responses for each CO₂
818 projection relative to fish mortality, resting metabolic rate and metabolic scope. Arrows show the
819 directionality of the responses: “↑” significantly positive; “↓” significantly negative; “↔” null; “x”
820 not applicable. See also Figures S1-S3 for full results.



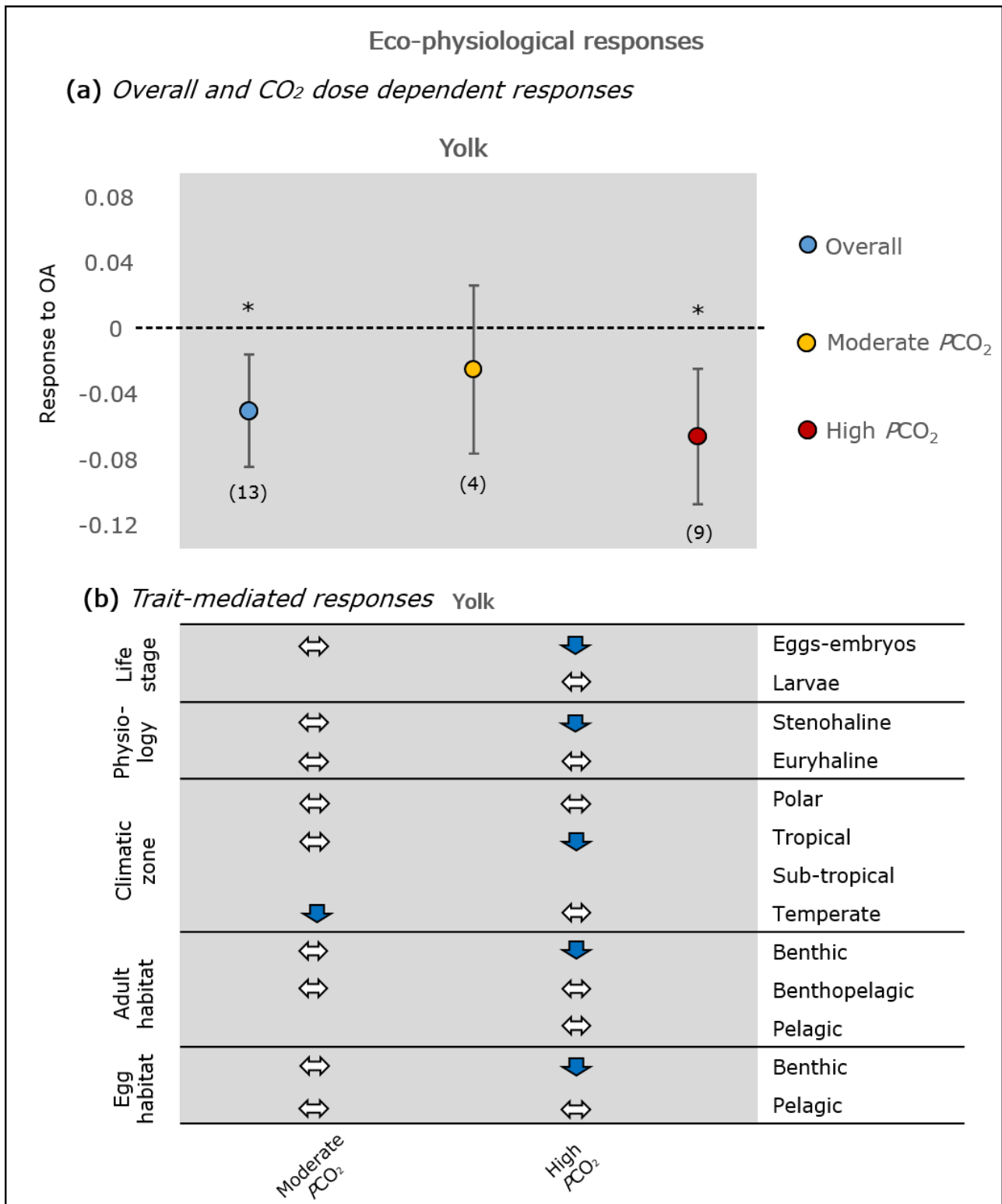
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822 **Figure 2** OA effects on fish eco-physiological responses (reproduction, growth and calcification).

823 **(a)** Overall and CO₂ dose-dependent responses and **(b)** summary of trait-mediated responses for

824 each CO₂ projection relative to fish reproduction, growth, and calcification. Symbols and type of
825 data as reported in Figure 1 legend , see also Figures S4-S6 for full results.

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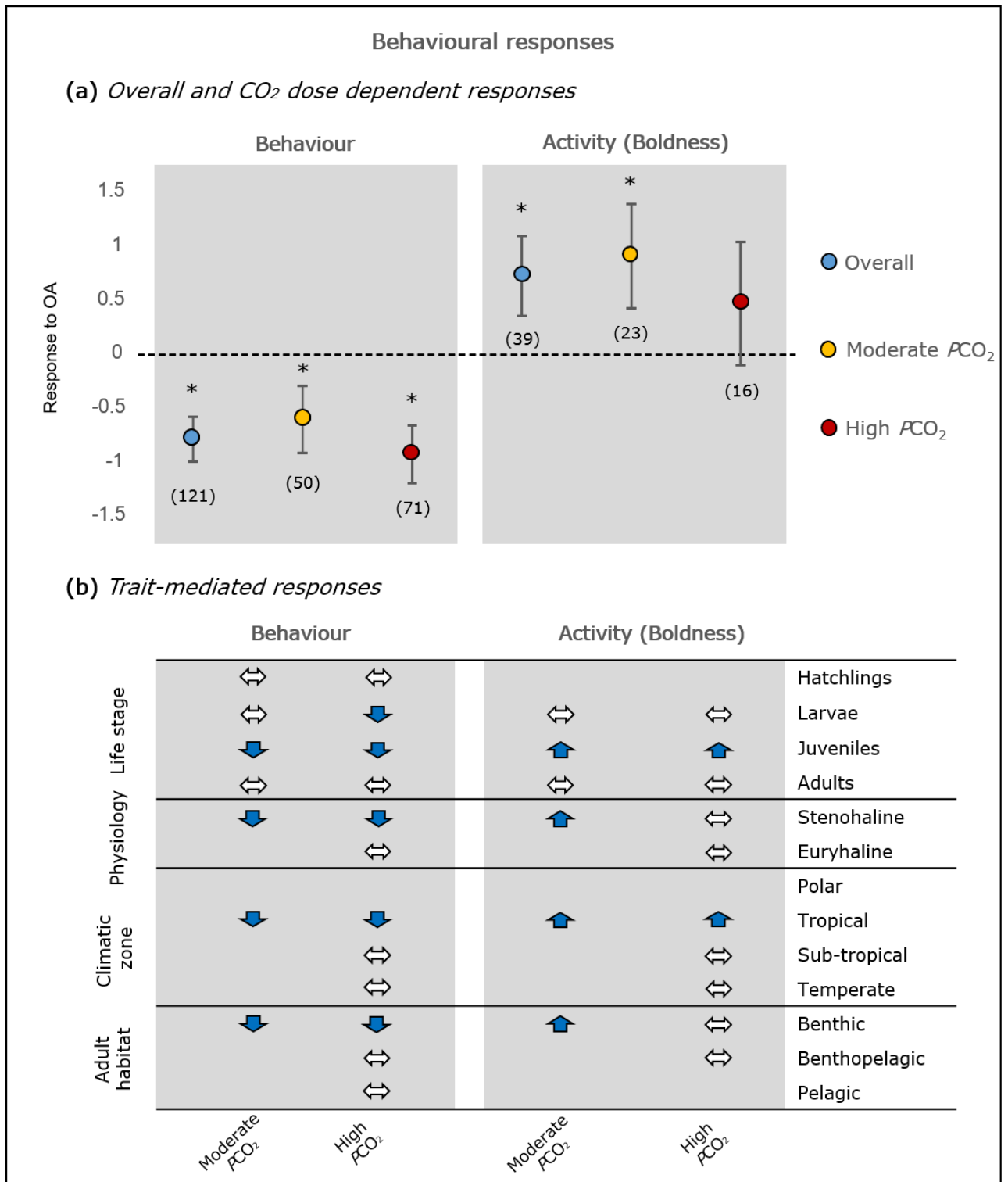


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828 **Figure 3** OA effects on fish development (yolk, hatching time and embryonic abnormalities). **(a)**

829 Overall and CO₂ dose-dependent responses and **(b)** summary of trait-mediated responses for each

830 CO₂ projection relative to yolk, hatching time and embryonic abnormalities. Symbols and type of
831 data as reported in Figure 1 legend, see also Figure S7 for full results.
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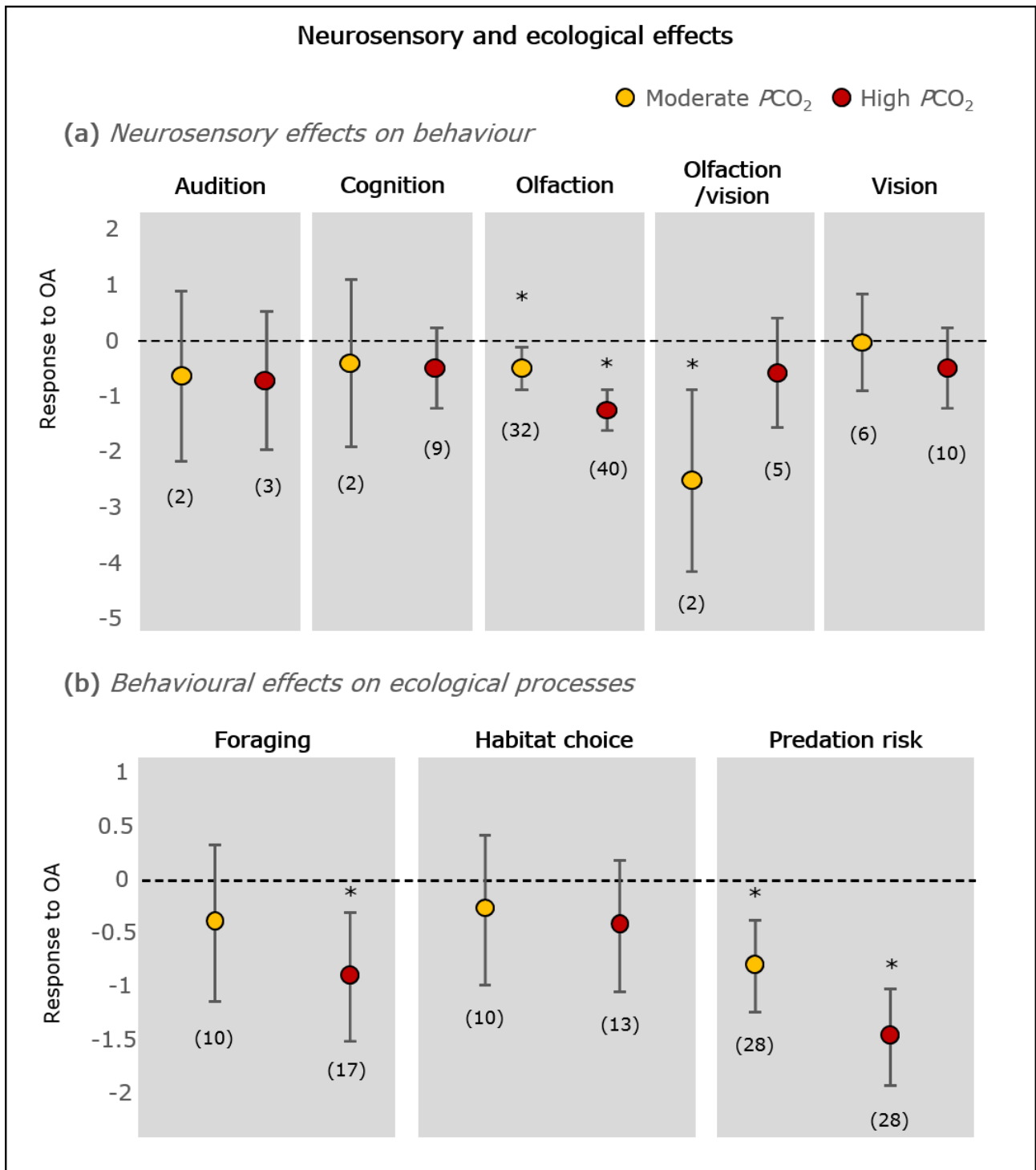


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835 **Figure 4** Effects of OA on behavioural responses (behaviour and activity) of fish. **(a)** Overall and

836 CO₂-dose dependent effects of OA on fish behaviour and **(b)** summary of trait-mediated responses

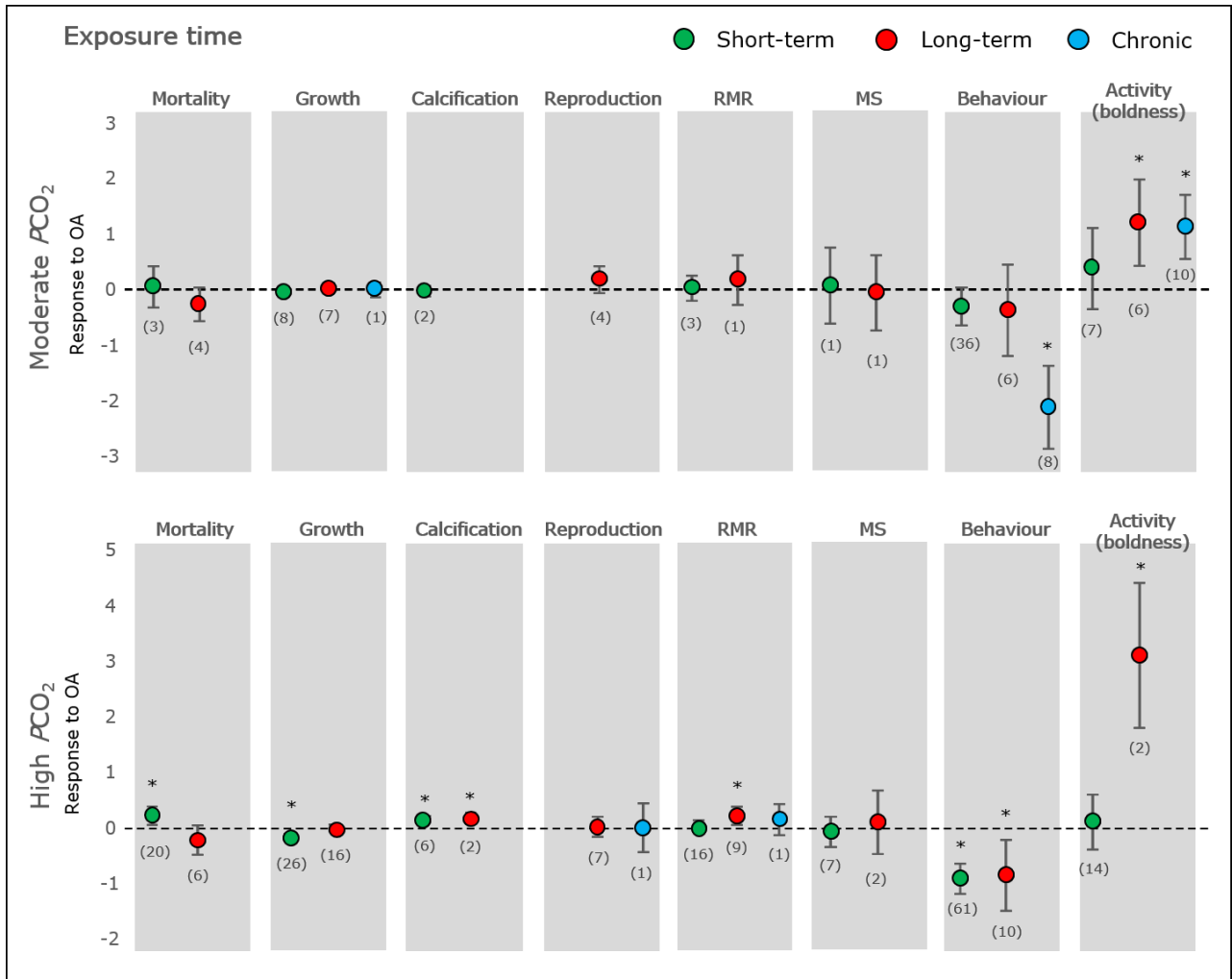
837 for each CO₂ projection relative to behaviour and activity. Symbols and type of data as reported in
838 Figure 1 legend , see also Figures S8-S9 for full results.
839



840

841 **Figure 5** Links between fish neurosensory, behaviour and ecological processes. **(a)** Neurosensory
 842 mediated responses of fish to OA in behavioural experiments and **(b)** behavioural effects on
 843 ecological processes (foraging, habitat choice and predation risk). Type of data as reported in
 844 Figure 1 legend.

845



846

847 **Figure 6** Effects of different CO_2 exposure times (short-term, long-term and chronic) on fish eco-
 848 physiology (mortality, growth, calcification, reproduction, resting metabolic rate, metabolic scope)
 849 and behavioural performance (behaviour and activity). Type of data as reported in Figure 1 legend.