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Living in a high CO2 world: a global meta-analysis shows multiple trait-mediated responses 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.

Keywords: Behaviour, Calcification, Development, Fish-traits, Growth, Heterogeneity, Metabolism, Survival, Teleosts.

INTRODUCTION

By absorbing >25% of the carbon dioxide (CO_2) and >90% of the heat accumulating in the atmosphere, the ocean plays a key role in regulating the Earth's climate (Gattuso et al., 2015). However, this occurs at a cost, as changes in the ocean's carbonate system resulting from increasing anthropogenic CO_2 emissions – a process termed ocean acidification (OA) – have been progressing since the industrial revolution at a historically unprecedented rate (Doney et al., 2009; Hönisch et al., 2012). OA is predicted to rise even more rapidly during this century, as average ocean PCO_2 (partial pressure CO_2) could reach ~1000 µatm (Meinshausen et al., 2011) leading to 0.3 units reduction in pH (Bopp et al., 2013; Stocker et al., 2013; Pörtner et al., 2014; McNeil & Sasse, 2016), well beyond the threshold expected to be detrimental to many marine organisms (Kroeker et al., 2013). This may have far-reaching consequences for marine ecosystems, including biodiversity loss and changes to marine community structure (Nagelkerken & Connell, 2015).

International organizations warn that global economy could risk losing as much as $1 trillion USD annually if countries do not take urgent steps to slow down anthropogenic CO_2 emissions by the end of the century (CBD, 2014). Reflecting mounting concerns, research attempts to predict biological impacts of OA have gone through a striking increase in publishing rate (with more than 500 articles published in 2013), which is unparalleled by any other scientific field (Riebesell & Gattuso, 2015).

Biological consequences of OA are fundamentally linked to the ecology and physiology of marine species (Pörtner et al., 2014; Heuer & Grosell, 2014). Existing quantitative syntheses have found that effects vary among life stages, species or broader taxonomic groups (Hendriks et al., 2010; Kroeker et al., 2010; Harvey et al., 2013; Kroeker et al., 2013; Wittmann & Pörtner, 2013). Fish were initially thought to be resilient to OA as they are able to defend their internal pH in a high CO_2 environment through active ion transport (Claiborne et al., 2002; Brauner & Baker 2009;

Melzner et al., 2009). Nevertheless, increased acid-base regulation activity may have energetic consequences that might affect physiology and development, especially in early life stages (Ishimatsu et al., 2008). More recently, the compensatory responses that occur during acid-base regulation were found to influence fish calcification, behaviour and ion transport (Nilsson et al., 2012; Heuer & Grosell, 2014; 2016). Calcification in fish occurs in the inner ear for formation of otoliths, carbonated structures playing a role in sound detection, acceleration and orientation, and in the intestinal lumen, where calcium carbonate is precipitated to excrete excess calcium ions (Heuer & Grosell, 2014). Fish potentially contribute with up to 45% of global calcium carbonate budget (Wilson et al., 2009), therefore playing an important role for ocean chemistry and carbon sequestration. Most notably, changes in the concentrations of acid-base relevant ions at higher CO_2 levels appear to impair the function of neurotransmitter receptors causing a range of behavioural changes (Nilsson et al., 2012; Hamilton et al., 2014; Heuer & Grosell, 2014). In fish, documented behavioural effects of OA involve impaired learning ability, sensory functions or decision-making, along with disrupted anti-predator responses, increased boldness, and altered homing behaviour (see Briffa et al., 2012; Heuer & Grosell, 2014; Clements & Hunt, 2015, for reviews). Several studies also documented that exposure to elevated CO_2 alters fish lateralization, the preference for left or right side during behavioural activity that confers benefits in terms of spatial orientation, reactivity, cognition and group coordination (Domenici et al., 2012; 2014 Jutfelt et al., 2013). Behavioural and sensory functions changes may affect key ecological processes such as predator-prey interactions, dispersal, settlement and habitat choice, with cascading implications at the community level (Nagelkerken & Munday, 2016).

To date, the large majority of studies documenting effects of high levels of PCO_2 on fish have been performed in laboratory conditions, showing variable and sometime contradictory results or in some instances underestimating the potential ability of fish to acclimate and adapt to predicted OA in the long term (Wittmann & Pörtner 2013). Indeed, species sensitivity to OA may depend on some life history traits that could confer tolerance to the expected changes in CO_2 levels. For instance, species living in pelagic environments, where CO_2 concentrations are stable over time could be more sensitive to OA than coastal benthic species that usually experience high fluctuations of CO_2 concentrations due to the alternation of respiration and photosynthesis processes by reef organisms (Munday et al. 2008; Hoffman et al. 2011).

To date, existing quantitative generalisations of OA consequences for fish under different CO_2 projections have been controversial, with uncertainty associated with the small number of experiments conducted (Harvey *et al*. 2013; Kroeker *et al*. 2013; Wittmann & Pörtner 2013). A more comprehensive picture of fish responses across different species characteristics and altered CO_2 concentrations is now possible, as research on the topic has progressed in the few last years. Indeed, this represents an opportunity to test the robustness of the patterns found in previous studies and to highlight more general insights.

Here, we used a mixed-effects meta-analytical approach to investigate the effects of projected CO_2 for 2050-2070 and 2100 under intermediate or high emission scenarios (RCP 6 and 8.5, respectively; Meinshausen *et al.* 2011; Pörtner *et al.* 2014), on several response variables extracted from 75 studies (327 contrasts) on 44 species, from polar to tropical regions. We classified and analysed potential effects of OA according to two broad types of fish responses: (1) the eco-physiological responses (mortality, reproduction, metabolism, growth, calcification and yolk dimension); and (2) the behavioural responses (i.e. effects on fish behaviour and activity levels). To assess potential differences in responses, we also analysed such response variables both in short and longer term CO_2 exposure experiments, as well as under chronic exposure in natural CO_2 seeps.

In addition to this, since fish responses to OA can vary among ontogenetic stages and species traits, we also examined the effects of elevated CO_2 on the above response typologies across different life-stages, physiological types (i.e. stenohaline or euryhaline), climatic zones, and habitats (see Appendix S1 for details). Specific hypotheses associated to these different fish characteristics were the following. 1) Due to their inefficient system for ionic regulation, we expected fish early life stages (i.e. embryos, larvae and juveniles) to be more affected by elevated CO_2 levels than the adult stage. 2) Stenohaline species should be more sensitive than euryhaline fish, as these latter experience highly fluctuating PCO_2 environments during their life cycle. 3) The eco-physiology and behaviour of tropical and polar species is more disrupted under OA conditions than that of temperate and sub-tropical ones, which evolved in a relatively more variable pH/CO_2 environment. 4) Similarly, being adapted to more stable CO_2 levels in offshore waters, pelagic species should be more sensitive than benthic and bentho-pelagic ones.

Several studies elucidated a clear relation between fish brain function, behaviour and ecological processes under expected OA levels (Nilsson *et al*. 2012; Chivers *et al*. 2014; Lai *et al*. 2015). Documented fish behavioural changes in fish are a consequence of (upstream) neurosensory disruptions (Heuer & Grosell 2014; 2016), and may lead to altered (downstream) ecological processes (recruitment, predation or competition; Nagelkerken & Munday 2016). Therefore, we also assessed to what extent fish behavioural responses were related to neurosensory (cognition, audition, olfaction, vision, and a combination among olfaction and vision) and ecological (foraging, predation risk and habitat choice) categories under elevated CO_2 levels (see Appendix S1 for details).

MATERIAL AND METHODS

Data collection and selection criteria

We searched for studies testing the effects of altered PCO_2 levels on marine teleost fish published online or printed through to January 2017 using Google Scholar and ISI Web of Science with the combination of the following three keywords: ocean acidification, fish and CO_2. We also 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; $\Delta PCO_2 \leq 350$ 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 metrics related to the same response typology (e.g., length and weight to estimate growth) we randomly chose one metric to avoid autocorrelation. For studies designed to assess interactive effects of multiple stressors (for instance food availability, oxygen, salinity, and temperature along with PCO_2) we included only responses to altered PCO_2 and considered control levels for the other factors. We included data from trans-generational acclimation experiments when parents were reared under control CO_2 conditions. The data on survival were transformed to mortality estimates (= 1 – survival). For the calcification, we included only studies dealing with otolith formation (e.g. otolith area). To assess OA effects on fish metabolism, we analysed the resting metabolic rate (RMR, i.e. oxygen consumption at resting) and its proxies, and the metabolic scope (i.e. aerobic scope - MS, i.e. the difference between maximum and resting metabolic rates) separately, as the directionality of each response under OA may be different. For instance, an increase of RMR may be related to an increase in the energy spent on acid base regulation and ventilation (Perry & Gilmour 2006), with this being potentially detrimental for the species performance as an increase in cost of acid-base regulation can shunt away the energy from growth (Ishimatsu *et al*. 2008) by decreasing MS. By contrast, a reduction in MS results in less energy being available for life-history processes, such as growth, foraging activity and reproduction (Pörtner & Farrell 2008) and can be due to a reduction in maximum metabolic rate even if RMR is not affected.

When assessing the fish behavioural response types to OA, we kept distinct those experiments showing behavioural changes (generally reported as reductions of behavioural performances, i.e. negative effects) from those experiments exhibiting changes in activity or boldness (which in turn often recorded increased effects, i.e. positive effects; but see Hamilton *et al*. 2014) and split these into behaviour and activity, respectively. Importantly, the definitions of positive or negative effects (see also effect size calculation) apply to the directionality (above or below the null effect) of each response variable separately and may not reflect the actual fish 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 information available in each paper considered. Moreover, to evaluate the effects of different time of exposure to OA experimental conditions, we compared data from short-term experiments (≤4 weeks), from long-term experiments (>4 weeks) and from experiments conducted along natural CO_2 vents where fish with limited mobility are chronically exposed to elevated CO_2 concentrations (i.e. chronic exposure experiments) following Nagelkerken & Connell (2015).

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

Data analysis

We used a weighted, random-effects meta-analysis to quantify the response of fish to OA and assess how the studied categories can mediate this response. For each combination of response type i, response variable j, category k, category level m and study l, the effect size of the fish response to elevated pCO_2 levels was calculated as log-ratios (Osenberg *et al*. 1997; Hedges *et al*. 1999):

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

where $\bar{X}_{I_{ijklm}}$ and $\bar{X}_{C_{ijklm}}$ are the mean values of response variable j, in response type i, in category k, and category level m from study in the impacted (elevated PCO_2) and control PCO_2 conditions, respectively.

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

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

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

RESULTS

Eco-physiological responses

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

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

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

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

Growth of larvae significantly decreased by 4% at high $P\mathrm{CO}_2$, and no effect was observed at moderate $P\mathrm{CO}_2$ (Fig. 1b; see Table S3, Fig. S2). This neutrality in growth response was also apparent on embryos and juveniles at both $P\mathrm{CO}_2$ levels and on adults at high $P\mathrm{CO}_2$ 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 $P\mathrm{CO}_2$ levels (Fig. 1b; see Table S3, Fig. S2). Growth of pelagic species significantly decreased by 9% at high $P\mathrm{CO}_2$ 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 $P\mathrm{CO}_2$ 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 $P\mathrm{CO}_2$ 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 $P\mathrm{CO}_2$ 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 increased their RMR at high PCO_2 levels, whereas benthopelagic and pelagic species resulted unaffected under elevated CO_2 conditions (Fig. 2b; see Table S3, Fig. S5).

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

Behavioural responses

Overall, OA conditions significantly and strongly affected behaviour (-54 %) and activity (+108 %) of fish (Fig. 4a, Table S1) and such responses did vary among PCO_2 levels (Fig. 4a, see Table S2). Moderate CO_2 concentrations decreased by 45% the behavioural performances of fish and increased their activity levels by 150% on average (Fig. 4a, see Table S2). At high PCO_2 levels fish displayed significant reductions of their behavioural performances (-60%), whereas no significant effects were evident on their activity (Fig. 4a, see Table S2). Behaviour of larvae was negatively affected at high PCO_2 levels, whereas juveniles showed significant reductions in behavioural performances at both PCO_2 levels considered (Fig. 4b; see Table S4, Fig. S8). Behavioural disruptions were also evident for tropical species exposed at moderate and high CO_2 concentrations (Fig. 4b; see Table S4, Fig. S8). OA did not seem to affect the behaviour of subtropical and temperate species, but the number of available studies is too low to detect a clear response pattern (Fig. 4b; see Table S4, Fig. S8). No data on OA effects on the behaviour of polar species were available. When considering fish physiology and habitat traits, most of the contrasts revealed OA effects on stenohaline and benthic species, showing severe behavioural disruptions at both moderate and high PCO_2 levels (Fig. 4b; see Table S4, Fig. S8).

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

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

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

Experimental CO_2 exposure time

Our analysis showed that fish sensitivity to OA scenarios differed according to experimental CO_2 exposure time and the PCO_2 ranges considered (Fig. 6; see Table S4). Short-term experiments at moderate $P\text{CO}_2$ levels did not affect any eco-physiological and behavioural responses, whereas short-term exposure to high $P\text{CO}_2$ levels significantly increased fish calcification (+6%) and mortality (+24%), and decreased growth (-3%) and behavioural performances (-60%) (Fig. 6; see Table S4). Long-term exposure at both moderate and high $P\text{CO}_2$ levels significantly increased fish activity/boldness (+232% and +2119%, respectively). High $P\text{CO}_2$ levels increased fish RMR (+20%) and decreased their behavioural performances (-58%). The effects of chronic exposure to elevated CO_2 concentrations were evident at moderate $P\text{CO}_2$ levels for behaviour (-88%) and for activity (+200%), no data are available for high $P\text{CO}_2$ levels .

DISCUSSION

New insights are gained from our findings by including the increasing number of studies published in recent years and the responses of fish to different CO_2 projections. This also allowed our analyses to identify a range of trait-based variations in fish sensitivity to OA, with eco-physiological and behavioural responses varying according to CO_2 scenarios, and fish ontogenetic phases and species characteristics. Specifically, we show that, if anthropogenic CO_2 emissions continue to rise both under the RCP 6 and RCP 8.5 scenarios, overall significant effects of elevated $P\text{CO}_2$ levels on fish metabolism, calcification (otolith formation), yolk consumption, behaviour and activity (boldness) are expected to occur in the next few decades. It is now well-established that under elevated CO_2 conditions, any ATP-demanding compensation activities for acid-base balance and enhanced transport of ions may incur elevated energetic costs, potentially leading to subtle fitness consequences for such response variables in marine fish (Sokolova 2013; Heuer & Grosell 2014). Hence, at the same time, we suggest that more subtle consequences of OA on mortality and growth of fish, as well as downstream ecological consequences, might occur.

There has been a clear call for addressing the important effects of species traits when making predictions about the impacts of environmental change (e.g. Estrada *et al*. 2016). Our work meets this call and sharpens the focus to fish. Previous meta-analyses did not take into account different fish traits and life stages instead focussing on a small amount of experiments available at that time (Harvey *et al*. 2013; Kroeker *et al*. 2013; Wittmann & Pörtner 2013; Lefevre, 2016). As an example, it has previously been argued that OA studies on fish were biased by the high number of experiments carried out on tropical species (Wittmann & Pörtner 2013), therefore suggesting that responses were predominantly focussed towards species with short life cycles, which are typical of this climate zone (Leis & McCormick 2002). By analyzing the body of literature through to January 2017 and by including data on additional 30 non-tropical species and 115 contrasts, here we were able to provide a clearer picture and show that fish responses to elevated CO_2 levels may be CO_2 dependent and vary according to different fish characteristics and traits.

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

We documented varying adverse responses when examining specific fish traits. In this regard, we did not find any specific fish characteristics which could potentially confer tolerance to fish under elevated CO_2 concentrations. These are important results as in addition to identifying the direction and quantifying the magnitude of these responses, we were able to show that, if fish traits are not taken into account, consequences of ocean acidification on marine animals might not be detected as in some cases species-specific responses could be antagonistic (Esbaugh, 2017). Our study adds on previous evidence showing that larvae are the most sensitive life stage as they displayed increased mortality and calcification (i.e. otolith formation), and decreased growth at elevated CO_2 conditions (~1000 µatm PCO_2). The observed higher sensitivity to elevated PCO_2 levels for early life stages of fish might be linked to their high surface to volume ratio affecting diffusive processes and to their acid-base balance system not being fully developed, with ionic exchanges occurring across the skin of larvae and the yolk of embryos until their gills can develop (Munday *et al*. 2009a; Baumann *et al*. 2012; Hurst *et al*. 2013).

Mortality at individual level affects population dynamics (Munday *et al*. 2010), which in turn can extend responses to higher community- and ecosystem-levels (Nagelkerken & Connell 2015). Since we only included experiments testing for the direct effects of elevated CO_2 on fish mortality, the increased mortality of larvae we recorded may be a conservative estimate, as more indirect effects can also occur leading to even higher mortality rates. As an example, we found that larval growth was significantly reduced at high CO_2 levels, and smaller larvae may exhibit a lower performance and survival in the wild as a result of their reduced swimming ability and less efficient predator avoidance (e.g. Miller *et al*. 1988), therefore leading to increased mortality through predation.

Heavily calcifying organisms like molluscs, echinoderms and corals are considered to be the most threatened marine taxa under OA (Kroeker *et al*. 2013; Wittmann & Pörtner 2013). Fish are not considered calcifying organisms despite producing calcium carbonate in the inner ear for otolith formation or precipitating $CaCO_3$ in the intestinal lumen to reduce osmotic pressure, release calcium ions excess in the environment and thus facilitate water absorption (Heuer & Grosell 2014). Here we found a significant increase in the size of otoliths in larvae exposed to high PCO_2 levels expected to occur by 2050 (following the business-as-usual RCP8.5 scenario) or by 2100 (following the intermediate RCP6.0 scenario). Our results also showed that otolith calcification in both benthic and bentho-pelagic species may increase at high PCO_2 levels. Recent studies shed light on the potential mechanisms involved in increased otolith formation under OA. Specifically, rising dissolved CO_2 concentrations in the ocean may lead to increased HCO_3^- levels in the fish plasma (which is used to compensate pH), while plasma PCO_2 levels remain elevated (Esbaugh et al., 2012). It appears that plasma HCO_3^- import in the alkaline endolymph and the hydration of CO_2 in the saccular epithelium lead to increased HCO_3^- and hence to increased otolith size when fish are exposed to elevated CO_2 levels (Heuer & Grosell, 2014). It is presently unknown whether larger otoliths may lower the ability of sound detection and the survival of fish in the wild, although we know that asymmetry between otoliths can be detrimental (Gagliano et al., 2008).

Despite the lack of effects of elevated CO_2 on the metabolism of fish early stages, adults experimentally exposed to high pCO_2 levels significantly increased their metabolism at rest. Similar responses were detected on polar fish species. At present, some studies suggest that temperature effects lead to higher costs for pH regulation in cold-adapted eurytherms compared to polar stenotherms (Pörtner, 2004), however existing knowledge on OA effects on the metabolism of cold environment fish species is scant. Although an increase in RMR may be expected to result in a decrease in metabolic scope, our results show that MS is not affected by elevated CO_2. This apparent contradiction could be due to either (1) a compensatory increase in maximum metabolic rate or (2) the fact that the number of studies on MS is much smaller than that for RMR and therefore the analysis for MS is less robust. A recent meta-analysis on warming and acidification effects on metabolism of marine organisms showed no significant effects of altered pCO_2 levels on resting metabolic rate and metabolic scope of teleost fish, highlighting high variability in the metabolic responses of fish (Lefevre, 2016). In this regard, our analyses suggest that fish metabolic responses might be trait-mediated as we mostly found elevated CO_2 effects on RMR of benthic, stenohaline and polar species. Earlier studies hypothesised that exposure to high PCO_2 levels could lead to increased fish resting metabolism due to the costs associated with internal CO_2 balance, however mixed responses were found, with some studies confirming this assumption both in tropical (Munday et al., 2009b; Couturier et al., 2013; Ferrari et al., 2015) and non-tropical species (Esbaugh et al., 2016; Flynn et al., 2015), whereas other studies revealed decreased resting metabolic rate after prolonged high-CO_2 exposure (Rummer et al., 2013; Pimentel et al., 2014; Heuer & Grosell, 2016).

Fish reproduction – indeed an energetically costly process – was unaffected by altered CO_2 levels, no matter which specific fish trait was considered. However, in this case, there were too few experiments to draw a clear picture of the effect of rising CO_2 on fish reproduction. In this regard, work on OA effects on fish reproduction *per se* should be a priority given its importance for population replenishment.

Marine habitats with different salinity levels also vary greatly in PCO_2 and thus euryhaline species – e.g. those fish having the ability to effectively osmoregulate across a broad range of salinities – are likely to experience highly variable PCO_2 environments during their life cycle. This suggests these organisms (i.e. marine-brackish fish) can be pre-adapted to OA conditions. Contrary to what we expected, we found a significantly higher mortality of euryhaline fish exposed to high PCO_2 levels. Mortality of stenohaline fish (which cannot tolerate a broad range of salinities) was unaffected by OA but these fish showed increased calcification rates (otolith formation) and metabolic rate at resting when exposed at high PCO_2 levels. To date, the impact of OA on fish osmoregulation at ecologically relevant CO_2 conditions is critically understudied (Heuer & Grosell 2014).

Overall, we found a negative effect on yolk consumption (i.e. decreased yolk size) in fish exposed to elevated CO_2. A decreased yolk size was evident after exposure to high PCO_2 levels for early developmental stages of benthic species, but not for pelagic ones. Similar to the trend proposed for stenohaline and euryhaline fish, some authors have hypothesised that embryos and larvae of pelagic spawning fish, which likely experience lower and more stable CO_2 conditions in the open ocean (Hofmann *et al*. 2011), might be more susceptible to CO_2 increases compared to the offspring of fish laying eggs in the benthic environment, which in turn might be better adapted to natural CO_2 fluctuations due to the alternation of photosynthetic and respiration processes by primary producers (Hofmann et al. 2011; Munday *et al*. 2011). Contrary to these expectations, when categorising yolk size responses according to the habitat of developing embryos, negative (i.e. decreased yolk size) responses have been noted at high PCO_2 levels only in benthic spawning fish, but not in offspring with pelagic development. It is known that through maternal provisioning, females may adjust eggs characteristics (e.g. offspring yolk size) to environmental conditions (Chambers 1997) and that yolk consumption is affected by elevated CO_2 conditions during the embryonic development (Chambers *et al*. 2014).

Our meta-analysis also confirmed that OA could strongly influence the behaviour of tropical fish, particularly that of larvae and juveniles. Indeed, these responses were rather consistent at environmentally relevant $P\mathrm{CO}_2$ levels expected by the next few decades. Importantly, the observed behavioural impairments were due to alterations of a range of different neurosensory functions. Olfaction was the most sensitive sensory function affecting fish behaviour even at $P\mathrm{CO}_2$ levels expected in 2050. At the same time, in many experiments, fish reared in elevated CO_2 conditions were bolder, displaying up to 1.5-fold increase in their activity rates (e.g. less time spent in a shelter, or increased distance ventured) and therefore increased vulnerability to predators (e.g. Munday *et al*. 2010).

As animals respond to environmental change through behavioural modifications, the downstream consequences of the observed behavioural and activity effects (particularly on larvae and juvenile fish) are expected to lower fish ecological performance (e.g. with respect to predator-prey dynamics, recruitment success, settlement, homing and habitat choice) and therefore to affect the outcomes at community level (Nagelkerken & Munday 2016). Consistently, our quantitative findings indicate that the behavioural disruptions we identified could result in a higher predation risk and a lowered foraging activity, which in turn may lead to increased mortality and decreased growth, therefore decreasing the ability of a species to persist in a given community. Unexpectedly the settlement and homing ability (i.e., habitat choice) of fish was unaffected by rising CO_2 levels. Certain effects, such as an increase in fish activity (boldness) and changes in fish lateralization, do not have an immediate bearing on ecological “fitness”, because in nature they represent a trade off in which for example, bold individuals can find food faster (Mamuneas et al., 2015) but are more exposed to predation (Ward et al., 2004) than shy individuals. Similarly, although a high degree of lateralization has been considered an advantage in terms of multitasking (escaping from predators and schooling; Bisazza & Dadda, 2005; Dadda & Bisazza, 2006; Dadda et al., 2010, Domenici et al., 2012), it was suggested to represent a trade off with the ability to deal equally well with stimuli of threats from all directions (Vallortigara & Rogers, 2005). In these cases, the effect of elevated CO_2 may be detrimental for fish because it alters the balance within the continuum of bold vs. shy and lateralized vs. non-lateralized individuals, with potential ecological consequences at population level (Sih et al., 2012).

Ocean acidification effects on fish under different experimental CO_2 exposure duration

To date, a great uncertainty associated with the consequences of different CO_2 projections on teleost fish, relies on whether such organisms will be able to cope with ongoing rapid changes in the carbonate chemistry of the ocean. Our analyses following different CO_2 exposure times from field- and lab-based experiments revealed that moderate PCO_2 levels have no effects on fish eco-physiology both in short- and long-term experiments, and that fish behavioural performances are disrupted particularly in chronically exposed fish populations. Despite consistent responses on fish calcification and behaviour, we found that high PCO_2 levels significantly affect mortality and growth of fish only after short- but not after long-term elevated CO_2 exposures. Indeed, our analyses suggests that further longer term and multi-generational experiments are needed to test for a potential adaptation of fish occurring at altered PCO_2 levels and we identify this issue as a critical gap of knowledge, which is further discussed in the next paragraph.

Future directions and conclusions

Acclimation and adaptation may have significant consequences for how marine organisms will respond to future high CO_2 oceans (Sunday et al., 2014) and some previous work argues that adaptation may be too slow for long-lived species (Pörtner et al., 2014). Due to the small number of experiments carried out so far, quantitative generalisations are not possible yet, and our investigation identified a critical need to increase the number of fish studies addressing such processes under OA. To date, experimental findings are not in line with conclusions made using fossil records and observations from paleo-studies which suggested that teleosts are very tolerant to elevated $P\mathrm{CO}_2$ levels when compared to invertebrate taxa (Wittmann & Pörtner, 2013). In this context, a few studies showed mixed potential for transgenerational acclimation (e.g. Allan et al., 2014; Welch et al., 2014) while others, examining OA effects on chronically CO_2 exposed fish populations (e.g. those from volcanic CO_2 seeps), documented changes in embryo metabolism, olfaction, spawning behaviour, and escape response (Munday et al., 2014; Nagelkerken et al., 2015; Cattano et al., 2016; Milazzo et al., 2016). Although much progress has been made in designing experiments to assess how elevated $P\mathrm{CO}_2$ can alter fish ecophysiology and behaviour, to date we are still far from understanding adaptive capacity to OA (Munday et al., 2013).

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

An additional unavoidable limitation of most OA studies on fish and other taxa is their un-representativeness of ecosystem effects since they do not take into account the complex species interactions acting at multiple levels of organisation in the real world. Yet, some attempts have succeeded in testing OA effects on key community ecological interactions (e.g. competition and predation) involving fish in their natural habitat (Nagelkerken & Munday, 2016). In this context, our study might also identify the need to expand the number of OA studies examining fish responses to community shifts and biogenic habitat modifications in naturally high CO_2 environments (Munday et al., 2014, Enochs et al., 2015; Nagelkerken et al., 2015; Sunday et al., 2016).

In summary, here we document negative effects on many fish eco-physiological and behavioural responses at CO_2 levels expected by the next few decades or by the end of this century depending on the CO_2 emission scenario considered. We suggest that some direct effects – particularly on fish mortality and growth – may be underestimated as we found increased predation risk and decreased foraging, confirm that larvae are the most sensitive life stages (Munday et al., 2009a), and advise that downstream ecological consequences can be very likely. Importantly, we do not find any traits potentially conferring fish tolerance to elevated CO_2, and thus reject previous hypotheses that some specific fish traits (e.g., those species characteristics associated to highly fluctuating PCO_2 environments) may mitigate such responses. Many explanations for documented eco-physiological and behavioural responses of marine fish to elevated CO_2 rely on changes expected to occur in fish brain function (Nilsson *et al*. 2012) and acid-base compensation (Heuer & Grosell 2014). Likely, effects on fish will be heaviest where ocean acidification, warming, and hypoxia regionally coexist, indicating that studies like the one we present focussing on an individual environmental drivers of change maybe conservative. However, some level of uncertainty remains over the long-term persistence of the observed effects on fish, particularly on some eco-physiological response variables. Given the importance of marine fish livelihoods in coastal communities, for ecosystem stability and food webs, it is fundamentally critical to slow down current rates of CO_2 emissions and deviate from the expected scenarios to mitigate OA effects on fish.

AUTHORS CONTRIBUTIONS

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

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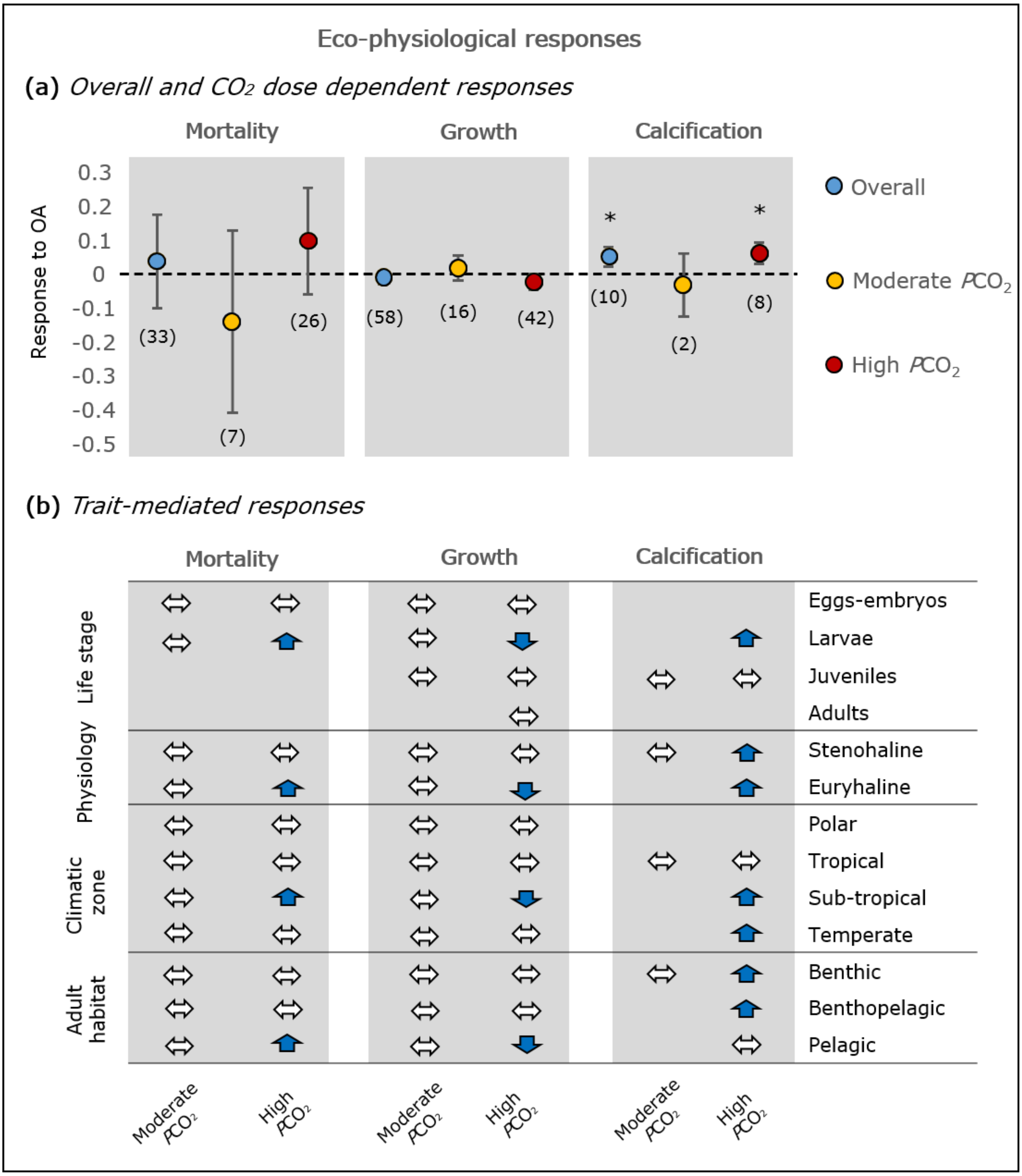


Figure 1 OA effects on fish eco-physiological responses (mortality, resting metabolic rate and metabolic scope). **(a)** Overall and CO_2 dose-dependent responses. Effect sizes (±95% CI) are shown for each response variable, overall and for each CO_2 projection (see Materials and methods for details).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

indicates significant difference from zero. **(b)** Summary of trait-mediated responses for each CO_2 projection relative to fish mortality, resting metabolic rate and metabolic scope. Arrows show the directionality of the responses: "↑" significantly positive; "↓"significantly negative; "↔" null; "x" not applicable. See also Figures S1-S3 for full results.

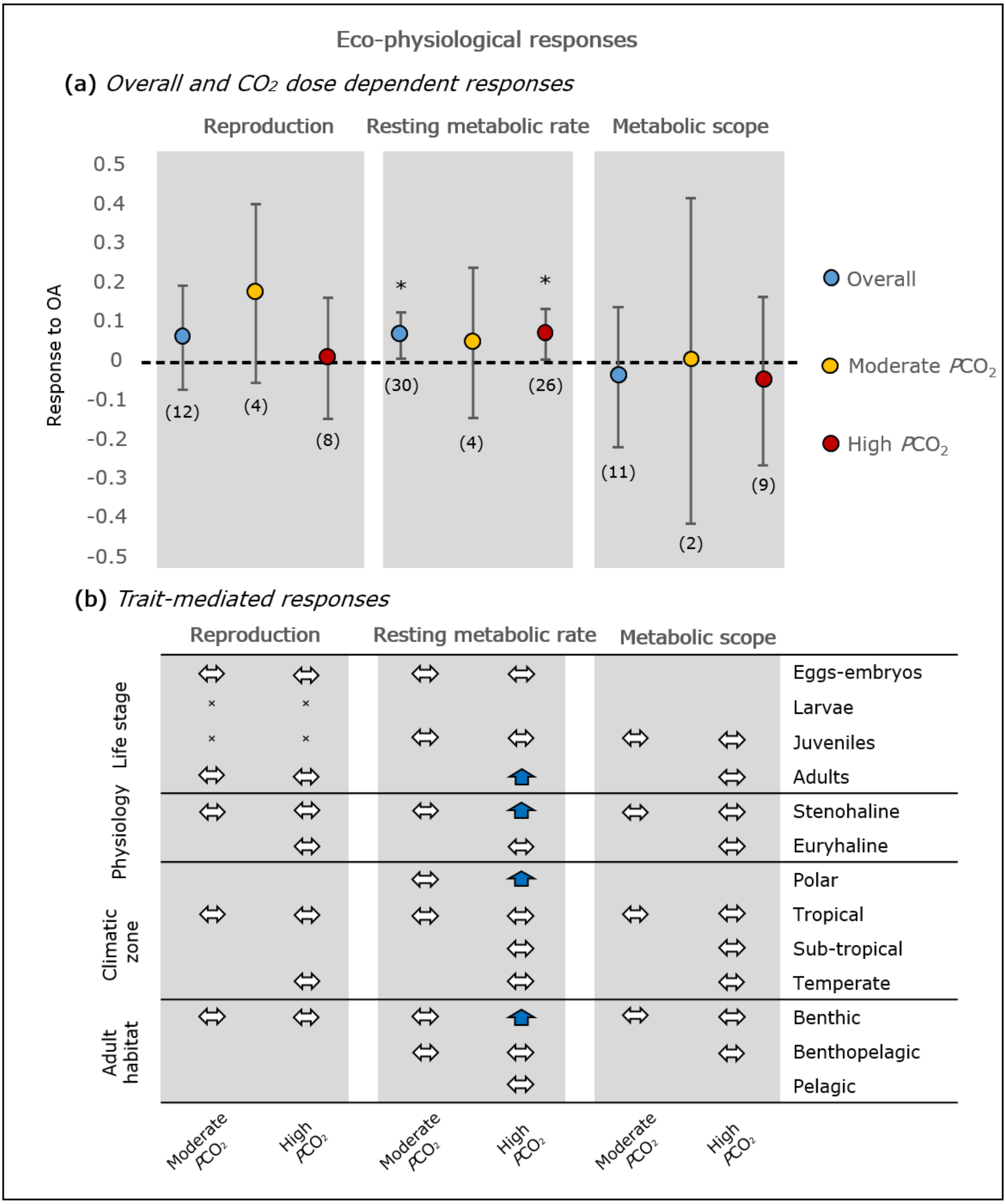
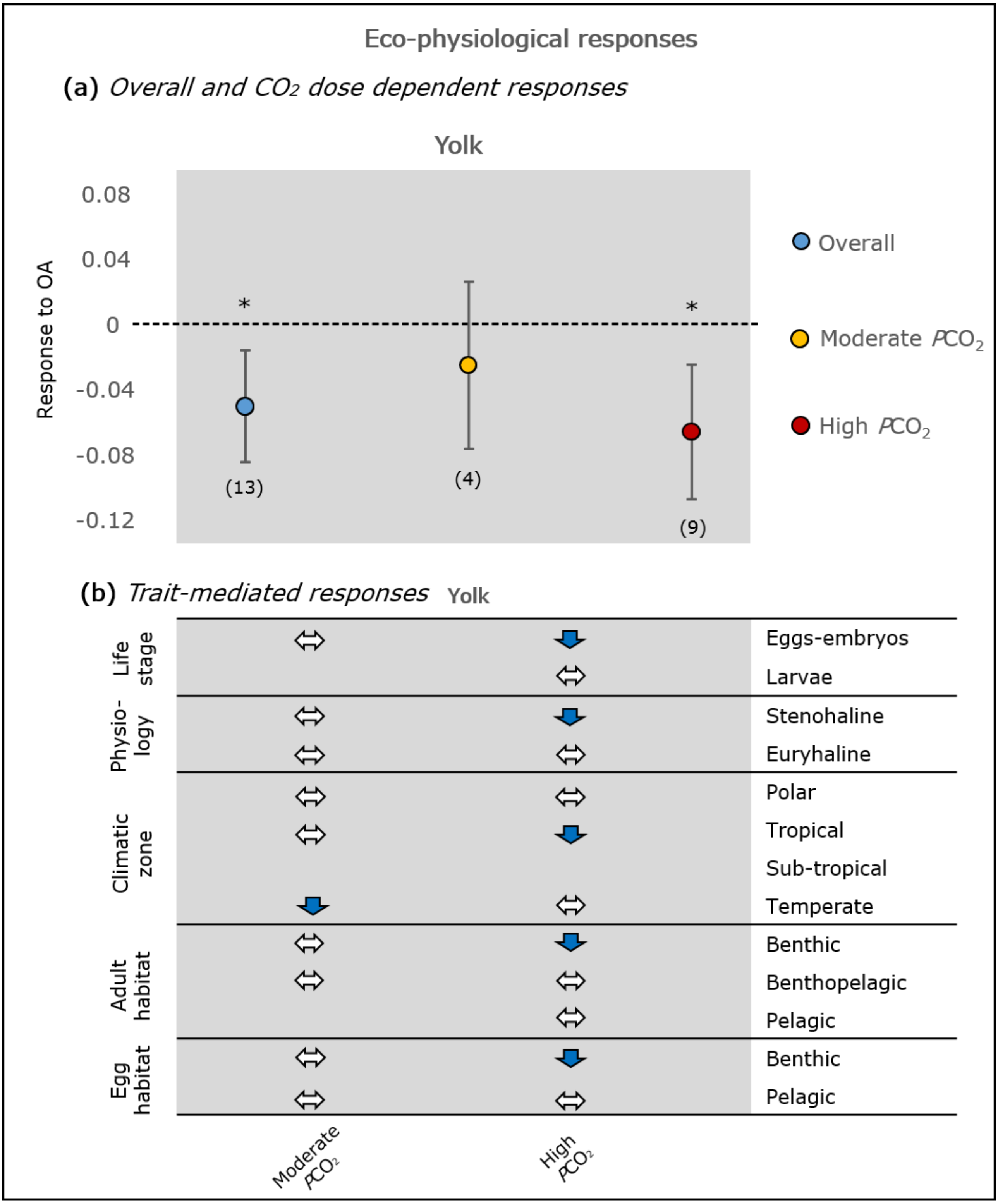


Figure 2 OA effects on fish eco-physiological responses (reproduction, growth and calcification). **(a)** Overall and CO_2 dose-dependent responses and **(b)** summary of trait-mediated responses for

each CO_2 projection relative to fish reproduction, growth, and calcification. Symbols and type of data as reported in Figure 1 legend , see also Figures S4-S6 for full results.



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

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

CO_2 projection relative to yolk, hatching time and embryonic abnormalities. Symbols and type of data as reported in Figure 1 legend, see also Figure S7 for full results.

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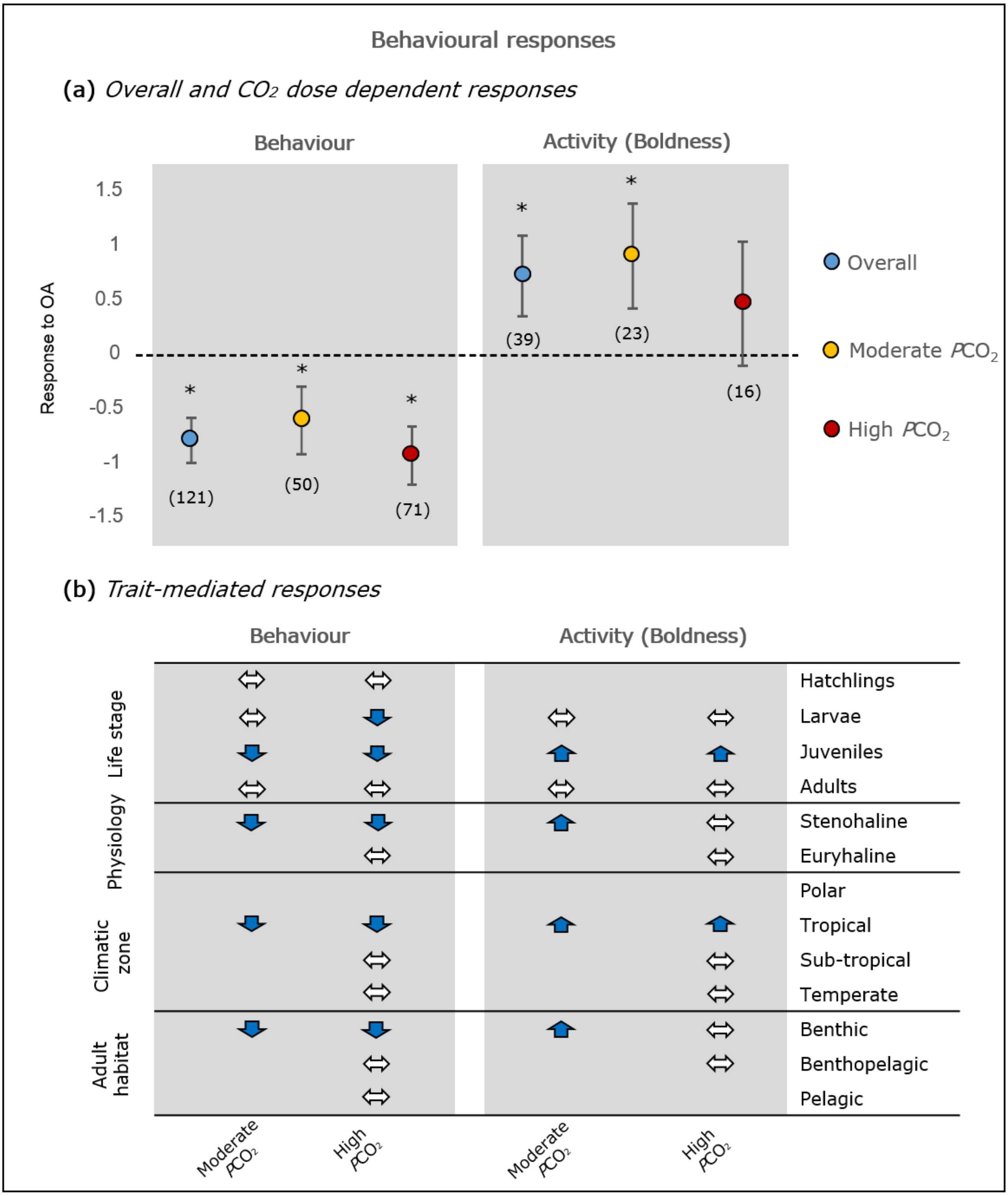

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835 **Figure 4** Effects of OA on behavioural responses (behaviour and activity) of fish. **(a)** Overall and

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

for each CO_2 projection relative to behaviour and activity. Symbols and type of data as reported in Figure 1 legend , see also Figures S8-S9 for full results.

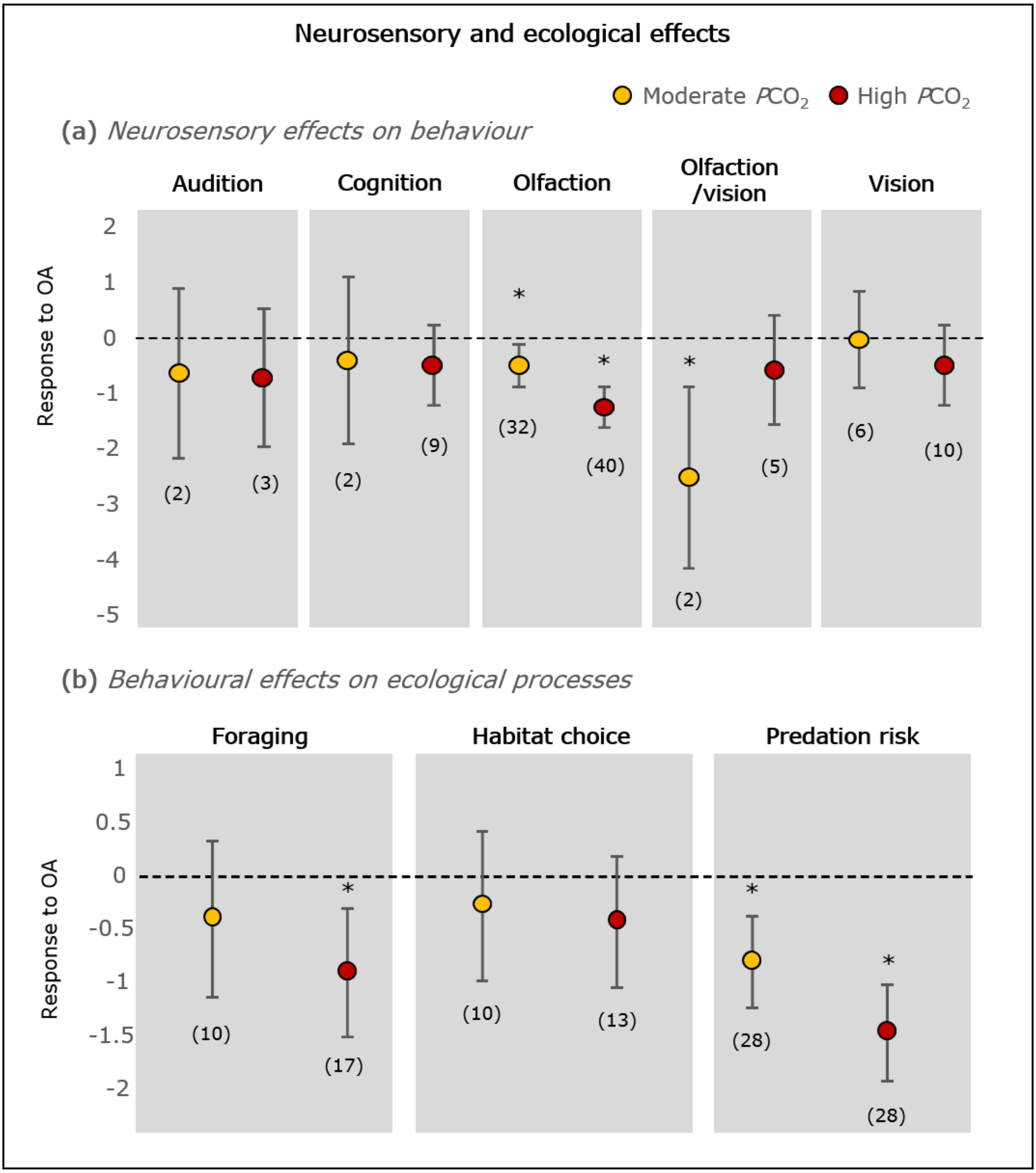

Figure 5 Links between fish neurosensory, behaviour and ecological processes. **(a)** Neurosensory mediated responses of fish to OA in behavioural experiments and **(b)** behavioural effects on ecological processes (foraging, habitat choice and predation risk). Type of data as reported in Figure 1 legend.

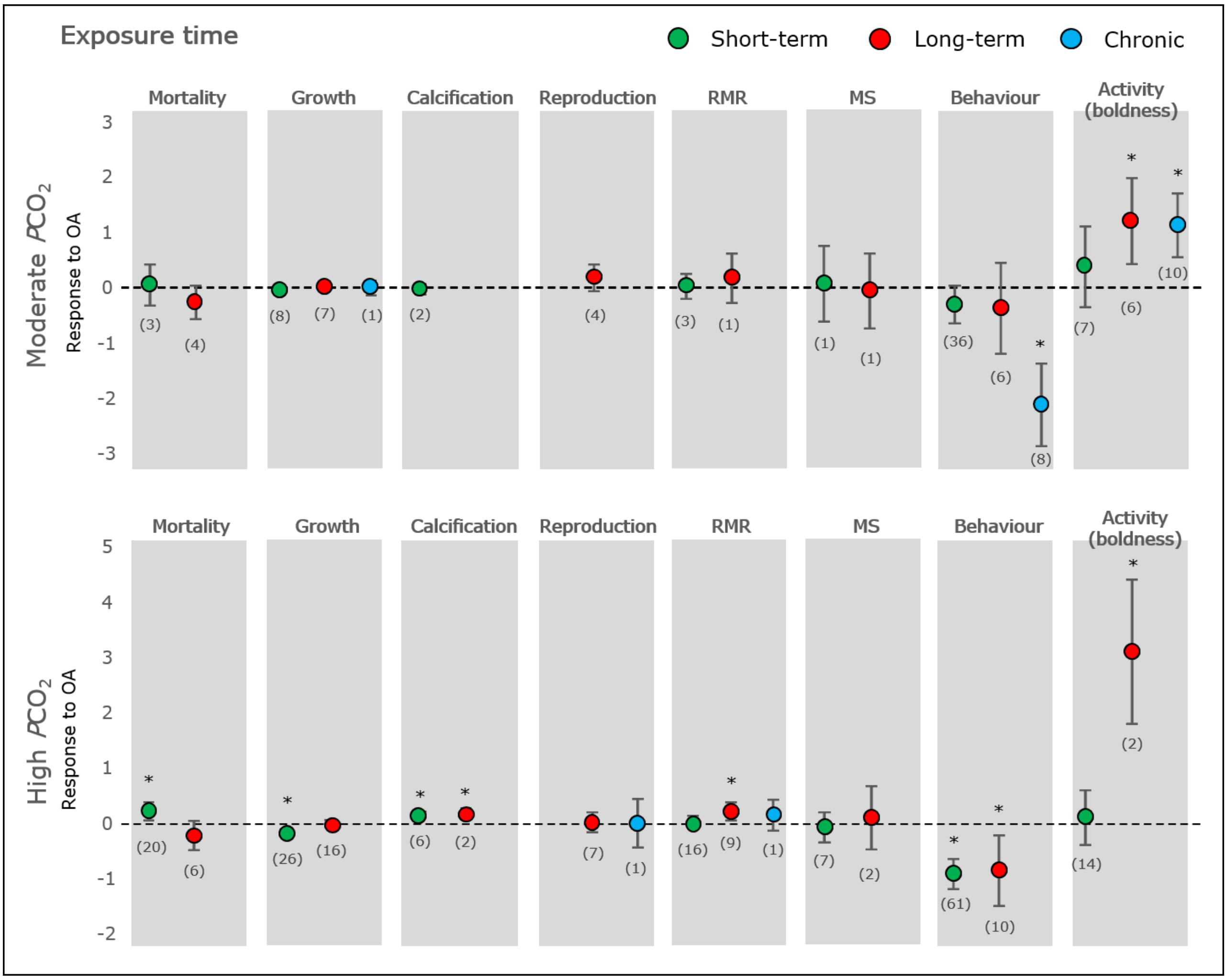

Figure 6 Effects of different CO_2 exposure times (short-term, long-term and chronic) on fish eco-physiology (mortality, growth, calcification, reproduction, resting metabolic rate, metabolic scope) and behavioural performance (behaviour and activity). Type of data as reported in Figure 1 legend.