Title: Temperature and O_2, but not CO_2, interact to affect aerobic performance of European sea bass (*Dicentrarchus labrax*)

Running title: Temperature & O_2, not CO_2, affect bass

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Abstract

Climate change causes warming, decreased O_2, and increased CO_2 in marine systems and responses of organisms will depend on interactive effects between these factors. We provide the first experimental assessment of the interactive effects of warming (14 to 22°C), reduced O_2 (~3 – 21 kPa O_2), and increased CO_2 (~400 or ~1000 µatm ambient CO_2) on four indicators of aerobic performance (standard metabolic rate, SMR, maximum metabolic rate, MMR, aerobic scope, and hypoxia tolerance, O_{2crit}), blood chemistry, and O_2 transport (P_{50}) of a marine fish, the European sea bass (*Dicentrarchus labrax*). Warming increased SMR and O_{2crit} (i.e. reduced hypoxia tolerance) as well as MMR in normoxia but there was an interactive effect with O_2 so that hypoxia caused larger reductions in MMR and aerobic scope at higher temperatures. Increasing CO_2 had minimal effects on SMR, MMR and O_{2crit} and did not show interactive effects with temperature or O_2 for any measured variables. Aerobic performance was not linked to changes in blood chemistry or P_{50}. Despite lack of effects of CO_2 on aerobic performance, increased CO_2 induced 30% mortality of fish exercised in low O_2 at 22°C indicating important threshold effects independent of aerobic performance. Overall, our results show temperature and O_2, but not CO_2, interact to affect aerobic performance of sea bass, disagreeing with predictions of the oxygen- and capacity-limited thermal tolerance hypothesis.

1. Introduction

Atmospheric CO_2 levels are increasing and could reach ~1000 µatm by the end of the century (IPCC, 2014). Rising atmospheric greenhouse gases increase ocean temperatures (Bopp et al., 2013), which reduces oceanic O_2 content and exacerbates the frequency and severity of hypoxic (low O_2) events (Breitburg et al.,

2018; Diaz & Rosenberg, 2008). As atmospheric CO_2 levels continue to rise so too does the concentration of CO_2 in marine systems (Caldeira & Wickett, 2003). Therefore, responses of marine organisms to climate change will be a result of simultaneous changes in temperature, O_2, and CO_2.

Changes in temperature, O_2, and CO_2 can individually impact physiological performance of fish. Concern has been raised that interactions between these three may occur in a non-linear manner, so that their combined impact cannot be predicted from responses to an individual variable (Côté et al., 2016; Crain et al., 2008; McBryan et al., 2013; Todgham & Stillman, 2013). As such, we need to understand how temperature, O_2, and CO_2 interact to affect the physiology of fish to enable accurate predictions of how climate change will influence fish species (Hollowed et al., 2013; Pörtner & Peck, 2010; Wernberg et al., 2012). One approach is to examine how these environmental factors affect the fish's range of aerobic metabolism (aerobic scope), defined as the difference between an animal's maximum rate of O_2 consumption (maximum metabolic rate, MMR) and the minimum rate needed to meet basal energy demands (standard metabolic rate, SMR) (Fry, 1971).

It has been proposed that aerobic scope provides a single metric of whole-animal performance in a particular environment. Therefore, aerobic scope can be directly linked to processes such as growth and reproduction and in turn overall organism fitness, through the concept of oxygen- and capacity- limited thermal tolerance, OCLTT (Pörtner, 2012; Pörtner et al., 2017). The OCLTT hypothesis assumes that an organism's overall fitness is maximised at an optimal temperature at which aerobic scope peaks. As such, anything that reduces aerobic scope will also diminish fitness, potentially changing the distribution of populations (Pörtner & Farrell, 2008). Hypoxia affects aerobic scope by limiting environmental O_2 availability

and, therefore, the maximum O_2 uptake rate possible by fish. Increased CO_2 has been proposed to affect aerobic scope both by increasing the SMR of fish (e.g. through increased cost of acid-base regulation) and by decreasing MMR (potentially because subsequent changes in internal acid-base chemistry can reduce O_2 transport capacity of the blood or impair tissue functioning). The OCLTT hypothesis therefore predicts that reduced O_2 and increased CO_2 will interact to reduce aerobic scope across the thermal performance curve of a species. This would result in a lower optimal temperature (where peak aerobic scope occurs) and reduced thermal tolerance. While this concept has been successfully used to explain changes in habitat suitability and population distributions of some fish species (Cucco et al., 2012; Del Raye & Weng, 2015), the assertion that it represents a universal framework to predict effects of climate change on all fish populations (Farrell, 2016) has been challenged (Jutfelt et al., 2018; Lefevre, 2016).

The proposal of the OCLTT hypothesis has led to numerous studies examining how O_2 or CO_2 interact with temperature to affect aerobic performance (for examples see Chabot and Claireaux, 2008; Rummer et al., 2013; Grans et al., 2014). However, to date no experimental work has sought to investigate how combined changes in all three factors (temperature, O_2, and CO_2) interact to affect aerobic performance in fish. Combining all three environmental variables is vital to accurately assess potential interactive effects for three reasons. Firstly, meta-analysis of multi-factor studies indicates that the prevalence of non-linear effects doubles when moving from studies that combine two factors to three factors (Crain et al., 2008). Secondly, the role of CO_2 as a limiting factor of aerobic scope was originally suggested to occur primarily in combination with hypoxia (Fry, 1971). Thirdly, low O_2 conditions in the environment always co-occur with increased CO_2 (Melzner et al., 2013). As such,

96 experiments investigating effects of O_2 or CO_2 and temperature on aerobic
97 performance may not accurately reflect interactive effects caused by all three
98 environmental factors.

99 In this study we investigated how temperature, O_2, and CO_2 interact to affect
100 aerobic performance of European sea bass (*Dicentrarchus labrax*), a species
101 showing recent northward range expansions thought to be related to warming
102 (Pawson et al., 2007). Two separate populations exist (Souche et al., 2015) and,
103 although the physiological responses of this species to environmental change have
104 been regularly examined, to date only one study has used fish from the Atlantic
105 population. As such, our experiment had three aims:

106 i. to assess how aerobic performance of sea bass from the Atlantic population
107 will respond to predicted future environmental changes;

108 ii. to determine whether combinations of hypoxia and increased CO_2 interact
109 with temperature to affect aerobic scope, as predicted by the OCLTT
110 hypothesis;

111 iii. to determine whether changes in aerobic performance are linked to blood
112 chemistry and O_2 transport capacity.

113 ## 2. Results

114 ### 2.1. Standard metabolic rate and hypoxia tolerance

115 The best supported model for SMR included both temperature and CO_2 as fixed
116 effects (Linear Mixed-Effects Model, marginal R^2 = 0.70, conditional R^2 = 0.70, Table
117 S6). Standard metabolic rate approximately doubled between 14 and 22ºC,
118 exhibiting a Q_{10} temperature coefficient of 2.09 (Figure 1). The best model indicated

that CO_2 had a small effect on SMR - reducing SMR by 7.4 $mgO_2\ kg^{-1}\ h^{-1}$, ~10 % of the smallest SMR, (95 % CI = -1.65 to 16.43 $mgO_2\ kg^{-1}\ h^{-1}$) across all temperatures at ~1000 µatm CO_2 (Figure 1). However, the model including temperature but not CO_2 had a ΔAICc <2 indicating that including the effect of CO_2 in the best model did not lead to a large improvement in model fit (Table S5). There was no evidence of an interactive effect between increasing temperatures and increasing CO_2.

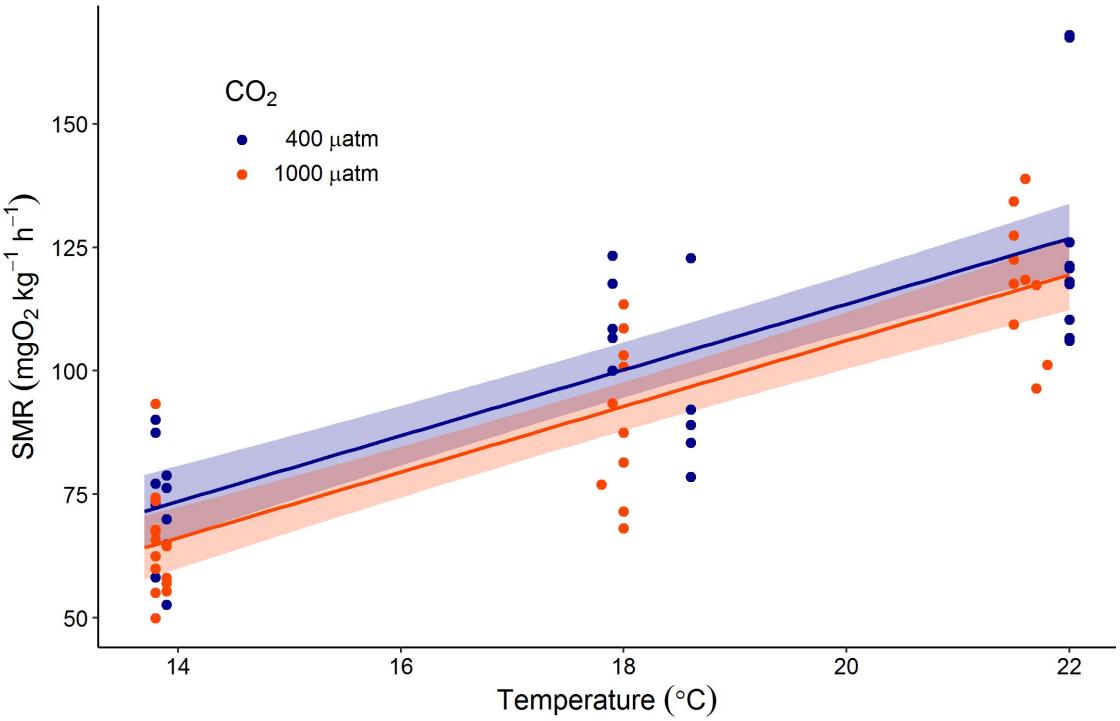


Figure 1: Impact of temperature and CO_2 on standard metabolic rates (SMR) of juvenile sea bass. The best supported model (marginal R^2 = 0.70, conditional R^2 = 0.70) to explain variation in SMR included temperature and CO_2 as explanatory variables but not their interaction. Points represent calculated SMR for individual fish, lines represented predicted SMR at two CO_2 levels (blue = present day ~400 µatm CO_2, orange = end of century ~ 1000 µatm CO_2) from the best supported model, and shaded areas represent bootstrapped 95 % CI of predictions (n = 1000).

133 The best supported model of O_{2crit} included the effects of temperature, CO_2 and
134 SMR but no interactions (Linear Mixed-Effects Model, marginal R^2 =0.72, conditional
135 R^2 = 0.77, Table S7). A doubling in SMR from 60 to 120 $mgO_2\ kg^{-1}\ h^{-1}$ is predicted to
136 increase O_{2crit} by 2.16 kPa O_2 (95 % CI = 1.66 to 2.66 kPa O_2). Independent of their
137 effects on SMR, both temperature and CO_2 were included in the best model of O_{2crit}.
138 The effect of temperature meant that for a given value of SMR O_{2crit} would reduce as
139 temperature increased. For instance, a fish at 14 ^oC is predicted to have an O_{2crit}
140 0.50 kPa O_2 (95 % CI = 0.13 to 0.87 kPa O_2) higher than a fish with the same SMR
141 at 18 ^oC (Figure 2A). Combined the effects of SMR and temperature mean warming
142 from 14 to 22 ^oC will increase O_{2crit} by 0.91 kPa O_2 (95 % CI = 0.53 to 1.29 kPa O_2,
143 Figure 2B). The additional effect of CO_2 is predicted to increase O_{2crit}. Despite this,
144 when taking into account the effect of CO_2 on SMR from the best model of SMR
145 (Figure 1), and the effect of SMR and temperature on O_{2crit}, the resulting effect of
146 CO_2 causes an increase in O_{2crit} of only 0.04 kPa O_2 (95 % CI = -0.32 to 0.40 kPa
147 O_2, Figure 2B).

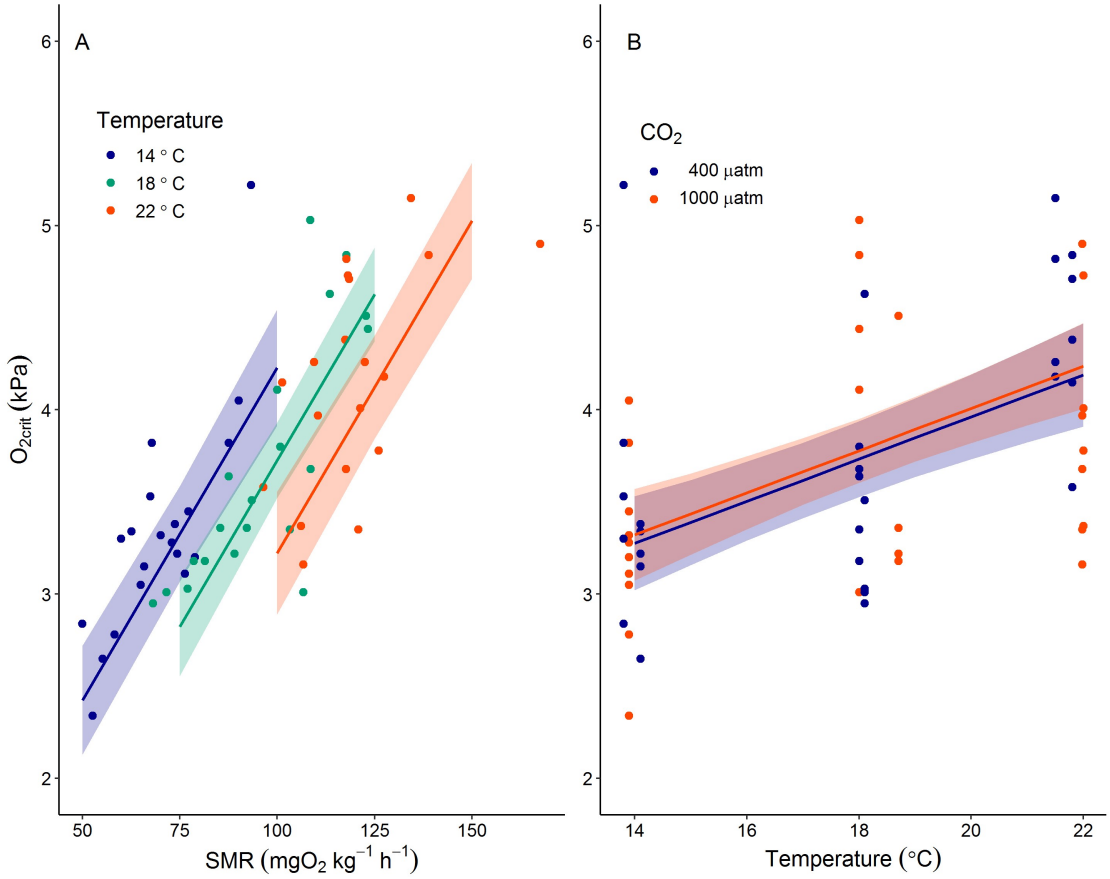


Figure 2: Combined impacts of SMR, temperature and CO_2 on hypoxia tolerance (O_{2crit}) of juvenile European sea bass. **A.** The best supported model (marginal R^2 = 0.72, conditional R^2 = 0.77) predicted a positive effect of increasing SMR on O_{2crit} with the effect of increased temperature resulting in lower O_{2crit} for a given value of SMR. **B.** Combined effects of SMR and temperature result in an increase in O_{2crit} between 14 ºC and 22 ºC. The predicted positive effect of increased CO_2 on O_{2crit} is small compared to changes in SMR and temperature. Points represent calculated O_{2crit} for individual fish, lines represented predicted O_{2crit} from the best supported model, and shaded areas represent bootstrapped 95 % CI of predictions (n = 1000).

2.2. Maximum metabolic rate and aerobic scope

Maximum metabolic rate of sea bass was affected by temperature, O_2, and CO_2 level. Warming from 14 to 22 ºC, in normoxia combined with normocapnia, caused a

50 % increase in MMR from 293.5 ± 10.4 to 435.9 ± 18.9 $mgO_2\ kg^{-1}\ h^{-1}$, with a Q_{10} of 1.64. In high CO_2 the temperature effect on MMR was very similar (Q_{10} = 1.60). The best supported model predicted that O_2 had a non-linear quadratic effect on MMR, so that a given reduction in O_2 caused a larger reduction in MMR at lower O_2 levels, as well as interactive effects between O_2 and temperature (Figure 3; Linear Mixed Model, marginal R^2 = 0.91, conditional R^2 = 0.96, Table S8). For example a 5 kPa reduction in O_2 at 18 ^oC (in normal CO_2) from air saturated levels (~ 20 to ~15 kPa O_2) results in a predicted decrease in MMR of 8.0 $mgO_2\ kg^{-1}\ h^{-1}$ (95 % CI = -7.2 to 23.2 $mgO_2\ kg^{-1}\ h^{-1}$) whereas the same reduction in O_2 from 10 to 5 kPa resulted in a 15-fold larger predicted decrease in MMR of 123.8 $mgO_2\ kg^{-1}\ h^{-1}$ (95 % CI = 115.1 to 132.5 $mgO_2\ kg^{-1}\ h^{-1}$). In addition, the non-linear O_2 effect interacted with the temperature effect so that the same reduction in O_2 caused a larger reduction in MMR as temperature increased (Figure 3A & B). This was particularly noticeable between 15 and 10 kPa O_2 where fish at 22 ^oC (in normal CO_2) exhibited a predicted decline in MMR that was 60 % larger than fish at 14 °C, 80.9 $mgO_2\ kg^{-1}\ h^{-1}$ (95 % CI = 63.2 to 98.6 $mgO_2\ kg^{-1}\ h^{-1}$) versus 50.8 $mgO_2\ kg^{-1}\ h^{-1}$ (95 % CI = 35.9 to 65.7 $mgO_2\ kg^{-1}\ h^{-1}$). Finally, environmental CO_2 level had a small, negative effect independent of interactions between temperature and O_2. As a result, an increase in CO_2 of 1000 µatm is predicted to reduce MMR by 8.5 $mgO_2\ kg^{-1}\ h^{-1}$ (95 % CI = -18.2 to 35.3 $mgO_2\ kg^{-1}\ h^{-1}$) irrespective of temperature and O_2 (Figure 3C, D, & E).

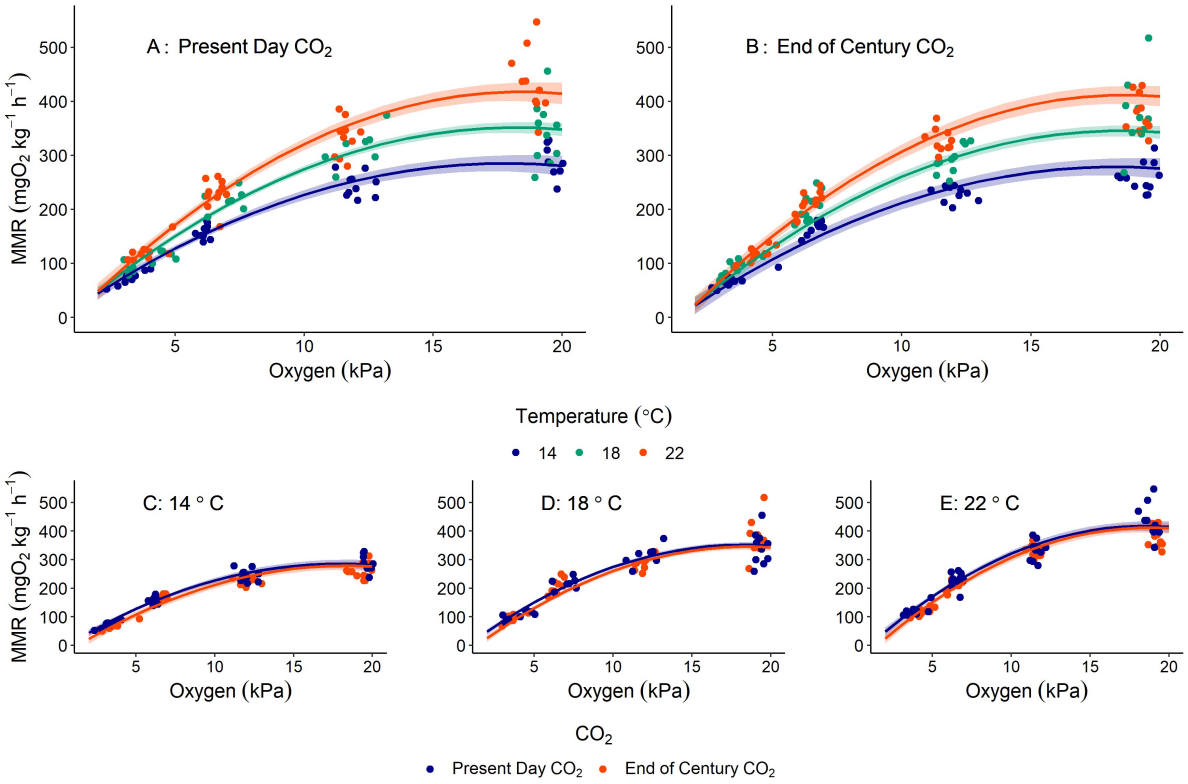
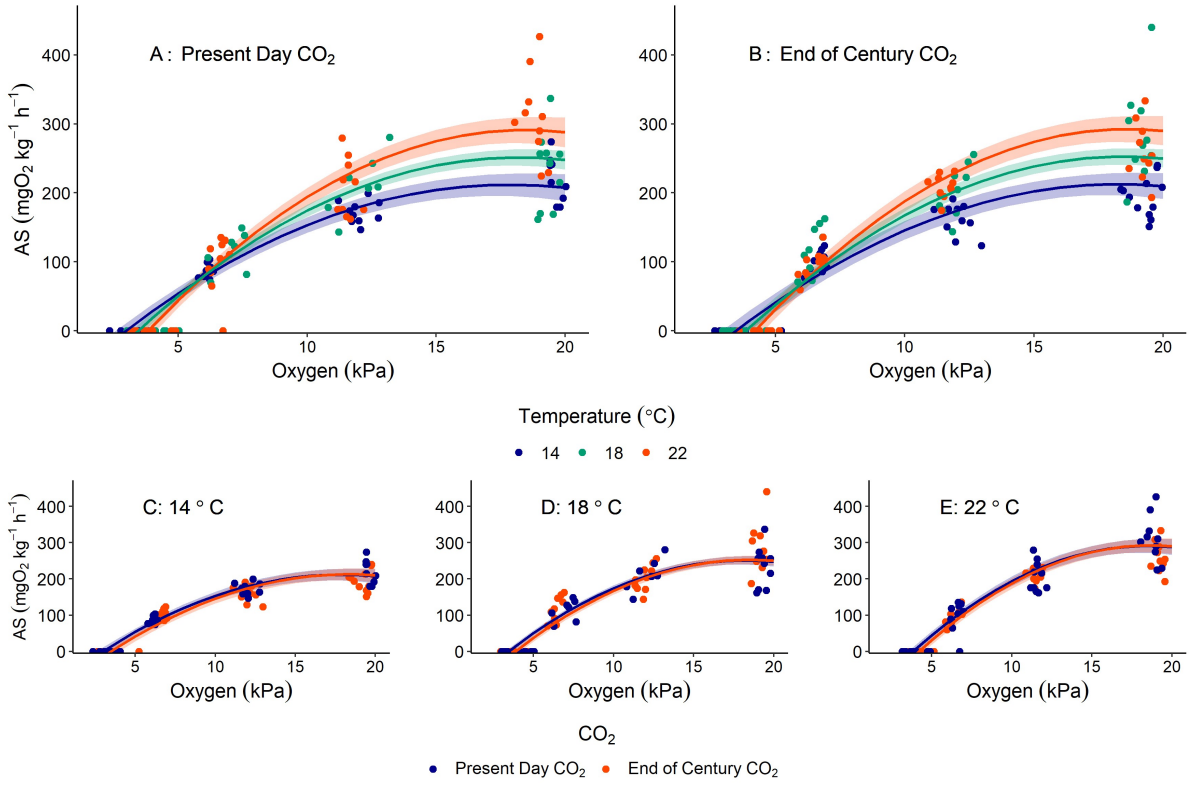


Figure 3: Effects of combinations of temperature, O_2, and CO_2 on the maximum metabolic rate (MMR) of European sea bass. There was a synergistic interactive effect of temperature and O_2 on MMR for sea bass exposed to both **A.** present day CO_2 conditions (present day CO_2 = 400 µatm at ~20 kPa O_2) and **B.** end of century CO_2 conditions (end of century CO_2 = 1000 µatm at ~ 20 kPa O_2). The additive effects of reduced O_2 and increased CO_2 levels are displayed for sea bass at **A.** 14 ^oC **B.** 18 ^oC and **C.** 22 ^oC. Points represent calculated MMR for individual fish, lines represent predicted MMR from the best supported model, and shaded areas represent bootstrapped 95 % confidence intervals (n = 1000).

We predicted the impacts of temperature, O_2, and CO_2 on aerobic scope from the best supported models fitted to measurements of SMR and MMR (Figure 4). At normoxia (~20 kPa O_2) aerobic scope is predicted to increase by 80.2 $mgO_2\ kg^{-1}\ h^{-1}$ (95 % CI = 24.8 to 135.7 $mgO_2\ kg^{-1}\ h^{-1}$) as temperature increases from 14 to 22 ^oC

195 independent of changes in CO_2 level (Figure 4A & B). Interactive effects between
196 temperature and O_2 on MMR are reflected in predictions of aerobic scope (Figure 4A
197 &B). As increasing CO_2 has the same direction of effect on both SMR and MMR the
198 impact of CO_2 on aerobic scope is minimal (Figure 4C, D, & E).



199

200 **Figure 4:** Effects of combinations of temperature, O_2, and CO_2 on the aerobic scope
201 (AS) of European sea bass. There was a non-linear interactive effect of temperature
202 and O_2 on aerobic scope for sea bass exposed to both **A.** present day CO_2
203 conditions (present day CO_2 = 400 µatm at ~20 kPa O_2) and **B.** end of century CO_2
204 conditions (end of century CO_2 = 1000 µatm at ~ 20 kPa O_2). The additive effects of
205 reduced O_2 and increased CO_2 levels are displayed for sea bass at **A.** 14 ^oC **B.** 18
206 ^oC and **C.** 22 ^oC. Points represent aerobic scope of individual fish derived from
207 calculated RMR and MMR of that individual, lines represent predicted aerobic scope
208 calculated by subtracting model predictions of RMR and MMR and shaded areas

represent 95 % confidence intervals calculated from bootstrapped standard errors of predicted RMR and MMR (n = 1000).

2.3. Blood chemistry & Hb-O_2 affinity

Increasing ambient CO_2 from ~400 to ~1000 µatm increased plasma pCO_2 (Two-way ANOVA, F = 15.84, df = 1, p < 0.001) whereas warming from 14 to 22 °C did not (F = 0.772, df = 2, p = 0.468), and there was no interactive effect noted (F = 2.067, df = 2, p = 0.138). Despite the significant overall effect of increased CO_2 on plasma pCO_2 pairwise comparisons (Figure 5) only revealed a significant increase in plasma pCO_2 in bass at 18 °C exposed to ~1000 µatm (0.53 ± 0.05 kPa pCO_2) compared to ambient conditions (0.29 ± 0.05 kPa pCO_2) (Pairwise comparisons of least square means, t = 3.688, df = 1, p = 0.008).

To compensate for increased plasma pCO_2 in ~1000 µatm CO_2 treatments sea bass accumulated ~3 mM extra HCO_3^- (Two-way ANOVA, F = 44.34, df = 1, p << 0.001) (Figure 5), compared to fish in ambient CO_2 conditions at 14 and 18 °C. Fish exposed to ~1000 µatm CO_2 at 22 °C showed a non-significant increase in HCO_3^- of just under 2 mM (95 % CI = 0.60 to 2.84 mM) when compared to fish at ~400 µatm CO_2 (t = 2.66, df = 1, p = 0.103). There was no temperature effect on plasma HCO_3^- (F = 2.538, df = 2, p = 0.0903) and no interactive effect between temperature and CO_2 (F = 0.969, df = 2, p = 0.387).

As a result of compensatory accumulation of HCO_3^-, blood extracellular pH (pH_e) was regulated in response to high CO_2 (F = 3.56, df = 1, p = 0.066) and sea bass did not show significant effects of temperature on pH_e (Two way ANOVA, F = 1.425, df = 2, p = 0.251). However, there was a significant interactive effect between temperature and CO_2 (F = 3.952, df = 2, p = 0.026). This interactive effect was

caused by a significant reduction in pH_e in sea bass exposed to ~1000 µatm CO_2 at 18 ºC (7.80 ± 0.03) when compared to fish at ambient CO_2 levels (7.97 ± 0.04) (pairwise comparisons of least square means, t = 3.242, df = 1, p = 0.026). We did not find significant differences between pH_e across all other treatment groups. Additionally, intracellular pH of red blood cells (pH_i) showed no significant differences between all treatments (Kruskal-Wallis test, $\chi^2 = 6.79$, df = 5, p = 0.237) (Figure 5).

There were no significant differences in plasma lactate levels across all treatments (Kruskall-Wallis, $\chi^2 = 6.40$, df = 5, p = 0.269), with mean lactate for all fish of 0.42 ± 0.06 mM (± S.E.). Plasma glucose levels (mean for all fish of 4.30 ± 0.14 mM, ± S.E.) were not significantly affected by temperature (Two-way ANOVA, F = 0.864, df = 2, p = 0.429), CO_2 (F = 0.0138, df = 1, p = 0.907) or the interaction between temperature and CO_2 (F = 1.052, df = 2, p = 0.358).

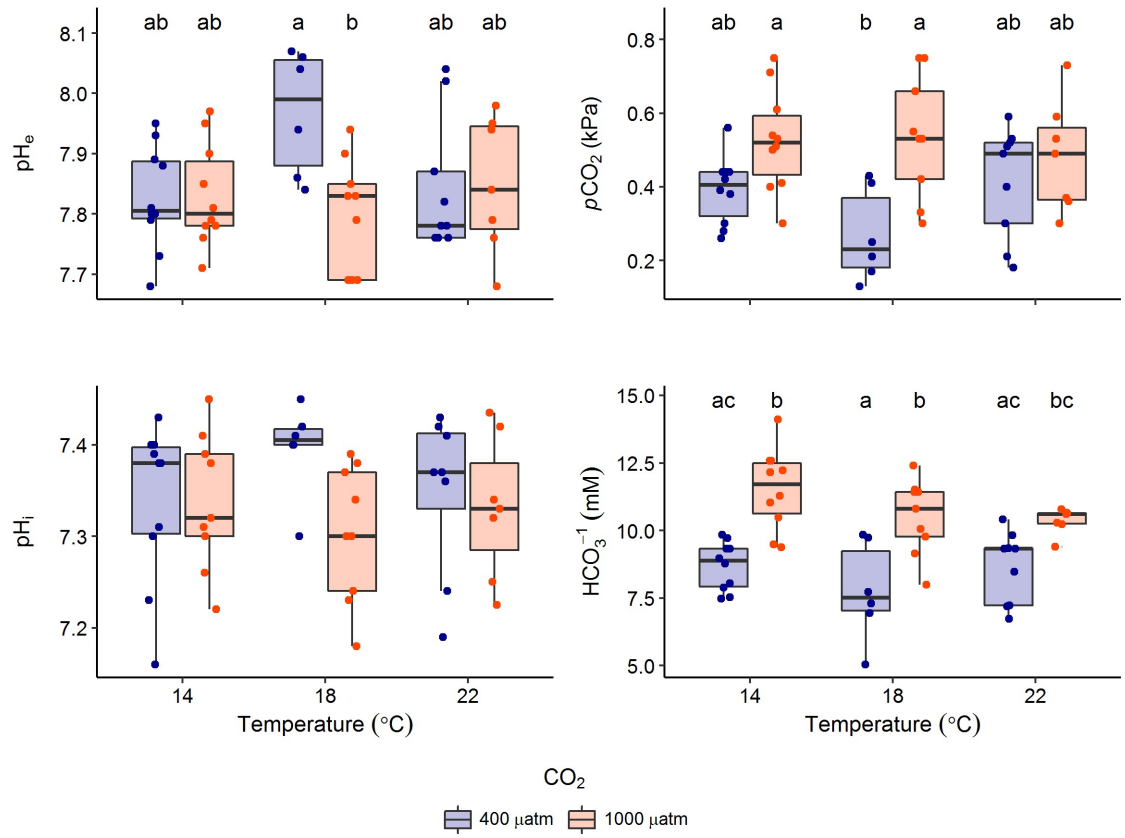


Figure 5: Impact of temperature and CO_2 on blood acid-base characteristics of European sea bass. Significant differences in pairwise comparisons of least square means between treatments are indicated by different letters (a,b,c). No significant differences between any treatments were noted for measurements of pH_i.

Our measurements of blood O_2 transport showed no consistent impacts of temperature or CO_2. Haemoglobin-O_2 binding affinity (P_{50}) (Kruskal-Wallis, χ^2 = 6.503, df = 4, p = 0.165) and Hills' number (One-way ANOVA, F = 0.878, df = 4, p = 0.487) were not significantly different between any treatments (Figure S1). Haematocrit (Hct) was not affected by temperature (Two-way ANOVA, F = 0.832, df = 2, p = 0.442) or CO_2 (F = 2.945, df = 1, p = 0.093) and no interactive effects were evident (F = 0.069, df = 2, p = 0.934). Haemoglobin (Hb) levels were affected by temperature (Two-way ANOVA, F = 7.094, df = 2, p = 0.002) with fish sampled at 22 °C having Hb levels 0.20 and 0.24 mM higher than fish sampled at 18 and 14 °C

respectively. Haemoglobin levels were not affected by CO_2 ($F = 0.690$, $df = 1$, $p = 0.411$) and there were no interactive effects ($F = 3.067$, $df = 2$, $p = 0.057$) (Figure S2).

2.4. Mortality

Three fish (30 %) died ~1 hour post-chase after exposure to ~6 kPa O_2 at 22 °C in the high CO_2 treatment. No fish died post-chase in any other treatment combinations.

3. Discussion

Our results demonstrate, for the first time, the interactive effects of temperature, O_2, and CO_2 on aerobic performance of an active predatory marine fish. We show that temperature and O_2 have a non-linear interactive effect on aerobic performance of European sea bass but CO_2's impact is minor and independent. Both SMR and MMR increased with temperature from 14 to 22 °C, but changes in MMR were greater leading to positive effects on absolute aerobic scope. These results suggest that European sea bass populations in the North-East Atlantic (typical temperatures are < 22 °C) could physiologically benefit from global warming. However, hypoxia tolerance reduced at higher temperatures, and hypoxia reduced MMR and aerobic scope by a greater amount at high temperatures, indicating that in warmer waters European sea bass will be more susceptible to hypoxia.

We confirm similar findings from previous research investigating effects of temperature and O_2 on European sea bass. For example, the increase in SMR with temperature we observed closely follows results from aquaculture sourced European sea bass (Claireaux & Lagardère, 1999). That is the Q_{10} temperature coefficient dropped from 2.33 between 14 and 18 °C to 1.82 between 18 and 22 °C. This drop in

Q_{10} as temperature increases provides further evidence that SMR does not increase exponentially after chronic vs acute exposure (Sandblom et al., 2014; Schulte et al., 2011). Responses of MMR to temperature showed similar Q_{10}'s indicating a linear increase in MMR between 14 and 22 ^{o}C. This also closely corresponds with observations from Claireaux and Lagardère (1999) who found that MMR increased approximately linearly between 14 and 22 ^{o}C, before peaking between 22 and 24 ^{o}C and declining at higher temperatures. Further work with sea bass from Mediterranean stock has shown that specific growth rate and feed conversion efficiency peak at ~25 ^{o}C and decline at higher temperatures (Person-Le Ruyet et al., 2004), similar to changes in metabolic scope shown by Claireaux and Lagardère (1999). Finally, aquaculture-produced sea bass display an Arrhenius break point of heart rate at ~21.5 ^{o}C and developed arrhythmia at ~26 ^{o}C (Crespel et al., 2019). The consistency in temperature of peak performance in sea bass from distinct sub-populations with vastly different environmental experiences supports the idea that fish face ceilings to physiological performance in the face of environmental change (Sandblom et al., 2016). Despite these similarities with previous research, SMR of fish in our study was higher at a given temperature than for fish from Mediterranean stocks (Claireaux & Lagardère, 1999). This may support the theory of metabolic cold adaptation, that basal energy demand in fish from warmer environments will be lower than in fish from cold environments when measured at the same temperature (Krogh, 1916). This has recently been supported by evidence from wild populations of three-spine stickleback (*Gasterosteus aculeatus*) (Pilakouta et al., 2020).

Declining O_2 caused a decrease in MMR along a limiting oxygen curve, similar to that seen previously in sea bass (Claireaux & Lagardère, 1999; Lagardère et al., 1998) and other fish species (Chabot & Claireaux, 2008; Claireaux et al., 2000;

309 Lefrancois & Claireaux, 2003; Mallekh & Lagardère, 2002). This result questions
310 recent predictions made by Seibel and Deutsch (2020) that MMR of fish should
311 decrease linearly from ~21 kPa O_2 to O_{2crit} values. The curved, rather than linear,
312 response of MMR we observed may occur as a result of compensatory mechanisms
313 (e.g. increased ventilation, cardiac output, gill lamellar perfusion and surface area,
314 and haematocrit) to maintain O_2 delivery (Farrell & Richards, 2009). While these
315 adjustments may limit reductions in MMR during mild to moderate hypoxia they may
316 reach their performance limits as O_2 approaches critical levels, resulting in a steeper
317 decline in MMR. For example, in moderate hypoxia rainbow trout (*Oncorhynchus*
318 *mykiss*) increase cardiac output via increased stroke volume but in severe hypoxia
319 cardiac output cannot be increased further leading to bradycardia (Sandblom &
320 Axelsson, 2005).

321 Temperature and O_2 interacted to affect metabolism of sea bass so that impacts
322 of hypoxia on MMR increased with temperature (Figure 3). This result supports
323 previous research by Claireaux & Lagardère (1999). However, sea bass in our study
324 displayed higher MMR at similar temperature and pO_2 when compared to bass from
325 Claireaux and Lagardère (1999). In addition, the O_{2crit} (the point at which SMR =
326 MMR) of our sea bass increased between 14 ºC and 22 ºC (Figure 2) whereas
327 results from previous work suggested that O_{2crit} increases or remains constant
328 across this temperature range (Claireaux & Lagardère, 1999). The reduction in
329 hypoxia tolerance of sea bass with warming was primarily a result of strong positive
330 correlation between O_{2crit} and SMR (Figure 1). This relationship has been shown for
331 numerous fish species, and most recently in work with black sea bass (*Centropristus*
332 *striata*) (Slesinger et al., 2019). However, our results also indicate that temperature
333 had a secondary effect which resulted in lower O_{2crit} at higher temperatures for a

given SMR. This suggests that temperature affects O_{2crit} via another mechanism (or mechanisms) independent of SMR. This is unlikely to be related to O_2 transport capacity of the blood as there were few consistent effects of temperature on Hct, Hb or P_{50} of sea bass (Figure 5, S1 and S2). It has previously been observed that improved hypoxia tolerance of fish after acclimation to increased temperature correlates to increased gill lamellar surface area (McBryan et al., 2016) or changes to heart structure (Anttila et al., 2015). Gomez Isaza *et al.* (2021) demonstrated that both of these cardiorespiratory responses can improve O_2 supply capacity. Combined, these results suggest that thermal acclimation can cause structural changes to the gills and the heart, improving performance in low O_2 conditions and mitigating the negative effect of temperature induced increases in SMR on hypoxia tolerance.

The independent effect of rising CO_2 reduced MMR. Previous research has not shown consistent effects of CO_2 on MMR or SMR of fish species, with the majority showing no effects of CO_2 (Lefevre, 2016, 2019). Interestingly the most recent research with European sea bass found that long term exposure to elevated CO_2 increased MMR. This may indicate that negative effects of short term exposures used in our study (i.e. weeks) can be overcome in the long term (i.e. years) (Crespel et al., 2019). As such, future work needs to determine whether interactions between CO_2 and temperature or O_2 occur over longer time scales. The effect of CO_2 on metabolic rate in our study was only identifiable by including the rise in CO_2 which co-occurs when environmental O_2 declines. Without the additional data from increased CO_2 exposures at lower O_2 levels the effect of ambient CO_2 on MMR at normoxia was not significant. Although the best model of SMR also included a negative effect of CO_2 this was predicted to be small with confidence intervals

overlapping zero (Figure 1). Additionally, removal of CO_2 as an explanatory variable from the model of SMR did not greatly impair model fit (ΔAICc < 2) which indicates that the effect of CO_2 on SMR was not critically important for overall model performance.

As CO_2 increases in the environment when O_2 declines the negative effect of CO_2 has a greater impact on MMR at lower O_2 levels (Figure 3 C, D, and E). Decreased MMR when fish are exposed to acutely increased CO_2 is usually thought to be a result of an internal acidosis causing a decrease in Hb-O_2 binding affinity, reducing the capacity of O_2 transport in the blood (Heuer & Grosell, 2016). However, fish have well developed acid-base regulatory mechanisms and blood sampling showed that sea bass in normoxia had fully compensated for the effects of increased environmental CO_2 on blood pH by compensatory accumulation of extra HCO_3^-, resulting in no changes in Hb-O_2 binding affinity (Figure 5 and S1). Additionally, we have recently shown that sea bass are able regulate blood pH when exposed to concurrent progressive hypercapnia during progressive hypoxia over the course of several hours and have higher Hb-O_2 binding affinity in these conditions when compared to fish exposed to progressive hypoxia with no concurrent hypercapnia (Montgomery et al., 2019). In addition, we did not see significant changes in Hb levels or Hct between CO_2 treatments (Figure 7). We conclude that the negative effect of increased CO_2 on MMR is unlikely to be related to changes in O_2 transport capacity in the blood. Whilst beyond the scope of the present study we can speculate that instead CO_2 may affect MMR via changes in mitochondrial metabolism (Leo et al., 2017; Strobel et al., 2012, 2013), cardiac performance (Crespel et al., 2019; Perry & Abdallah, 2012) or via shifts from aerobic to anaerobic metabolic pathways (Michaelidis et al., 2007). Indeed, recent results show that CO_2

384 impacts mitochondrial function of sea bass from the Atlantic population in
385 combination with acute warming (Howald et al., 2019)

386 While the effect of CO_2 on MMR was part of the best supported model it is
387 uncertain whether it would cause biologically relevant impacts. Previous research
388 has linked declines in MMR caused by increased CO_2 with decreased swimming
389 performance (Lefevre, 2019) but it is unknown if the relatively small changes in MMR
390 shown in the present study would translate to other aspects of whole animal
391 performance. This is especially the case as predictions of aerobic scope from the
392 combined best supported models of MMR and SMR essentially show no effect of
393 CO_2 on aerobic scope at O_2 levels >10 kPa (Figure 4 C, D, and E). This occurs
394 because predicted effects of CO_2 act in the same direction for both SMR and MMR.
395 As changes in aerobic scope typically predict environmental impacts on processes
396 such as growth and reproduction (Clark et al., 2013; Pörtner et al., 2017) we would
397 predict that climate change relevant CO_2 increases have negligible effects on these
398 endpoints. However, the effect of CO_2 may have important consequences not
399 reflected in changes in aerobic scope. In our most extreme treatment (22 ^oC, ~30 %
400 air saturation, end of century CO_2) we observed 30 % mortality of sea bass after
401 exhaustive exercise. Fish exercised at the same temperature and O_2 levels in
402 ambient CO_2 conditions showed no mortality (and no mortality was observed in any
403 other treatment combinations) – consequently it appears the elevated CO_2 during
404 hypoxia in a future ocean scenario (which was approximately 1100 µatm higher than
405 in the present day CO_2 scenario) may impair recovery from exercise when O_2 is
406 limiting. Mortality in fish post-exercise has been theorised to result from intracellular
407 acidosis generated during anaerobic respiration (Wood et al., 1983). Therefore, the
408 greater increase in CO_2 during hypoxia in the future ocean CO_2 scenario may either

409 exacerbate the intracellular acidosis caused by anaerobic activity or impair the ability
410 of fish to process anaerobic end products.

411 ### 3.1. Evidence to support OCLTT?

412 The OCLTT hypothesis suggests climate change will affect fish because
413 combined effects of reduced O_2 and increasing CO_2 will synergistically interact,
414 lowering aerobic scope across its thermal performance curve (Pörtner & Peck,
415 2010), and that changes in aerobic scope can be used as a single metric for
416 predicting whole animal performance (Pörtner, 2012). Our data provides some
417 support for the OCLTT hypothesis as the observed interactions between increased
418 temperature and reduced O_2 on aerobic scope would be expected to result in
419 changes to the thermal performance curve as predicted by Pörtner and Farrell
420 (2008). However, our highest temperature treatment did not decrease MMR or
421 aerobic scope and so we cannot confirm whether interactive effects between
422 temperature and hypoxia would follow predictions of the OCLTT above the optimum
423 temperature of aerobic scope. In contrast to hypoxia, the effects of CO_2 did not
424 follow predictions from the OCLTT as CO_2 did not interact with either temperature or
425 hypoxia and had minimal impacts on aerobic scope.

426 The interactive effects of O_2 and temperature on the MMR of sea bass in this
427 study closely resemble predictions of the metabolic niche framework which Ern
428 (2019) proposed as an update to the OCLTT hypothesis. In particular the concept of
429 aerobic scope isopleths (where aerobic scope remains constant across a range of
430 temperatures as a result of changes in O_2 or vice versa) is supported by our data,
431 showing that aerobic scope of sea bass would be expected to remain constant
432 across an 8 ^{o}C temperature range at an O_2 level of ~6 kPa (Figure 3 A and B). As

433 such, we would support Ern's suggestion to experimentally assess how aerobic
434 scope affects important processes, such as growth, independently of changes in
435 temperature and O_2 by utilising these isopleths. Although increased aerobic scope
436 has long been linked to improved individual fitness there is a still a lack of evidence
437 to confirm this relationship occurs – as such the assumption that peak physiological
438 fitness occurs when aerobic scope peaks (central to the OCLTT) may be erroneous.

439 Alternatively, Deutsch *et al.* (2015) have suggested a framework which aims to
440 predict impacts of climate change on the physiological suitability of a habitat for a
441 species via a metabolic index, relating the ratio of O_2 supply to resting metabolic
442 demand (i.e. SMR), rather than aerobic scope. This metabolic index appears to
443 provide a tool for predicting the biogeographical distributions of species with the
444 biogeographical distribution limits of many marine species corresponding to a
445 metabolic index of ~2-5 (Deutsch et al., 2015). This approach has been supported by
446 experimental work with black sea bass, *Centropristis striata,* (Slesinger et al., 2019)
447 and Roman sea bream, *Chrysoblephus laticeps*, (Duncan et al., 2020) which showed
448 that the metabolic index can accurately predict changes in population distributions of
449 these species . Applying the principle of the metabolic index to our data suggests
450 that temperatures of 22 ^{o}C are close to the upper temperature limits of European sea
451 bass from the North-East Atlantic sub-population (metabolic index at 22 ^{o}C and 20
452 kPa O_2 was ~5).

453 4. Conclusion

454 In summary, our research shows that aerobic scope of European sea bass will
455 increase with expected warming in the North-East Atlantic, and that even extreme
456 summer temperatures (~22 ^{o}C) at the end of the century will positively impact on the

457 absolute aerobic performance of sea bass. However, synergistic interactions
458 between warming and reduced O_2 indicate that hypoxic conditions will have greater
459 impacts on sea bass in future oceans. Increased CO_2 levels showed no interactions
460 with either temperature or O_2 changes but were predicted to cause a small decline in
461 MMR – although this had little impact on aerobic scope because increased CO_2
462 caused a trend for decreased SMR. Sea bass fully compensated blood pH for
463 increased CO_2 levels and increases in SMR and reductions in MMR with
464 temperature were not linked to changes in O_2 transport. Despite end of century CO_2
465 levels having minimal effects on aerobic scope, they did cause increased mortality of
466 fish recovering from exercise in the more extreme hypoxic scenario (~30 % air
467 saturation) at 22 ^oC. This effect would not have been observed without including
468 expected increases in CO_2 as O_2 declines in hypoxia treatments. Thus,
469 environmentally relevant changes in CO_2 during hypoxia may lead to important
470 threshold effects which could be missed if experiments only consider changes in
471 CO_2 related to atmospheric concentrations. Interactive effects of temperature and O_2
472 support predictions from the oxygen-and temperature-limited metabolic niche
473 framework proposed as an update to the OCLTT hypothesis by Ern (2019), however
474 the effect of CO_2 did not support predictions of the OCLTT (Pörtner & Farrell, 2008).
475 Changes in the metabolic index proposed as a physiological constraint by Deutsch *et*
476 *al.* (2015) suggest that despite increases in MMR and aerobic scope future climate
477 change may result in conditions which will begin to constrain growth and
478 reproduction of sea bass in areas where temperatures increase above 22 ^oC.
479 However, there is a vital need for increased research to link changes in aerobic
480 scope to population relevant metrics such as growth and reproduction to better

assess what environmental impacts on aerobic performance may mean for wider populations.

5. Materials & Methods

5.1. Animal Collection and Husbandry

We collected juvenile sea bass from estuaries and coastal lagoons on the south Dorset coast and Isle of Wight in June 2017. Fish were held in a marine recirculating aquaculture system (RAS) at the University of Exeter for 332 days before experimental work began (see water chemistry data in Table 1). Sea bass were fed a diet of commercial pellet (Horizon 80, Skretting) at a ration of ~1-2 % body mass three times per week and supplemented with ~1 % body mass of chopped mussel (*Mytilus edulis*) once per week. All experimental procedures were carried out under a UK Home Office licence (P88687E07) and approved by the University of Exeter's Animal Welfare and Ethical Review Board.

Table 1: Water chemistry parameters of the recirculating aquaculture system in which sea bass were held prior to experimental work beginning (means ± S.D. shown). Fish were initially held at 15 ºC before stock systems were raised to 18 ºC approximately 6 months prior to experimental work beginning. The temperature shown in the table represents the mean temperature for the entire time fish were held in the RAS prior to experimental trials beginning.

Time in system (days)	**Temperature (ºC)**	**pH (NBS scale)**	**Salinity**	**Total Alkalinity (µM)**	***p*CO$_2$ (µatm)**
332	17.1 ± 1.4	8.02 ± 0.05	32.98 ±	2072.0 ±	534.0 ±

	0.65	130.2	60.2

5.2. Treatment conditions

Sea bass were transferred to an experimental RAS, in a temperature-controlled room for a minimum of 14 days acclimation to treatment conditions (Figure 6). Six treatment conditions were used combining three temperatures and two CO_2 levels in a three x two factorial design (Table 2). Temperature treatments (14, 18 and 22 ^oC) were chosen to reflect temperature ranges in coastal UK waters from spring to autumn as well as potential future summer temperatures at the end of the century (IPCC, 2014; Tinker et al., 2020). CO_2 treatments (~400 & ~1000 µatm) were chosen to reflect annual average ambient atmospheric CO_2 levels currently and possible end-of-century ambient atmospheric levels according to an RCP 8.5 scenario (IPCC, 2014). Sea bass were transferred to the experimental RAS at 18 ^oC before temperatures were adjusted at a rate of 2 ^oC per day to reach treatment conditions (i.e. 14 or 22 ^oC). A header tank (~500 L) in the experimental RAS was used to adjust CO_2 to the desired level for each treatment before entering the treatment tank which contained the sea bass. For present day CO_2 treatments (~400 µatm) the header tank was aerated using CO_2 scrubbed air to remove excess CO_2 added to the RAS by the biological filters. For end of century CO_2 treatments (~1000 µatm) an Aqua Medic pH computer was used to adjust RAS water to an appropriate pH (7.8). Additionally, treatment tanks were aerated with a gas mix with the appropriate CO_2 content for each treatment.

Measurements of treatment tank pH (NBS scale), salinity, temperature, and a 12 mL water sample to measure Dissolved Inorganic Carbon (DIC) were taken every 2-

3 days. Seawater DIC analysis was conducted using a custom built system described in detail by Lewis *et al.* (2013). Data for pH, salinity, temperature and DIC were then input into the seawater carbon calculator programme, CO2SYS (Pierrot et al., 2006) to calculate pCO_2 based on the equilibration constants refitted by Dickson and Millero (1987), and KSO_4 dissociation constants from Dickson (1990) (Table 2).

Table 2: Mean ± S.D. of water chemistry parameters in treatment tanks during treatments at 3 temperature (14, 18, and 22 °C) and 2 CO_2 levels (~400 and ~1000 µatm). Treatment order represents time course of treatments (i.e. treatment 1 was conducted first and treatment 6 last). Total Alkalinity (TA) varied somewhat over time as a result of biological activity and so was adjusted periodically by addition of 1.0 M $NaHCO_3$ to restore TA levels back to >2000 µM.

	Treatment					
Parameter	1	2	3	4	5	6
Temperature (°C)	17.9 ± 0.0	22.0 ± 0.0	13.9 ± 0.1	21.7 ± 0.5	13.9 ± 0.1	18.0 ± 0.1
pCO_2 (µatm)	460 ± 34	375 ± 28	333 ± 38	1065 ± 172	1057 ± 65	973 ± 114
pH (NBS)	8.06 ± 0.03	8.12 ± 0.02	8.14 ± 0.04	7.79 ± 0.03	7.81 ± 0.04	7.83 ± 0.05
Salinity	32.9 ± 0.6	32.5 ± 2.9	33.3 ± 0.3	32.6 ± 0.3	34.6 ± 0.5	33.8 ± 0.6
TA (µM)	1935 ± 93	1745 ± 26	1861 ± 88	2147 ± 292	2393 ± 188	2170 ± 20

5.3. Respirometry measurements (SMR, MMR, and O_{2crit})

Rates of oxygen consumption ($\dot{M}O_2$) were made as a proxy of metabolic rate using an intermittent-flow respirometer system, details of which can be found in Montgomery *et al.* (2019), set-up following recommendations by Svendsen *et al.* (2016). Sea bass were starved for 72 hours prior to the start of measurements to ensure that metabolism was not affected by the specific dynamic action of digestion (Chabot et al., 2016). Individual sea bass were then transferred to respirometer chambers and left to acclimate for a minimum of 12 hours overnight before measurements of $\dot{M}O_2$ began. For each treatment all respirometry measurements were conducted in two groups (hereafter referred to as respirometry group), with five fish being measured simultaneously for each group. Following the 12 hour acclimation period we measured $\dot{M}O_2$ of each sea bass for ~3-4 hours (from ~6 am to ~10 am) before hypoxia tolerance was assessed using a critical O_2 tension (O_{2crit}) trial (Figure 6), following protocols set out in Montgomery *et al.* (2019). Carbon dioxide levels in the water were simultaneously increased as O_2 declined during O_{2crit} trials to reflect the natural rise in CO_2 during hypoxic events in aquatic systems (Melzner et al., 2013; Montgomery et al., 2019). During O_{2crit} trials water pH, temperature, salinity, and DIC were measured every hour to calculate water carbonate chemistry. Changes in system pCO_2 and pH during O_{2crit} trials for each treatment are given in supplementary materials (Table S1).

O_{2crit} trials were stopped once a minimum of three consecutive $\dot{M}O_2$ measurements showed a transition from an oxy-regulating to oxy-conforming state for each fish. Following completion of O_{2crit} trials the respirometer system was aerated with ambient air (CO_2 ~400 µatm) or a 0.1 % CO_2 in air gas mix (CO_2 ~1000 µatm) to rapidly restore O_2 levels to normoxia and CO_2 to the appropriate treatment

level. Sea bass were left to recover in respirometers, for a minimum of one hour post-trial, until O_2 levels reached ~ 21 kPa O_2 (~100 % air saturation) before removing the fish and measuring background respiration for a minimum of one hour (six measurement cycles) for all respirometers immediately post trial. Each sea bass was then placed in an individual ~10 L isolation tank which was subsequently fed by the respirometry system sump (at a rate of ~4 L min^{-1}) to maintain treatment conditions (with overflowing water from the isolation tanks recirculating back to the sump).

After fish had rested overnight in isolation tanks, MMR was measured for each fish (using an exhaustive chase protocol; Norin and Clark, 2016) on three consecutive days (with overnight recovery in between) at three different levels of O_2 (100, 60 and 30 % air saturation) with increasing CO_2 levels for each O_2 level as detailed for O_{2crit} trials (Figure 6). The appropriate O_2 and CO_2 level was achieved by aerating isolation boxes with a mix of N_2, O_2 and CO_2 (G400 Gas mixing system, Qubit Biology Inc.) at a rate of 5 L min^{-1}. Fish were exposed to the new O_2 and CO_2 conditions for ~2 hours before chase protocols were conducted. Chase protocols and subsequent respirometry measurements were conducted at the appropriate temperature, O_2, and CO_2 conditions for each treatment. Measurements of water pH, temperature, salinity, and DIC were taken for each isolation tank, the chase tank (after all fish were chased) and the respirometer system (during $\dot{M}O_2$ measurements) to calculate water carbonate chemistry. Temperature, O_2, and CO_2 conditions for all MMR trials are given in Table S2.

For all $\dot{M}O_2$ measurements dissolved O_2 concentration (% air saturation) was measured continuously (frequency ~1 Hz) in respirometer chambers using a fibre optic O_2 optode mounted in the recirculation loop of the respirometer chamber.

These optodes were linked to two Firesting Optical Oxygen Meters (Pyro Science, Aachen, Germany) which were connected to a PC running AquaResp 3 software which automatically logged all measurements.

$\dot{M}O_2$ was automatically calculated by the AquaResp3 software by fitting a linear regression to the O_2 versus time data for each measurement period. The slope (s) of this regression (kPa O_2 h^{-1}) was then used to calculate $\dot{M}O_2$ (mg O_2 kg^{-1} h^{-1}) using the equation outlined by Svendsen *et al.*(2016):

$$\dot{M}O_2 = sV_{resp}\alpha m^{-1}$$

where V_{resp} is the respirometer volume minus the volume of the fish (L), α is the solubility of O_2 in water (mgO_2 L^{-1} kPa^{-1}) for the relevant salinity and temperature, and m is the mass of the fish (kg). For the purpose of establishing the impacts of reduced O_2 on MMR and determining O_{2crit} values, the O_2 level of each measurement period was defined as the mean dissolved O_2 measurement over the measurement period. The mean background respiration for each respirometer over the 1 hour post-trial measuring period (average was < 2 % of fish $\dot{M}O_2$) was subtracted from $\dot{M}O_2$ measurements. Background corrected $\dot{M}O_2$ was then scaled to an average individual mass of 120 g using a mass exponent of 0.89 prior to subsequent analysis (Jerde et al., 2019).

We calculated SMR of each fish as the mean of the lowest 10 $\dot{M}O_2$ measurements from the ~3-4 hour period prior to O_{2crit} trials in which mean dissolved O_2 saturation was >80 % air saturation. The critical O_2 tension (O_{2crit}) of each individual fish was then calculated using $\dot{M}O_2$ measurements from O_{2crit} trials with

function 'calcO2crit' from package 'fishMO2' (Chabot *et al.* 2016) in R v.3.6.3 (R Core Team, 2020), using the estimated SMR of each individual, as detailed in the supplementary material of Claireaux & Chabot (2016). Finally, we defined MMR as the single highest measurement of $\dot{M}O_2$ in the one hour period immediately following exercise to exhaustion (Norin & Clark, 2016). This point usually occurred during the first measurement period immediately after each fish was moved to the respirometer chamber i.e. ~ 2-5 minutes after the cessation of the chase protocol. However, for some fish in normoxia spontaneous activity inside the respirometer chamber during SMR or O_{2crit} trials resulted in instantaneous measurements of $\dot{M}O_2$ higher than those noted following chase protocols. In these occasions this higher value of $\dot{M}O_2$ was used as the estimated MMR for that fish (n = 2 out of 65 fish).

5.4. Blood chemistry and Hb-O_2 affinity measurements

Following MMR measurements, sea bass were left overnight in the isolation boxes before blood samples were taken (Figure 6), following methods outlined in Montgomery *et al.* (2019), from each fish in normoxic conditions and at the relevant treatment temperature and CO_2 level (Table S3). We then measured extracellular pH (pH_e), haematocrit (Hct), TCO_2, haemoglobin content (Hb), plasma glucose, and plasma lactate and calculated pCO_2 and HCO_3^- following methods detailed in Montgomery *et al.* (2019). We also followed the freeze-and-thaw method to measure intracellular pH of RBCs (pH_i) as described by Zeidler and Kim (1977), and validated by Baker *et al.* (2009). All measurements or storage of blood for subsequent analysis occurred within 10 minutes of blood sampling. Finally, we measured Hb-O_2 affinity using a Blood Oxygen Binding System (BOBS, Loligo systems), detailed in general in Oellermann *et al.* (2014) and specifically for fish blood in Montgomery *et al.* (2019).

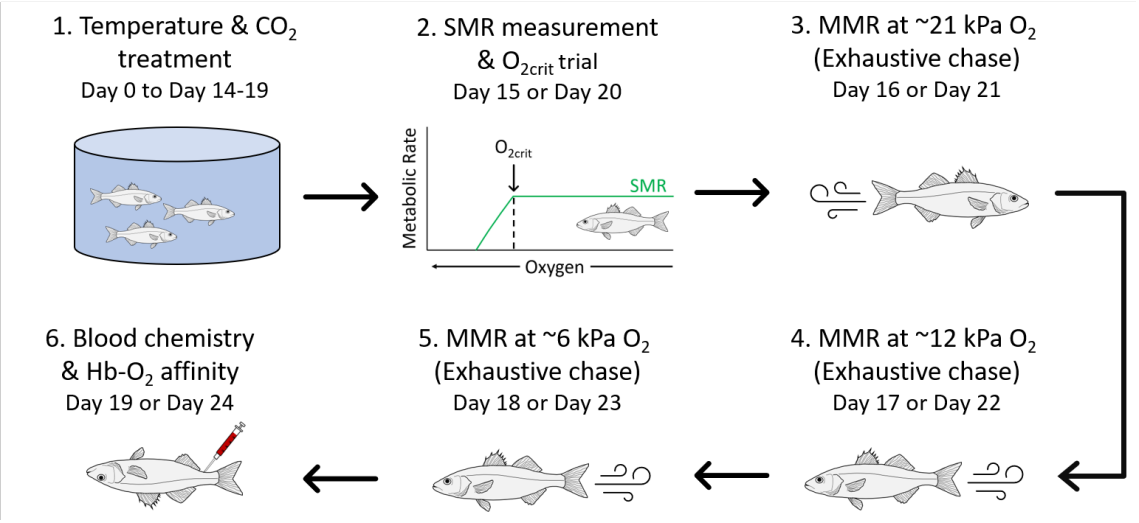


Figure 6: Summary of the timeline over which experimental end points were measured. Sea bass were acclimated for a minimum of 14 days to a temperature (14, 18, 22 ºC) and CO_2 (~400 µatm or ~1000 µatm ambient CO_2) treatment before measurements of SMR/O_{2crit}, MMR (at ~21 kPa, ~12 kPa, and ~6 kPa O_2), and blood chemistry/Hb-O_2 affinity were obtained on consecutive days for each individual.

5.5. Statistical Analysis

5.5.1. Respirometry data analysis

We conducted all statistical analysis in R v3.6.3 (R Core Team, 2020). Results are reported as mean ± S.E unless otherwise stated. Sample sizes for respirometry data can be seen in Table S4. The effects of temperature, O_2, and CO_2 on individual physiological performance metrics (SMR, MMR, and O_{2crit}) were analysed using separate general linear mixed-effects models (GLMM) in package 'lme4' (Bates et al., 2015; Pinheiro et al., 2018). All models included respirometry group as a random intercept term to account for potential tank effects introduced during respirometry measurements. For each physiological metric the best supported model was determined as the model with the lowest corrected Akaike's information criterion, AICc (Burnham & Anderson, 1998; Hurvich & Tsai, 1989). Residual diagnostic plots

of each GLMM were then assessed using package 'DHARMa' to confirm validity of model fit (Hartig, 2020). Once the best supported model for each physiological parameter was identified (see Table S5 for model comparisons) predictions were made across a range of temperatures, O_2 levels, and CO_2 levels to visualise combined effects of these variables on the physiology of seabass. We then used function bootMer from lme4 (Pinheiro et al., 2018) to calculate 95 % confidence intervals of model predictions.

5.5.2. Blood chemistry and Hb-O_2 affinity data analysis

Measurements of blood chemistry parameters (pH_e, pH_i, pCO_2, HCO_3^-, P_{50}, Hills' number, Hct, Hb, lactate, and glucose) were analysed using the ambient water temperature of the treatment and using a categorical CO_2 level of low (i.e. ~400 µatm treatment) or high (i.e. ~1000 µatm treatment). Measurements were analysed using a type III sum of squares two-way ANOVA (to account for unequal sample sizes). Post hoc-tests were then conducted on least-square means generated by package 'emmeans' (Lenth, 2020), with Tukey adjusted p-values for multiple comparisons. Model residuals from analysis of plasma lactate and glucose measurements did not meet the assumptions of normality or equal variances required by two-way ANOVA, as such this data was analysed using the alternative non-parametric Kruskal-wallis test.

Blood-oxygen binding parameters (P_{50} and Hills' number) could not be obtained for fish in the 14 ^oC and high CO_2 treatment as a result of an equipment failure. As such these data were analysed using a one-way ANOVA. If statistical assumptions of one-way ANOVA were not met then data were analysed using the non-parametric Kruskal-Wallis test. Sample sizes for blood chemistry data can be seen in Table S4.

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Competing Interests

The authors declare no competing interests.

Data availability

Data will be made publicly available via the University of Exeter's online repository if the manuscript is accepted: https://ore.exeter.ac.uk/repository/

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995 **Supplementary materials**

996 **Table S1:** Measurements of pCO_2 (µatm, top row) and pH (NBS scale, bottom row)
997 as O_2 declined during O_{2crit} trials for each treatment. All values are mean ± S.D. apart
998 from for Treatment 3 at ~30% dissolved O_2 where only one measurement of pCO_2
999 and pH was made and calculations of mean and S.D. were not possible

	Treatment					
~Dissolved O_2	14 °C	18 °C	22 °C	14 °C	18 °C	22 °C
(% air saturation)	Present CO_2	Present CO_2	Present CO_2	Future CO_2	Future CO_2	Future CO_2
100	377 ± 23	483 ± 20	455 ± 35	1019 ± 50	1070 ± 10	1172 ± 89
	8.14 ± 0.02	8.09 ± 0.02	8.10 ± 0.04	7.79 ± 0.01	7.78 ± 0.00	7.80 ± 0.03
80	418 ± 9	508 ± 10	486 ± 47	1149 ± 2	1156 ± 86	1209 ± 18
	8.10 ± 0.01	8.07 ± 0.01	8.07 ± 0.04	7.74 ± 0.01	7.75 ± 0.02	7.79 ± 0.02
60	477 ± 25	613 ± 90	497 ± 23	1383 ± 125	1315 ± 73	1335 ± 92
	8.05 ± 0.02	7.99 ± 0.01	8.07 ± 0.02	7.67 ± 0.02	7.69 ± 0.02	7.75 ± 0.03
40	625 ± 44	746 ± 40	577 ± 46	1637 ± 83	1488 ± 24	1658 ± 237
	7.94 ± 0.03	7.92 ± 0.02	8.01 ± 0.04	7.59 ± 0.01	7.64 ± 0.00	7.66 ± 0.05
30	822	1104 ± 155	647 ± 18	1973 ± 159	1964 ± 134	2179 ± 63
	7.83	7.76 ± 0.06	7.96 ± 0.01	7.51 ± 0.04	7.53 ± 0.02	7.55 ± 0.01
20	1162 ± 2	2103 ± 79	808 ± 35	2491 ± 238	2416 ± 265	2547 ± 266
	7.69 ± 0.00	7.50 ± 0.02	7.88 ± 0.02	7.42 ± 0.05	7.45 ± 0.03	7.48 ± 0.06
15	1734 ± 92	2431 ± 663	1318 ± 108	3099 ± 448	2579 ± 4	3380 ± 354
	7.53 ± 0.02	7.45 ± 0.11	7.68 ± 0.04	7.32 ± 0.07	7.41 ± 0.00	7.36 ± 0.06

Table S2: Temperature (°C), pCO_2 (µatm), and O_2 (kPa) conditions sea bass were exposed to pre-chase, during chase, and when recovering from exercise during measurements of MMR. All values are presented as mean ± S.E. - indicates measurements for which data was not collected. For the 14 °C present day CO_2 treatment missing pre-chase values would be expected to be extremely similar to values recorded during chase as shown by values for all other treatments.

		Treatment																	
		14 °C Present CO_2			18 °C Present CO_2			22 °C Present CO_2			14 °C Future CO_2			18 °C Future CO_2			22 °C Future CO_2		
		Pre-chase	During chase	Recovery	Pre-chase	During chase	Recovery	Pre-chase	During chase	Recovery	Pre-chase	During chase	Recovery	Pre-chase	During chase	Recovery	Pre-chase	During chase	Recovery
~100 % air saturation	Temp (°C)	13.9 ± 0.1	13.9 ± 0.1	13.9 ± 0.1	-	18.3 ± 0.5	18.3 ± 0.5	22.0 ± 0.0	21.8 ± 0.1	22.0 ± 0.0	13.9 ± 0.0	14.2 ± 0.5	13.9 ± 0.1	18.0 ± 0.1	18.5 ± 0.0	18.0 ± 0.2	21.6 ± 0.1	22.3 ± 1.1	21.9 ± 0.1
	pCO_2 (µatm)	-	-	364 ± 12	-	522 ± 105	522 ± 105	-	-	404 ± 0	1056 ± 27	1148 ± 171	1040 ± 4	1085 ± 57	1129 ± 44	1195 ± 44	1092 ± 27	1168 ± 177	1100 ± 52
	O_2 (kPa)	-	-	19.6 ± 0.1	20.8	20.7	19.3 ± 0.2	-	-	18.8 ± 0.2	20.0 ± 0.3	20.7 ± 0.2	19.2 ± 0.6	20.8 ± 0.4	20.4 ± 0.6	19.1 ± 0.3	20.5 ± 0.2	20.3 ± 0.1	19.2 ± 0.9
~60 % air saturation	Temp (°C)	13.3 ± 0.1	13.6 ± 0.1	13.9 ± 0.1	-	17.7 ± 0.1	18.7 ± 0.1	20.5 ± 0.1	21.4 ± 0.2	22.0 ± 0.0	13.7 ± 0.2	13.8 ± 0.4	14.0 ± 0.2	17.5 ± 0.2	17.7 ± 0.2	18.0 ± 0.1	21.2 ± 0.4	21.2 ± 0.3	21.9 ± 0.1
	pCO_2 (µatm)	542 ± 67	-	501 ± 69	-	619 ± 98	674 ± 37	554 ± 25	-	579 ± 93	1533 ± 62	1577 ± 67	1528 ± 44	1457 ± 137	1486 ± 168	1540 ± 38	1468 ± 67	1436 ± 58	1468 ± 60
	O_2 (kPa)	13.9 ± 0.3	14.5 ± 0.2	12.0 ± 0.1	14.3 ± 0.3	14.3 ± 0.6	11.8 ± 1.0	14.3 ± 0.2	14.8 ± 0.0	11.6 ± 0.2	14.5 ± 0.2	14.4 ± 0.0	12.0 ± 0.1	14.4 ± 0.3	14.4 ± 0.2	11.9 ± 0.2	14.5 ± 0.2	14.5 ± 0.1	11.5 ± 0.1
~30 % air saturation	Temp (°C)	13.1 ± 0.2	13.4 ± 0.0	13.8 ± 0.1	-	17.6 ± 0.5	18.7 ± 0.1	20.5 ± 0.1	21.4 ± 0.1	22.0 ± 0.0	12.9 ± 0.2	13.1 ± 0.6	13.9 ± 0.1	17.6 ± 0.0	17.6 ± 0.1	18.3 ± 0.2	20.9 ± 0.1	20.9 ± 0.1	21.9 ± 0.1
	pCO_2 (µatm)	1173 ± 95	-	839 ± 53	-	854 ± 40	793 ± 118	929 ± 85	-	898 ± 101	2575 ± 243	2467 ± 110	2219 ± 216	2213 ± 135	2278 ± 179	2123 ± 272	2400 ± 126	2399 ± 34	2027 ± 100
	O_2 (kPa)	8.3 ± 0.3	8.8 ± 0.0	6.1 ± 0.1	8.4 ± 0.5	8.6 ± 0.3	6.8 ± 0.8	8.5 ± 0.3	8.8 ± 0.2	6.6 ± 0.2	9.1 ± 0.3	8.9 ± 0.0	6.7 ± 0.1	8.8 ± 0.4	9.1 ± 0.1	6.4 ± 0.3	8.7 ± 0.5	8.7 ± 0.3	6.4 ± 0.5

Table S3: Temperature and pCO_2 levels fish were exposed to when they were anaesthetised immediately prior to blood sampling. All measures are given as mean ± S.D.

	Treatment					
Parameter	14 °C Present CO_2	18 °C Present CO_2	22 °C Present CO_2	14 °C Future CO_2	18 °C Future CO_2	22 °C Future CO_2
Temperature (°C)	13.1 ± 0.1	17.4 ± 0.1	21.2 ± 0.3	13.3 ± 0.3	17.8 ± 0.1	21.3 ± 0.2
pCO_2 (µatm)	350 ± 13	445 ± 52	398 ± 28	1130 ± 33	1103 ± 47	1221 ± 35

Table S4: Samples sizes for each measurement in all treatments. We were unable to make measurements of P_{50} or Hills number for the 14 °C future CO_2 scenario due to an equipment failure.

	Treatment					
Measurement	14 °C Present CO_2	18 °C Present CO_2	22 °C Present CO_2	14 °C Future CO_2	18 °C Future CO_2	22 °C Future CO_2
SMR	10	10	10	10	10	10
O_{2crit}	10	10	10	10	10	10
MMR at ~21 kPa O_2	9	10	10	12	10	10
MMR at ~12 kPa O_2	10	7	10	10	10	10
MMR at ~6 kPa O_2	10	7	10	10	10	10
Blood pH_e	10	6	9	10	9	7
Plasma pCO_2	10	6	9	10	9	7
Plasma HCO_3^-	10	6	9	10	9	7
RBC pH_i	10	6	8	9	9	7
P_{50}	10	6	9	NA	8	7
Hills number	10	6	9	NA	8	7
Haematocrit	10	6	9	10	9	7
Haemoglobin	7	6	9	10	9	7
Plasma lactate	9	6	9	7	8	7
Plasma glucose	9	6	10	7	8	7

Table S5: Structure of models and model comparison results explaining variation in SMR, O_{2crit}, and MMR of sea bass exposed to differing combinations of temperature, O_2, and CO_2. The best supported model for each response variable is highlighted in bold.

Response Variable	Explanatory Variables Structure	AICc	ΔAICc	d.f.
SMR	**Temperature + CO_2**	**546.3**	**0**	**5**
	Temperature	548.2	1.9	4
	Temperature*CO_2	548.7	2.4	6
	CO_2	578.4	32.1	4
O_{2crit}	**Temperature + CO_2 + SMR**	**72.7**	**0**	**6**
	Temperature * SMR + CO_2	75.2	2.5	7
	Temperature * CO_2 + SMR	75.2	2.5	7
	Temperature + CO_2 * SMR	75.3	2.6	7
	Temperature + SMR	76.2	3.5	5
	Temperature*SMR	78.6	5.9	6
	Temperature * CO_2 * SMR	83.0	10.3	10
	SMR	84.0	11.3	4
	SMR + CO_2	84.8	12.1	5
	SMR*CO_2	87.2	14.5	6
	Temperature	134.2	61.5	4
	Temperature + CO_2	136.4	63.7	5
	Temperature*CO_2	138.8	66.1	6
	CO_2	144.0	71.3	4
MMR	**CO_2 + Temperature*O_2 + Temperature*$(O_2)^2$**	**2240.8**	**0**	**12**
	CO_2*Temperature*O_2 + Temperature*$(O_2)^2$	2243.0	2.2	15
	CO_2 + Temperature*O_2 + $(O_2)^2$	2247.5	6.7	11
	CO_2*Temperature*O_2 + $(O_2)^2$	2248.8	8.0	14
	Temperature + CO_2*O_2 + CO_2*$(O_2)^2$	2280.1	39.3	12
	CO_2*Temperature*O_2	2349.8	109.0	13
	Temperature + CO_2*O_2	2360.7	119.9	10
	CO_2 + Temperature*O_2	2362.8	122.0	10
	CO_2*O_2	2365.1	124.3	9
	Temperature*CO_2 + O_2	2371.4	130.6	10
	Temperature + CO_2 + O_2	2379.7	138.9	9
	Temperature + CO_2	2464.3	223.5	8
	Temperature + O_2	2464.7	223.9	8
	Temperature *CO_2	2465.4	224.6	9
	Temperature*O_2	2467.0	226.2	9

CO_2	2478.3	237.5	7
O_2	2488.8	248.0	7
Temperature	2638.1	397.3	7

Table S6: General linear mixed-effects model outputs for analysis of standard metabolic rate. The best supported model was fitted using a Gaussian distribution and included the parameters temperature and CO_2 as explanatory variables and group ID as a random intercept term. Parameter effects are compared against a reference level where temperature and CO_2 are 0. Marginal R^2 = 0.70, Condition R^2 = 0.70.Confidence intervals for each parameter were determined from function confint in package lme4. Marginal and conditional R^2 of the model were determined using function r.squaredGLMM from package MuMIn.

Parameter	Estimate	Standard Error	Confidence Interval (95%)	t-value	Variance	Standard Deviation
Best supported model <- lmer(SMR ~Temperature + CO_2 + (1|Group)						
Intercept	-14.77	11.62	-37.35 – 7.81	-1.27		
Temperature	6.66	0.57	5.55 – 7.78	11.61		
CO_2	-0.012	0.006	-0.024 – -0.001	-2.04		
Group					0.0	0.0

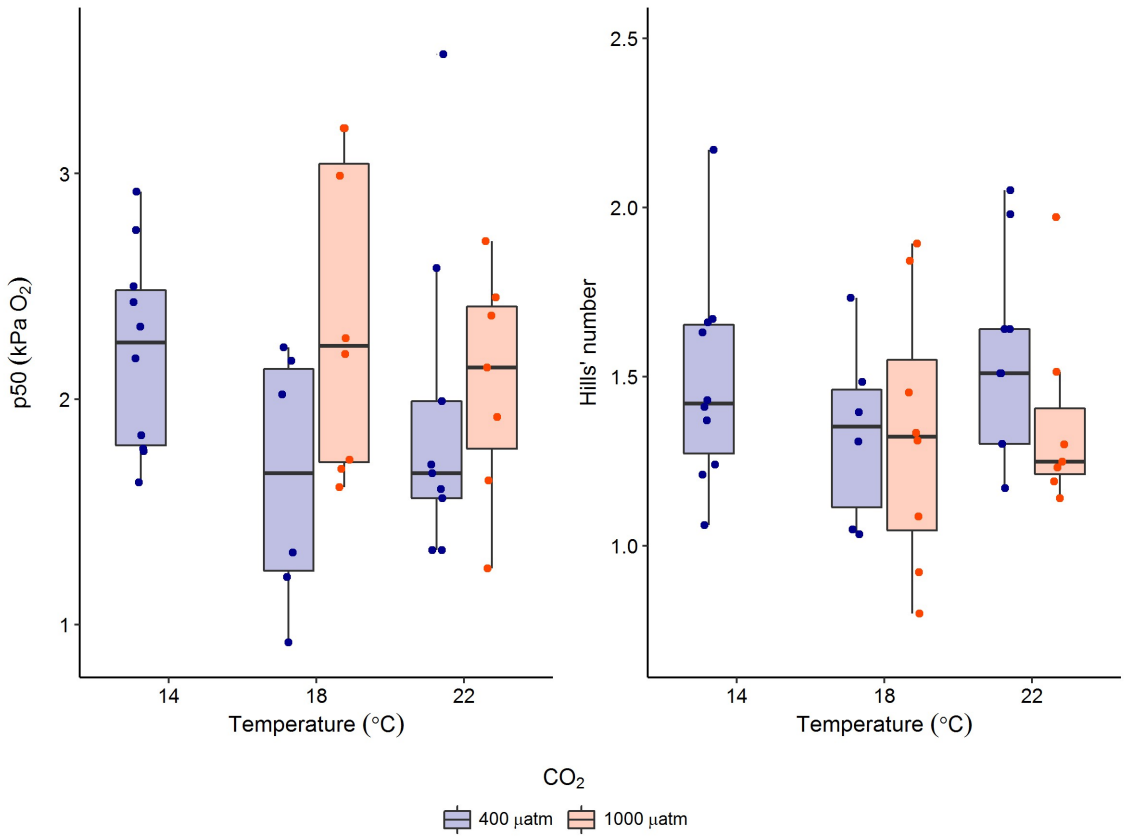
Table S7: General linear mixed-effects model outputs for analysis of O_{2crit}. The best supported model was fitted using a Gaussian distribution and included the parameters temperature,CO_2, and SMR as explanatory variables and group ID as a random intercept term. Parameter effects are compared against a reference level where temperature, CO_2, and SMR are 0. Marginal R^2 = 0.72, Condition R^2 = 0.77. Confidence intervals for each parameter were determined from function confint in package lme4. Marginal and conditional R^2 of the model were determined using function r.squaredGLMM from package MuMIn.

Parameter	Estimate	Standard Error	Confidence Interval (95%)	t-value	Variance	Standard Deviation
Best supported model < - lmer(O2crit~ Temperature+CO_2+SMR) + (1|Group)						
Intercept	2.174	0.421	1.401 – 2.945	5.16		
Temperature	-0.126	0.031	-0.185 – -0.068	-4.00		

CO_2	0.0005	0.0002	0.0001 – 0.0009	10.75		
SMR	0.036	0.003	0.030 – 0.043	2.37		
Group					0.03	0.18

Table S8: General linear mixed model outputs for analysis of maximum metabolic rate. The best supported model was fitted using a Gaussian distribution and included the parameters temperature and CO_2 as explanatory variables and group ID as a random intercept term. Parameter effects are compared against a reference level where temperature and CO_2 are 0. Marginal R^2 = 0.91, Condition R^2 = 0.96.Confidence intervals for each parameter were determined from function confint in package lme4. Marginal and conditional R^2 of the model were determined using function r.squaredGLMM from package MuMIn.

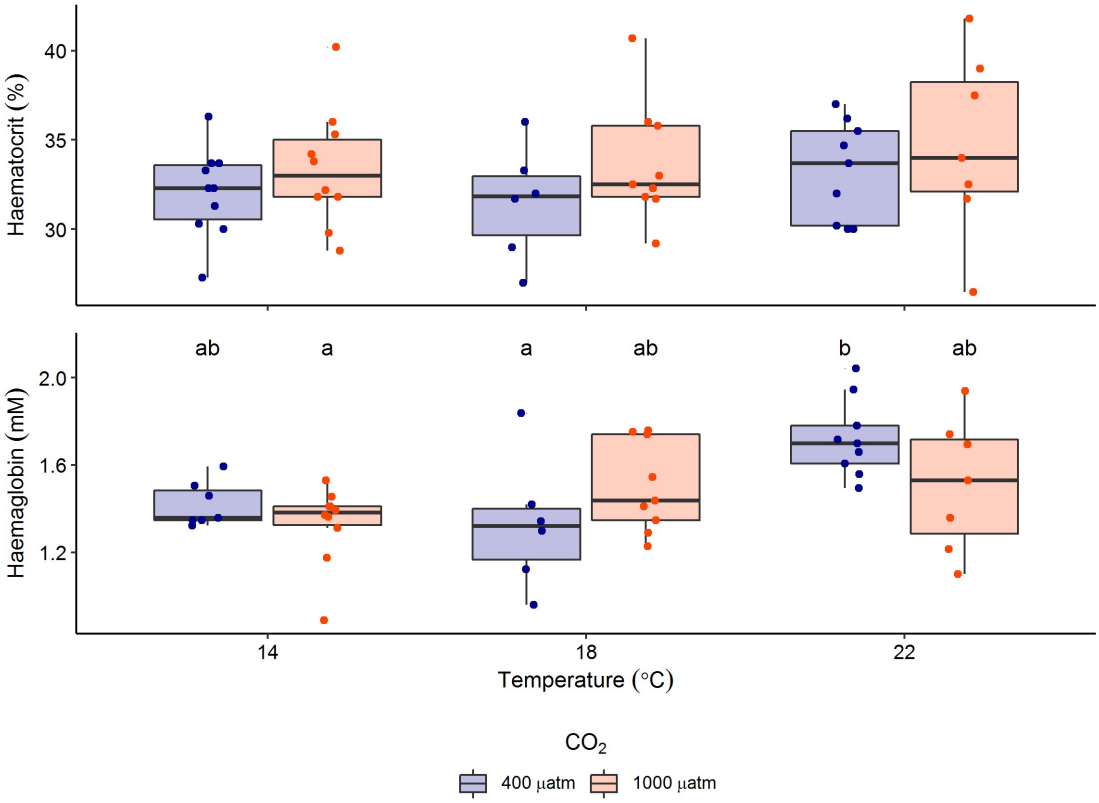
Parameter	**Estimate**	**Standard Error**	**Confidence Interval (95%)**	**t-value**	**Variance**	**Standard Deviation**
Best supported model < - lmer(MMR ~ scale(Temperature)*scale(O_2) + scale(Temperature)*scale(O_2^2) + scale(CO_2) + (O_2|Fish ID) + (1|Group)						
Intercept	231.99	2.58	227.01 – 236.98	89.96		
scale(Temperature)	32.53	2.55	27.60 – 37.46	12.76		
scale(O_2)	240.35	9.50	221.92 – 258.74	25.31		
scale(Temperature*O_2)	41.95	8.60	25.26 – 58.62	4.88		
scale(O_2^2)	-157.69	8.68	-174.50 – -140.85	-18.17		
scale(Temperature*O_2^2)	-25.10	8.36	-41.31 – -8.86	-3.00		
scale(CO_2)	-7.52	2.58	-12.08 – -2.97	-3.20		
Fish ID (Intercept)					90.02	9.49
Fish ID (O_2)					6.53	2.56
Group					4.78×10^{-6}	0.00



1048

1049 **Figure S1:** No impacts of temperature and CO_2 were observed for
1050 measurements of haemoglobin-O_2 binding affinity (measured using P_{50}) and
1051 Hills' number. Due to an equipment failure no measurements were possible
1052 during the original experimental period for fish at 14 ºC exposed to ~1000 µatm
1053 CO_2.

1054



1055

1056 **Figure S2**: Impact of temperature and CO_2 on haematological parameters of
1057 sea bass. No significant difference in haematocrit were observed between any
1058 treatments. Significant difference in haemoglobin content were noted between
1059 fish sampled at different temperature and CO_2 treatments and are represented
1060 by different lower case letters.

1061