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

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2 **of fish to ocean acidification.**

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ABSTRACT

1. Understanding how marine organisms will be affected by global change is of primary importance to ensure ecosystem functioning and human well-being through nature's contribution to people. This study meets the call for addressing how life-history traits mediate effects of ocean acidification on fish.

2. We built a database of overall and trait-mediated responses of teleost fish to future CO_2 levels by searching the scientific literature. Using a meta-analytical approach, we investigated the effects of projected CO_2 for 2050-2070 and 2100 on fish eco-physiology and behaviour from 327 experiments on 44 species, stemming from polar to tropical regions.

3. With anticipated CO_2 emission scenarios, multiple CO_2-dose dependent effects on calcification, resting metabolic rate, yolk, behavioural performances along with increased predation risk and decreased foraging will occur, particularly for larvae. Importantly, no traits confer fish tolerance to elevated CO_2.

4. Far-reaching ecological consequences on fish population replenishment and community structure are likely to occur.

5. Fish are a key resource for livelihoods in coastal communities and a key component for ecosystem stability. Given the multiple CO_2-dose dependent effects evidenced here, especially for larvae, we stress the need to expand the number and duration of ocean acidification studies to multi-generational, multiple stressor (e.g. warming, hypoxia, fishing) and species interactions experiments to elucidate complex ecosystem-level changes and how these changes alter ecosystem services supply.

50

51   **INTRODUCTION**

52   By absorbing >25% of the carbon dioxide (CO_2) 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 CO_2 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 $P\mathrm{CO}_2$ (partial pressure CO_2) 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 CO_2 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 CO_2 environment through active ion transport (Claiborne et al., 2002; Brauner & Baker 2009;

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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 CO_2

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 CO_2 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

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100 depend on some life history traits that could confer tolerance to the expected changes in CO_2

101 levels. For instance, species living in pelagic environments, where CO_2 concentrations are stable

102 over time could be more sensitive to OA than coastal benthic species that usually experience high

103 fluctuations of CO_2 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 CO_2 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 CO_2 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 CO_2 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 CO_2 exposure experiments, as well as under chronic exposure in natural

121 CO_2 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 CO_2 on the above response typologies

124 across different life-stages, physiological types (i.e. stenohaline or euryhaline), climatic zones, and

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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 CO_2 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/CO_2 environment. 4) Similarly, being adapted to more stable CO_2 levels in offshore

133 waters, pelagic species should be more sensitive than benthic and bentho-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 CO_2

142 levels (see Appendix S1 for details).

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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 CO_2. We also

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used the reference lists of the retrieved papers. In addition, we searched for OA papers in the news stream managed by the Ocean Acidification International Coordination Centre (OA-ICC; http://news-oceanacidification-icc.org). Papers not reporting mean responses, error estimates and sample sizes in at least one control CO_2 condition and one elevated CO_2 treatment were discarded (in few instances, corresponding authors were contacted to obtain missing information).

As seawater carbonate chemistry varies regionally and between ecosystems (Hofmann *et al*. 2011), control CO_2 levels used in the retrieved experiments spanned from 300 to 800 µatm PCO_2. Therefore, we considered for each study a PCO_2 range (ΔPCO_2) – expressed as the absolute difference between the PCO_2 values in the elevated and in the control CO_2 condition – that was assigned to one of the following OA projections under intermediate or high emission scenarios (RCP 6 and 8.5, respectively; Meinshausen *et al*. 2011): (1) moderate PCO_2 expected by 2050-2070 following the RCP 8.5 (business-as-usual) scenario or expected by 2100 following the RCP 6 scenario (~500-700 µatm; ΔPCO_2 ≤350 µatm); (2) high PCO_2 expected by 2100 following the RCP 8.5 scenario or beyond 2100 following the RCP 6 scenario (~1000 µatm; ΔPCO_2 between 350 and 700 µatm) (Meinshausen *et al*. 2011). We have not included in the analyses studies using PCO_2 levels beyond the predictions for 2100 under the business as usual scenario (i.e. PCO_2 > 1300 µatm), but a few studies (n=3) where control CO_2 conditions were above-average values as they reflected the conditions of specific ecosystems where acidification is predicted to be heavier (Hoffmann *et al.* 2011). Studies manipulating carbonate chemistry using acid addition were not considered as they do not mimic the expected changes in HCO_3^- concentrations and dissolved CO_2.

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

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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 ($= 1 -$ 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

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performance outcome. As an example, in some experiments, fish may have exhibited either a behavioural (negative) change (i.e. a decreasing time percentage spent in the home site cue; Devine *et al*. 2012) or an increased (positive) activity (i.e. venturing at an increasing distance from a shelter; Munday *et al*. 2013) under elevated CO_2, although both types of response could theoretically lead to an increased mortality (hence decreased performance).

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

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

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

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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 pCO_2 levels was calculated as log-ratios (Osenberg *et al*. 1997; Hedges *et al*.

239 1999):

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

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 PCO_2) and control PCO_2

243 conditions, respectively.

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

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

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247 where $\sigma^2_{I_{ijklm}}$ and $\sigma^2_{C_{ijklm}}$ are the variances associated with the means $\bar{X}_{I_{ijklm}}$ and $\bar{X}_{C_{ijklm}}$,

248 respectively, where $n_{I_{ijklm}}$ and $n_{IC_{ijklm}}$ 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).

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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;

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see Table S3, Fig. S1). When considering climatic zones, mortality increased only for sub-tropical species exposed to high $P\text{CO}_2$ (+38%), and no effects were evident for polar, tropical and temperate species exposed at moderate and high $P\text{CO}_2$ levels (Fig. 1b; see Table S3, Fig. S1). Mortality of pelagic fish increased at high $P\text{CO}_2$ levels, whereas benthic and benthopelagic species did not show significant effects at the two of $P\text{CO}_2$ level ranges considered (Fig. 1b; see Table S3, Fig. S1).

Growth of larvae significantly decreased by 4% at high $P\text{CO}_2$, and no effect was observed at moderate $P\text{CO}_2$ (Fig. 1b; see Table S3, Fig. S2). This neutrality in growth response was also apparent on embryos and juveniles at both $P\text{CO}_2$ levels and on adults at high $P\text{CO}_2$ levels (Fig. 1b; see Table S3, Fig. S2). We did not find any OA effects on fish from different climatic zone, except for subtropical species that showed decreased growth (-14%) at high $P\text{CO}_2$ levels (Fig. 1b; see Table S3, Fig. S2). Growth of pelagic species significantly decreased by 9% at high $P\text{CO}_2$ conditions, whereas benthic and bentho-pelagic fish were neutrally affected by altered CO_2 (Fig. 1b; see Table S3, Fig. S2).

Calcification (otolith formation) responses to OA differed between fish life stages, physiologies, climatic zones, and habitats (Fig. 1b; see Table S3). Exposure to high $P\text{CO}_2$ levels significantly increased calcification of fish larvae (+6%), and of sub-tropical (+7%), temperate (+6%) and benthic (+6%) species (Fig. 1b; see Table S3, Fig. S3). In addition to this, the calcification of benthopelagic species was also affected but only one contrast was available for this analysis.

Fish reproduction and metabolic scope (MS) were unaffected at both $P\text{CO}_2$ levels, no matter the different fish traits considered (Fig. 2; see Table S3, Fig. S4, S6). Resting metabolic rate (RMR) increased in adult (+13%) and stenohaline (+8%) fish exposed to high $P\text{CO}_2$ levels. Interestingly, polar species exposed to high CO_2 concentrations showed a RMR 33% higher than control conditions. Trait-mediated responses were also evident among fish habitats, as benthic fish

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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

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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 $P\text{CO}_2$ 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 $p\text{CO}_2$ levels, whilst neutral effects were recorded for larval and adult stages at the

328 two ranges of $P\text{CO}_2$ levels (Fig. 4b; see Table S4, Fig. S9). Fish activity increased only in

329 stenohaline and benthic species exposed to moderate $P\text{CO}_2$ 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 $P\text{CO}_2$ 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 $P\text{CO}_2$ (only 2 contrasts included in the analyses) and no

337 effects at high $P\text{CO}_2$ 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%) $P\text{CO}_2$ levels. Feeding activity and detection of

340 prey (i.e., the foraging activity of fishes) also decreased by 59% at high $P\text{CO}_2$ 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 $P\text{CO}_2$ 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 $P\text{CO}_2$ ranges considered (Fig. 6; see Table S4). Short-term experiments

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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

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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 CO_2 levels may

381   be CO_2 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 CO_2 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 CO_2 conditions (~1000 µatm PCO$_2$). The observed higher sensitivity to elevated PCO$_2$

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).

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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 CO_2 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 CO_2 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 $CaCO_3$ 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   PCO_2 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 bentho-pelagic species may increase at high PCO_2 levels. Recent

415   studies shed light on the potential mechanisms involved in increased otolith formation under OA.

416   Specifically, rising dissolved CO_2 concentrations in the ocean may lead to increased HCO_3^- levels

417   in the fish plasma (which is used to compensate pH), while plasma PCO_2 levels remain elevated

418   (Esbaugh et al., 2012). It appears that plasma HCO_3^- import in the alkaline endolymph and the

419   hydration of CO_2 in the saccular epithelium lead to increased HCO_3^- and hence to increased otolith

420   size when fish are exposed to elevated CO_2 levels (Heuer & Grosell, 2014). It is presently

421   unknown whether larger otoliths may lower the ability of sound detection and the survival of fish

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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 CO_2 on the metabolism of fish early stages, adults

425 experimentally exposed to high pCO_2 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 CO_2. 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 pCO_2 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 CO_2 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 CO_2

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-CO_2 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 CO_2

446 levels, no matter which specific fish trait was considered. However, in this case, there were too

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447 few experiments to draw a clear picture of the effect of rising CO_2 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 PCO_2 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 PCO_2 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 PCO_2 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 PCO_2 levels. To date, the impact of

458 OA on fish osmoregulation at ecologically relevant CO_2 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 CO_2. A decreased yolk size was evident after exposure to high PCO_2 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 CO_2 conditions in

465 the open ocean (Hofmann *et al*. 2011), might be more susceptible to CO_2 increases compared to

466 the offspring of fish laying eggs in the benthic environment, which in turn might be better adapted

467 to natural CO_2 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 PCO_2 levels only in benthic spawning

471 fish, but not in offspring with pelagic development. It is known that through maternal

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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 CO_2 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 CO_2

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 CO_2 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

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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   CO_2 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 CO_2 exposure duration*

506   To date, a great uncertainty associated with the consequences of different CO_2 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 CO_2 exposure times from

509   field- and lab-based experiments revealed that moderate PCO_2 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 PCO_2 levels significantly affect mortality and

513   growth of fish only after short- but not after long-term elevated CO_2 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 PCO_2 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 CO_2 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 $P\text{CO}_2$ 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 $P\text{CO}_2$ 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.

22

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 CO_2

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 CO_2 levels expected by the next few decades or by the end of this century

558　depending on the CO_2 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 CO_2, and

563　thus reject previous hypotheses that some specific fish traits (e.g., those species characteristics

564　associated to highly fluctuating $P\mathrm{CO_2}$ environments) may mitigate such responses. Many

565　explanations for documented eco-physiological and behavioural responses of marine fish to

566　elevated CO_2 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

23

572   marine fish livelihoods in coastal communities, for ecosystem stability and food webs, it is

573   fundamentally critical to slow down current rates of CO_2 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.

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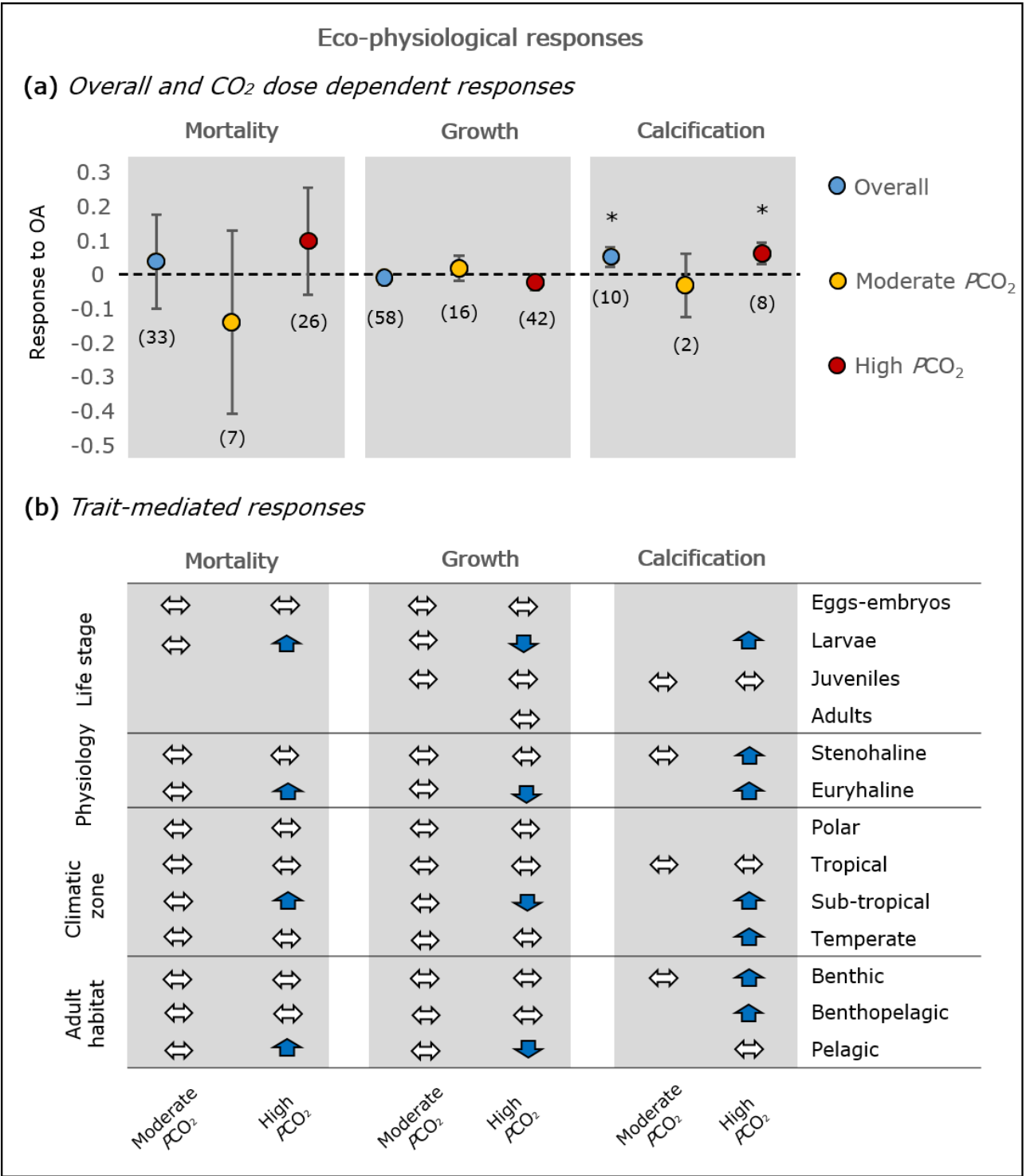
Figure 1 OA effects on fish eco-physiological responses (mortality, resting metabolic rate and metabolic scope). **(a)** Overall and CO_2 dose-dependent responses. Effect sizes (±95% CI) are shown for each response variable, overall and for each CO_2 projection (see Materials and methods for details). 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 CO_2

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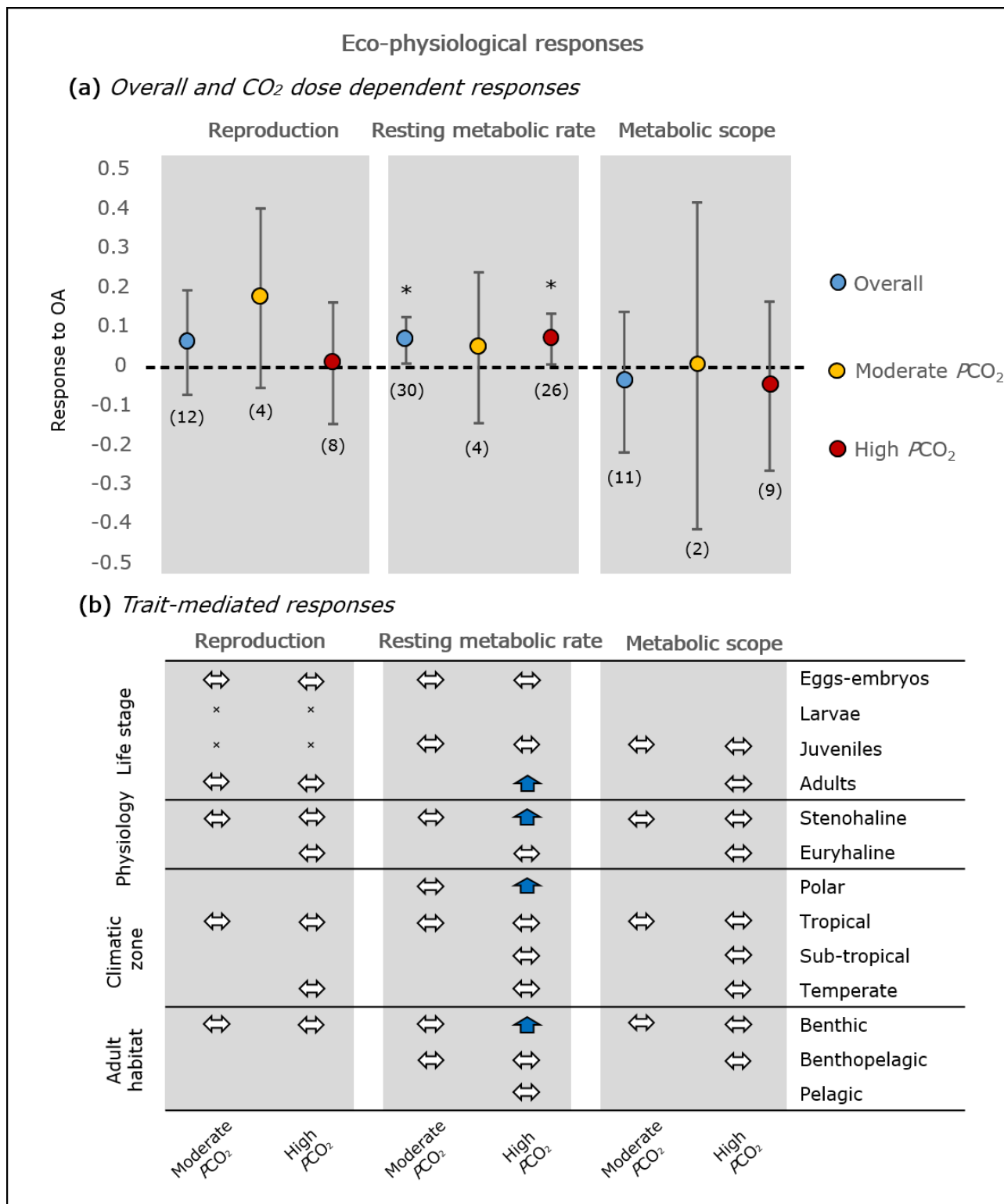
Figure 2 OA effects on fish eco-physiological responses (reproduction, growth and calcification).

(a) Overall and CO_2 dose-dependent responses and **(b)** summary of trait-mediated responses for

824   each CO_2 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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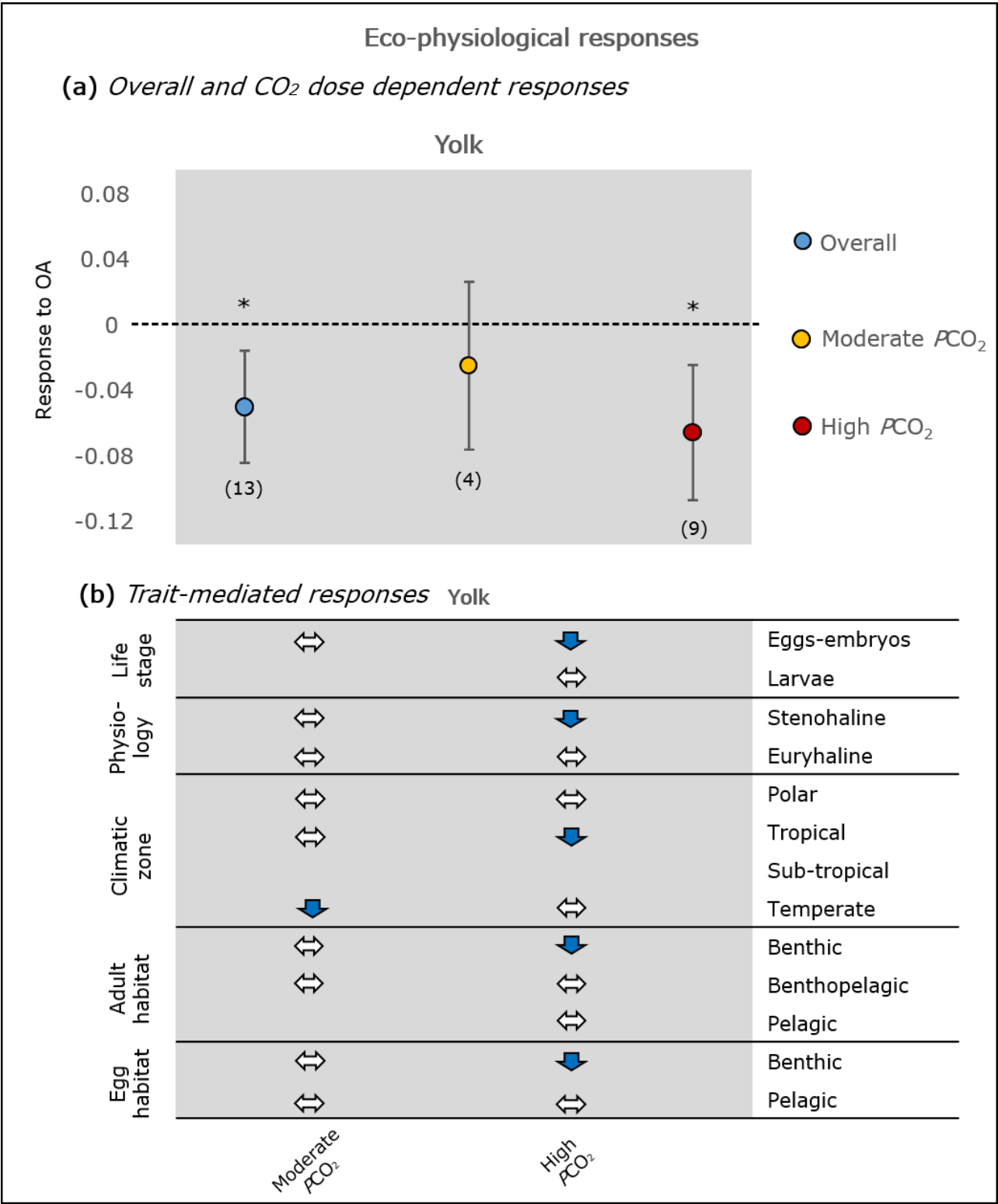
Figure 3 OA effects on fish development (yolk, hatching time and embryonic abnormalities). (a) Overall and CO_2 dose-dependent responses and (b) summary of trait-mediated responses for each

830   CO_2 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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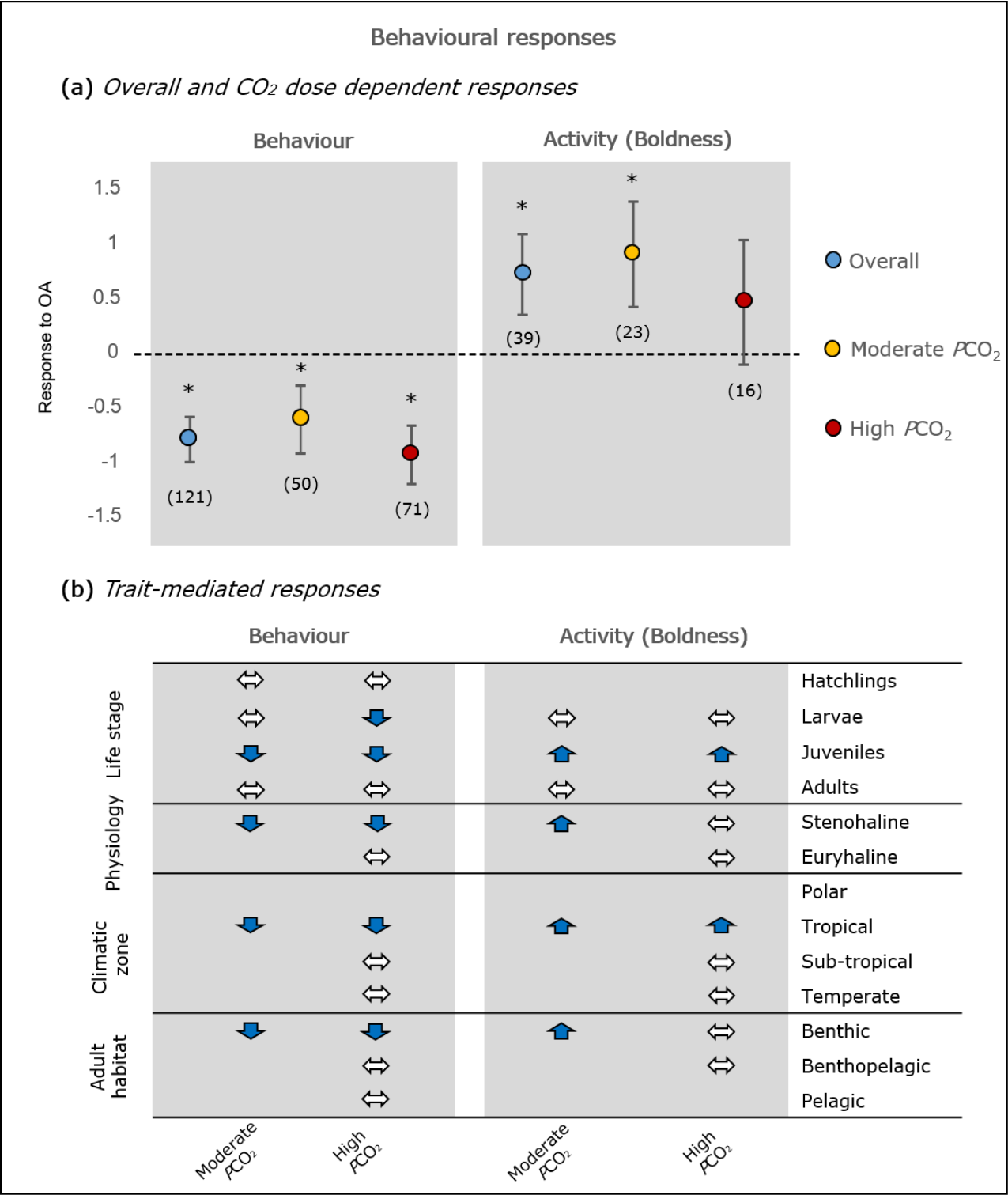
Figure 4 Effects of OA on behavioural responses (behaviour and activity) of fish. **(a)** Overall and

CO_2-dose dependent effects of OA on fish behaviour and **(b)** summary of trait-mediated responses

837   for each CO_2 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.

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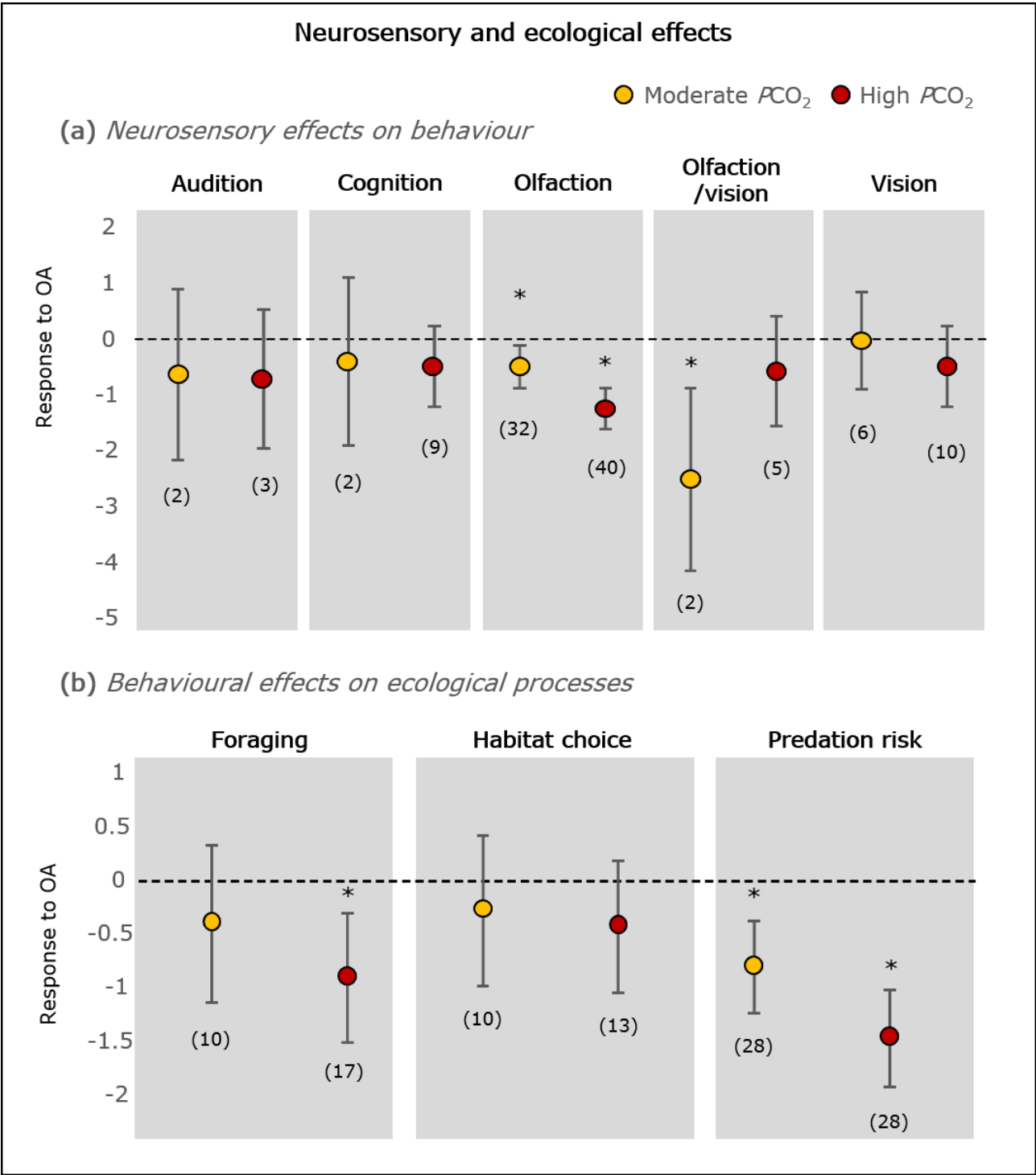
Figure 5 Links between fish neurosensory, behaviour and ecological processes. **(a)** Neurosensory mediated responses of fish to OA in behavioural experiments and **(b)** behavioural effects on ecological processes (foraging, habitat choice and predation risk). Type of data as reported in Figure 1 legend.

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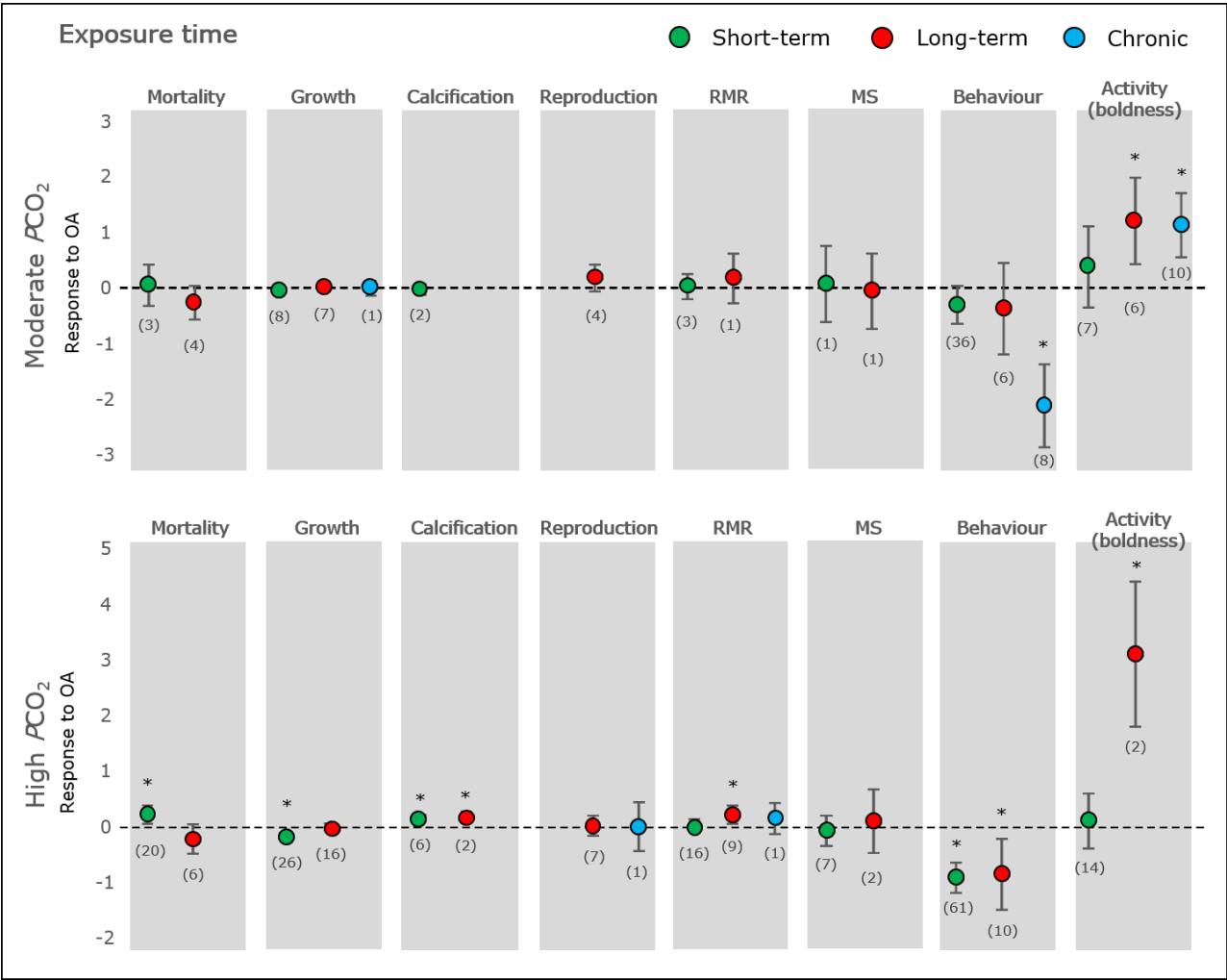
Figure 6 Effects of different CO_2 exposure times (short-term, long-term and chronic) on fish eco-physiology (mortality, growth, calcification, reproduction, resting metabolic rate, metabolic scope) and behavioural performance (behaviour and activity). Type of data as reported in Figure 1 legend.