Respiratory plasticity during acclimation to hypoxia and following a recovery in normoxia

Yangfan Zhang, Florian Mauduit, Luca Pettinau, Hélène Ollivier, Frédéric Lancien, Katja Anttila, Anthony Farrell, Guy Claireaux

Canadian Journal of Zoology
September 2023, Volume 101, Issue 9, Pages 794-806
https://doi.org/10.1139/cjz-2022-0158 https://archimer.ifremer.fr/doc/00839/95129/

Respiratory plasticity during acclimation to hypoxia and following a recovery in normoxia

Zhang Yangfan [1, *], Mauduit Florian [2], Pettinau Luca [3], Ollivier Helene [4], Lancien Frederic [4], Anttila Katja [3], Farrell Anthony Peter [5], Claireaux Guy [6]

[1] Harvard University, 1812, Organismic and Evolutionary Biology, Cambridge, Massachusetts, United States;
[2] University of California Davis, 8789, Davis, California, United States;
[3] University of Turku, 8058, Turku, Varsinais-Suomi, Finland;
[4] Université de Bretagne Occidentale, 27002, Brest, Bretagne, France;
[5] University of British Columbia, Zoology Department, Vancouver, British Columbia, Canada;
[6] Université de Brest, LEMAR (UMR-6539), Plouzané, France;

* Corresponding author : Yangfan Zhang, email address : yangfan_zhang@fas.harvard.edu

Abstract :

Phenotypic plasticity manifested after acclimatization is a very important source of biological variability among fish species. We hypothesized that hypoxic acclimation, besides potentially generating a temporary hypoxic respiratory phenotype, would also manifest as a continued benefit after re-acclimation to normoxia. Hence, we holistically characterized the respiratory phenotype of European sea bass (Dicentrarchus labrax) acclimated to normoxia with or without prior acclimation to hypoxia. Compared with the original normoxic phenotype, prior acclimation to hypoxia and return to normoxia produced a 27% higher absolute aerobic scope (AAS), a 24% higher citrate synthase activity in red muscle and a 28% lower excess post-exercise O2 consumption. Additional testing of hypoxia-acclimated fish under normoxia explored the specific effects of hypoxic acclimation. The hypoxic phenotype, when compared with the original normoxic phenotype, had a lower standard metabolic rate, a better hypoxia performance and a lower minimum PO2 for supporting 50% AAS. Given this respiratory malleability, general predictions for marine fish exploiting a more hypoxic future should better consider respiratory plasticity and prolonged effects of hypoxic exposures.

Introduction

Animals respond to environmental change either by moving to a more favourable location, by changing their phenotype (acclimatization), or potentially by evolving over multiple generations (genetic adaptation). Yet, the drastic decrease in dissolved oxygen (O_2) availability (*i.e.,* hypoxia) during the Permian era (299-251 million years ago), which extirpated over 90% of marine fish species (Graham *et al*., 1995; Clack, 2007), illustrates potential limits to such coping strategies. Nonetheless, fish species subsequently radiated into the most specious vertebrate

group, suggesting that successful and broad environmental adaptations did occur among the survivors of the Permian era.

Today, the Anthropocene is presenting extant marine fishes with another hypoxic challenge. Associated with global warming, the increased frequency of hypoxic episodes can be one of the key driving forces well into the future, reshaping the distribution and evolution of marine species (Deutsch *et al*., 2015). The hypoxia-induced redistribution can be affected by the ability of aquatic ectotherms to obtain O_2 in the hypoxia (Seibel and Deutsch, 2020; Seibel *et al*., 2021). With this prospect, a key question concerning the biodiversity of marine fishes is how plastic is the respiratory phenotype of marine fishes? We define respiratory phenotype as a suite of respiratory performance metrics that characterize whole-animal aerobic and glycolytic metabolism.

In nature, ample opportunities exist for fishes to manifest new respiratory phenotypes that preserve their capacity to sustain their activities. These opportunities are the daily and seasonal environmental cycles experienced by fish in freshwater (Morash *et al*., 2018), intertidal (Somero, 2002) and marine (Drinkwater *et al*., 2003) ecosystems, as well as diurnal or seasonal migrations into hypoxic zones of certain marine fishes for foraging (Douglas *et al*., 1976; Gjøsaeter, 1984; MacKenzie and Mariani, 2012). In the present study, we were particularly interested in examining the aerobic performance of fish, the development of a hypoxic phenotypic and its reversibility, *i.e.*, would a fish restore its original normoxic respiratory phenotype after being returned to normoxia following hypoxic acclimation, or would a new phenotype emerge?

Phenotypic plasticity in response to hypoxia can occur rapidly in fishes and enhances aerobic performance under hypoxic conditions (*see summaries* by Wang *et al*., 2009; Gamperl and Driedzic, 2009; Richards, 2009). The high-latitude minnow (*Rhynchocypris lagowskii*

Dybowski, 1869), for example, remodels its entire gill structure after just a 30-min hypoxic exposure (Yang *et al*., 2021). Similarly, the mangrove rivulus (*Kryptolebias marmoratus* Poey, 1880) remodels epidermal ionocytes and respiratory traits after just 24 h of air exposure (Blanchard *et al*., 2019; Dong *et al*., 2021). Moreover, a brief ischemic period can precondition fish cardiac myocytes to help maintain stroke volume and cardiac output, can induce cardiac hypertrophy and can enhance the sarcolemmal ATP-sensitive K^+ channels, helping fish to perform better in a subsequent hypoxic episode (Gamperl *et al*., 2001b; Gillis and Johnston, 2017; Carnevale *et al*., 2021). Hence, we hypothesized that hypoxic acclimation, besides generating a hypoxic respiratory phenotype, would also provide respiratory benefits upon re-acclimation to normoxia. Our broader objective was to contribute to understanding how marine fishes might respond to seasonal O_2 cycling and thereby better predict future distributions of marine fish species.

Our model species was European sea bass (*Dicentrarchus labrax* Linnaeus, 1758) because it has an active lifestyle and naturally exploits hypoxic habitats. Adults require a high aerobic capacity to capture prey in hydraulically dynamic coastal waters (Pickett and Pawson, 1994), while juveniles successfully exploit hypoxic estuaries and coastal lagoons. Indeed, their hemoglobin-O_2 affinity (P_{50} = ~ 1.7 kPa; Pichavant *et al*., 2003), which lies between that of the hypoxia-tolerant common carp (P_{50} = 0.9 kPa; Roy and Lykkeboe, 1978) and that of the active, hypoxia-sensitive rainbow trout (P_{50} = 2.9 kPa; Weber *et al*., 1976), suggests a moderate hypoxia tolerance. Furthermore, when ambient O_2 was cycled between 8.3 and 17.8 kPa (Thetmeyer *et al*., 1999), growth rate and feed conversion efficiency were preserved over a 4-week period. In fact, sea bass held at 10.4 kPa ambient O_2 [50 % saturation (% sat.) at 20 °C] displayed a full

postprandial peak O_2 uptake (Zambonino-Infante *et al*., 2017). Thus, hypoxia acclimation of the present study used 50 % sat. for the maintenance of normal growth during hypoxia.

Our hypothesis was tested by returning European sea bass that had been acclimated to a stable hypoxic environment back into their original normoxic environment. We asked whether they simply reverted to the original normoxic phenotype, or whether a new normoxic phenotype would emerge (Fig. 1). Also, to provide clues to the mechanisms underlying any new respiratory phenotype, we tested hypoxia-acclimated fish under normoxic and hypoxic conditions. Thus, by holistically characterizing a normoxic and hypoxic phenotype might provide insights into the O_2 cost of breathing hypoxic water as well as establishing the nature of hypoxic acclimation. We holistically characterized the individual respiratory phenotype of sea bass using whole-animal respirometry (Claireaux and Lagardère, 1999; Svendsen *et al*., 2016; Zhang *et al*., 2019) and measured muscle enzymes activities of citrate synthase (CS) and lactate dehydrogenase (LDH) (Childress and Somero, 1979; Dalziel *et al*., 2012). In addition, we generated individual hypoxic performance curves (Zhang *et al*., 2022).

Materials and methods

(a) Experimental animals and acclimation procedures

Before experiments started, a stock of juvenile European sea bass (*Dicentrarchus labrax*, Linnaeus 1758; n = 150; Aquastream, Lorient, France) was reared for 12 weeks under normoxic laboratory conditions in a 2000-L indoor tank in Ifremer research facilities (Plouzané, France). They were fed *ad libitum* twice weekly (Le Gouessant, Lamballe, France). Individual radio-frequency identification tags were subcutaneously implanted under anaesthesia (100 mg L^{-1} MS-222) at the end of the sixth week of the rearing period. Fish holding and all experimental

procedures followed the guidelines of current animal care rules and regulations in France (Apafis 2018040916374437).

Acclimation prior to respirometry measurements involved equally redistributing fish into two acclimation tanks (500-L) that received flow-through (300 L h^{-1}) and thermoregulated seawater (16 °C; the average summer temperature of the species experience in the region). Photoperiod was synchronized to the natural regional cycle with an adjustment to the diurnal cycle each week. One acclimation tank contained control normoxic fish that remained at a partial pressure of O_2 level of ~20.4 kPa (dissolved O_2 of ~98 % sat.). Some of these fish were tested after 4 weeks of acclimation to on-going normoxic conditions (N-N: 61.5 ± 2.0 g, n = 28). The other acclimation tank had been made progressively hypoxic at a rate of 10 % sat. h^{-1} to 50 % sat. (10.4 kPa) using a custom-built, 50-L gas-equilibration column that was situated upstream of the aquarium and received the thermoregulated seawater into the top while nitrogen gas was injected at the bottom. The hypoxic water was maintained for six weeks for the hypoxia-acclimated group. Thereafter, some fish respirometry tests were performed either under the same hypoxic conditions (10.4 kPa; H-H; 63.6 ± 5.7 g; n = 16; Suppl. Mat.) or under normoxic conditions (H-N: 74.7 ± 4.5 g, n = 16). The remainder were returned to normoxia for four weeks for re-acclimation before being tested under normoxic conditions (HN-N: 63.3 ± 6.1 g, n = 13) and comparison with the N-N group.

A common acclimation period for temperate fish species is 3-4 weeks under normoxic conditions (*e.g.* Fangue *et al*., 2009); a new cardiac phenotype can begin to appear even after 8 h of 4 °C acclimation (Sutcliffe *et al*., 2020; Gilbert *et al*., 2022). Therefore, we assumed that a steady-state phenotype would emerge after a normoxic acclimation period of four weeks and be stable thereafter. The respiratory phenotype of a temperate fish can be stable for 9–18 weeks

under controlled laboratory conditions (Table S1; Zhang *et al*., 2019; Polinski *et al*., 2021; Zhang, 2021). Hypoxia, however, could slow the acclimation processes. Therefore, as a precaution, we used a 6-week hypoxic acclimation period in the event of a slower acclimation process in an oxygen limiting environment. Our reasoning was partly based on a 6-week hypoxic (~40 % sat., 8.4 kPa) acclimation period being previously used for a hypoxic phenotype of Atlantic cod (*Gadus morhua*, Linnaeus, 1758) at a colder temperature of 10 °C (Petersen and Gamperl, 2010; Petersen and Gamperl, 2011).

(b) Protocol used to characterize the individual respiratory phenotypes

We followed simultaneously individual $\dot{M}O_2$ for eight, fasted (for 48 h) fish over a 3-day period using an automatic respirometry system (Steffensen, 1989), as previously validated and described for the Integrated Respiratory Assessment Protocol (IRAP; Zhang *et al*., 2016; 2019). Phenotyping of each treatment group (n=~16) consequently involved two sets of measurements over a 5-day period using eight 2.25-L Loligo®-type respirometer chambers (water volume: fish ratio = 36:1) that were immersed in a 500-L seawater bath at an ambient water temperature of 16 ± 0.5 °C. This outer bath was connected via a pump to the gas-equilibrium column used to control water PO_2. Water from the outer bath (normoxic or hypoxic depending on the test conditions) was supplied to each respirometry chamber via a dedicated individual water pump. Water PO_2 in each of the eight respirometers was continuously measured using an optical O_2 probe (Robust O_2 Probe OXROB2, PyroScience GmbH, Aachen, Germany). $\dot{M}O_2$ of each individual fish was reported on-line every 10 min (*see* Fig. 2a) by AquaResp software (Svendsen *et al*., 2019), which used a sequential interval regression analysis for a 420-s period when the respirometer was sealed (*see* Suppl. Mat.). The remainder of the 10-min $\dot{M}O_2$ measurement cycle

was taken up by a 120-s flush period (the respirometer open) and a 60-s stabilization period (the respirometer closed) prior to the actual 420-s $\dot{M}O_2$ measurement period.

Each respirometry chamber was equipped with a customized chasing device (a 14-cm soft, flexible plastic strip located at the mid-point of the chamber; Zhang *et al*., 2020). This device individually agitated fish after a ≥ 30-min period of habituation to the respirometer. During the 10-min agitation period each fish become refractory and during this agitation the on-line monitoring of $\dot{M}O_2$ revealed peaks and plateaus in $\dot{M}O_2$ associated with activity and rest periods. Peaks in $\dot{M}O_2$ were occasionally seen in the 10-min measurement cycle when the fish was no longer being agitated and $\dot{M}O_2$ was in a declining phase. Maximum O_2 uptake ($\dot{M}O_{2max}$) was determined from these peaks in $\dot{M}O_2$ ($\dot{M}O_{2peak}$) using a more precise, off-line analysis (Zhang *et al*., 2019; Zhang and Gilbert, 2017 an iterative algorithm applied to 2-min measurement windows (Fig. S1); *see* Appendix). This method of generating an $\dot{M}O_{2max}$ was previously validated for rainbow trout because $\dot{M}O_{2max}$ was higher compared with a protocol that chased rainbow trout outside of the respirometer (Zhang *et al*., 2020). Indeed, sea bass chased to exhaustion at 16 °C outside of the respirometer at the Ifremer laboratory (Brest, France) had a numerically lower $\dot{M}O_{2max}$ (~400 mg O_2 h^{-1} kg^{-1}; Zhang *et al*., 2017) when compared with our $\dot{M}O_{2max}$ measurements (see *Results*).

After the agitation, we followed the decline in $\dot{M}O_2$ of fish for about 10 h to calculate the total O_2 consumed during the recovery (Zhang *et al*. 2018) and estimate the excess post-exhaustion O_2 consumption (EPOC; see calculation in Appendix). After this recovery, each fish remained undisturbed (except for a daily visual check) for the ensuing two-day quiescent period that yielded ~240 measurements of routine $\dot{M}O_2$ per fish, from which standard metabolic rate (SMR) and routine metabolic rate (RMR) were estimated using established analytical

192 procedures. SMR was analysed off-line with a quantile algorithm (q0.2) (Chabot *et al*., 2016)
193 applied to the ~240 $\dot{M}O_2$ measurements. Absolute aerobic scope (AAS) was derived from the
194 numerical difference between $\dot{M}O_{2max}$ and SMR, while factorial aerobic scope (FAS) was
195 derived from the quotient of $\dot{M}O_{2max}$ and SMR. RMR was determined as the average of the ~240
196 $\dot{M}O_2$ measurements and the standard deviation of an individual's RMR was used as an index of
197 spontaneous activity, *i.e.* the more active a fish, the greater the variability of RMR measurements
198 for an individual fish. All $\dot{M}O_2$ values were corrected for the background $\dot{M}O_2$, which was
199 measured for 20 min in each respirometer without a fish, both before and immediately after
200 every trial. A logarithmic microbial growth model was applied to background measurement over
201 the entire period of respirometry so that the background $\dot{M}O_2$ could be subtracted from each
202 relevant $\dot{M}O_2$ measurement.
203 IRAP ended with a hypoxia challenge test (HCT) when the gas-equilibration column
204 reduced PO_2 in the outer bath initially to ~6.25 kPa (DO = ~30 % sat.) within 45 min (*i.e.,* 0.313
205 kPa min^{-1} or ~1.5% sat. min^{-1}) and then at a slower rate of deoxygenation (0.0313 kPa min^{-1} or
206 ~0.15% sat. min^{-1}) until the fish lost its dorso-ventral equilibrium. The incipient lethal O_2 partial
207 pressure (ILOP; Claireaux *et al*., 2013) was assigned to the partial O_2 pressure (PO_2) when the
208 fish first lost equilibrium. At this point, fish were immediately removed from the respirometer
209 and successfully revived before returning them to their holding aquaria. The off-line analysis of
210 $\dot{M}O_2$ during the HCT and as a fish became progressively hypoxic yielded the PO_2 level at which
211 SMR could no longer be maintained, the critical O_2 partial pressure (P_{crit}; see calculation in
212 Appendix). The scope for O_2 deficit (SOD) was assigned to the difference between P_{crit} and
213 ILOP.

214 Our holistic respiratory phenotyping was based, therefore, on 10 measured or derived
215 respiratory indices for individual fish. The respiratory phenotype of the H-N group was
216 compared with the N-N group to ascertain the nature of the that had emerged for the hypoxic
217 phenotype. The H-H and H-N groups were also compared (Suppl. Mat.) to understand the
218 limiting effect of ambient hypoxia (Fry, 1971).
219 Respiratory indices were statistically compared among treatment groups (N-N, H-N and
220 HN-N) with general linear effect models and body mass as a covariate. Logarithm
221 transformations were needed for comparisons of the variance of RMR to meet the assumptions of
222 normality of residuals and homoscedasticity of the variance.
223
224 **(c) Protocol used for measuring an individual hypoxic performance curve**
225 A hypoxic performance curve (HPC) can quantify the constraint of a progressive decrease of
226 ambient water PO_2 on $\dot{M}O_{2max}$, *i.e.*, the relationship between $\dot{M}O_{2peak}$ and water PO_2. Previous
227 studies have generated and validated an HPC for group activity of fish (Lefrancois and
228 Claireaux, 2003) and for individual fish (Zhang *et al*., 2021; Zhang *et al*., 2022). We generated
229 HPC on individual fish after normoxic acclimation (N-N; n=8) and after hypoxic acclimation (H-
230 N; n=8). These sea bass were tested following a 7-day recovery (5 days feeding and 2 days
231 fasting) from their IRAP test and assumed full recovery from the exhaustive and the HCT would
232 take < 24 h (Milligan, 1996; Zhang *et al*., 2018).
233 Fish were placed in individual respirometers, as described above, for the HPC. They
234 habituated to the respirometer in a flush mode and received normoxic seawater (with DO =
235 ~95 % sat., 19.8 kPa, 16 ˚C) for 30 min. The $\dot{M}O_2$ measurement cycle was 5 min: a 120-s $\dot{M}O_2$
236 recording period, a 150-s flush period and a 30-s stabilization period to better capture $\dot{M}O_{2peak}$.

An initial agitation for 10 min generated a $\dot{M}O_{2peak}$ under normoxic condition (again using off-line analysis; *see* Appendix). During the ensuing 25 min, while the water PO_2 was progressively reduced with the respirometer in flush mode (*see* above), a fish would partially recover. The next $\dot{M}O_2$ measurement cycle started 25 min after the previous measurement and at a lower PO_2, which was maintained while the fish was again agitated to generate a new $\dot{M}O_{2peak}$. This procedure was then repeated every 10 min at a progressively lower PO_2 down to 4.2 kPa (DO = 20% sat.) (*i.e.,* slightly higher than our measured P_{crit}). The total test time of an HPC was ~125 min and yielded 11 $\dot{M}O_{2peak}$ values at progressively lower levels of water PO_2. After an HPC test, the fish were removed from the respirometer and returned to a well-aerated aquarium where they all recovered.

An individual-based HPC was based on a one-phase association regression equation (Eqn. 1), which best modelled the relationship between the measured $\dot{M}O_{2peak}$ and the ambient PO_2 (Mueller and Seymour, 2011). We only used individual regression models that had $0.65 \leq R^2 \leq 0.99$ (three fish were rejected). Those satisfying this level of quality assurance were pooled for averaged HPCs of the normoxia-acclimated (n=6) and hypoxia-acclimated (n=7) test groups. Individual variation among the individual HPCs was accounted for by normalizing $\dot{M}O_{2peak}$ as a percentage of the individual AAS (derived from the individual $\dot{M}O_{2peak}$ measured at normoxia). This normalized HPC was then used to interpolate the minimum PO_2 at which a fish could generate 50% of its normoxic aerobic scope, P_{AAS-50} (Zhang *et al*., 2022). These individual data were used to statistically compare P_{AAS-50} normoxia-acclimated and hypoxia-acclimated test groups with an independent sample t-test.

$$y = I + (Asymptote - I) * [1-\exp(-K * x)] \qquad \text{One-phase association equation (Eqn. 1)}$$

Where *I* is the intercept at the y-axis, *Asymptote* is a line that the curve continues to approach at infinity. *I* and *Asymptote* are expressed in the same unit as y. *K* is the rate constant for a hyperbolic increase.

(d) Organ and enzyme activity measurements

Additional fish were directly sampled by removing them directly from the acclimation tanks (normoxic, hypoxic and re-aerated hypoxic fish) to provide representative measurements of organ size, hematology and metabolic enzyme activity of each acclimation phenotype. They were sacrificed with a blow on the head (N: n = 23; H: n = 13 and HN: n = 12). Blood was removed immediately by caudal puncture into a heparinized syringe to determine hematocrit (Sigma 201m microhematocrit centrifuge) and hemoglobin concentration [Hb]. The [Hb] was calculated as described by Clark *et al*. (2008) from the absorbance measured in triplicates (PerkinElmer EnSpineTM 2300 Multilabel plate reader, Perkin Elmer, Turku, Finland) at 540 nm for 10 µl of blood diluted to 1 ml with a solution containing: 50 mg $K_3Fe(CN)_6$ (Merck, Espoo, Finland), 12.5 mg KCN (Pharmakon Inc, NJ, USA), 40 mg KH_2PO_4 (MilliporeSigma, Darmstadt, Germany) in 175 ml H_2O. The ventricle and liver were removed and weighed to calculate relative liver and ventricular masses as a percentage of fish body mass. Samples of red and white skeletal muscle (7-8 mm thickness) were removed from the cross-section of the second dorsal fin and caudal fin. They were flash-frozen with liquid nitrogen before storage at -80°C until analysis. We reasoned that, because skeletal muscle is the largest and most active organ in fish, citrate synthase (CS, EC 2.3.3.1) and lactate dehydrogenase (LDH, EC 1.1.1.27) activities from the red and white muscle of fish are useful index of oxidative and substrate-level energy

283 metabolic capacity of the fish. These muscle samples were homogenized in 19 and 6 vol.
284 homogenization buffer (0.1% Triton, 50 mM Hepes, 1 mM EDTA, pH 7.4) for CS activity, and
285 in 19 and 40 vol. homogenization buffer for LDH. Both assays were performed in triplicate
286 (randomized) at room temperature measuring the maximal activity for three minutes with the
287 EnSpire 2300 Multilabel Reader and subtracting the background reaction rate (Dalziel *et al.*,
288 2012). The concentration of protein in muscle homogenates was analyzed with a BCA protein
289 assay kit (ThermoFisher, Waltham, MA, USA) to express enzyme activity as g^{-1} protein. Organ,
290 hematocrit, haemoglobin and enzyme activity metrics were statistically compared among
291 treatment groups using ANOVA with Tukey-Kramer *post-hoc* tests. Statistical significances for
292 all analyses were assigned when $\alpha \leq 0.05$.
293

294 **Results**

295 **(a) Re-acclimation to normoxia of hypoxia-acclimated sea bass produced a new normoxic**
296 **respiratory phenotype, one with an improved aerobic capacity**

297 Hypoxia-acclimated sea bass returned to normoxia for 4 weeks did not fully return to their
298 original normoxic phenotype. Notably, aerobic performance was significantly improved. While
299 SMR was similar compared with the N-N test group, the HN-N test group had an 18% higher
300 $\dot{M}O_{2max}$ (503.4 ± 27.2 vs. 427.3 ± 11.7 mg O_2 h^{-1} kg^{-1}, $F_{2,53} = 6.4$, $p = 0.003$), which contributed
301 to a 27% higher AAS (402.5 ± 23.3 vs. 318.0 ± 13.7 mg O_2 h^{-1} kg^{-1}, $F_{2,53} = 7.2$, $p = 0.001$) and a
302 28% higher FAS (5.1 ± 0.2 vs. 4.0 ± 0.2, $F_{2,53} = 7.1$, $p = 0.003$; Fig. S6). The HN-N phenotype
303 also had a 24% higher citrate synthase activity in red muscle ($F_{2,47} = 3.2$, $p = 0.049$, Fig. 3a) than
304 the N-N phenotype.

Furthermore, the HN-N group had a 10% lower P_{crit} ($F_{2, 53} = 18.1$ $p = 0.001$; Fig. 4a) than the N-N phenotype, indicating an improved hypoxia tolerance. The relative liver mass of the HN-N phenotype was significantly lower ($F_{2, 25} = 4.5$, $p = 0.016$, Fig. S3d) and their EPOC was 28% lower ($F_{2, 46} = 5.4$, $p = 0.013$; Fig. 4d) compared to hypoxia-acclimated fish.

While a new normoxic phenotype certainly emerged after the hypoxia-acclimated sea bass were re-acclimated to normoxia, SMR, ILOP and SOD remained statistically the same as those of the original N-N group ($F_{2, 53} \leq 7.7$, $p \geq 0.059$, power ≥ 0.71, Fig. 2c & Fig. 4b, c).

(b) Hypoxic acclimation of sea bass produced a new respiratory phenotype with improved hypoxia tolerance and hypoxic performance

As anticipated (Zambonino-Infante *et al*., 2017), hypoxic acclimation at 10.4 kPa (50% sat.) did not affect body size (Fig. S2). Likewise, the maximum lactate dehydrogenase activities for both red and white muscles ($F_{2, 46} \leq 0.27$, $p \geq 0.93$, Fig. S7), hematocrit, [Hb] and relative masses of the ventricle and liver were similar for the hypoxia-acclimated fish ($F_{2, 25} \leq 3.5$, $p \geq 0.07$; Fig. S3) when compared to the normoxia-acclimated fish.

All the same, a new hypoxic phenotype was confirmed by testing the hypoxic and normoxic phenotypes in normoxia (*i.e.* H-N and N-N test groups) and revealing significant differences in their respiratory indices. Notably, SMR was 19% lower in the H-N test group when compared with N-N fish (88.0 ± 2.1 vs. 109.3 ± 3.5 mg O_2 h^{-1} kg^{-1}, $F_{2, 53} = 6.0$, $p = 0.003$; Fig. 2c). This hypometabolic state was also reflected in the RMR of the H-N test group. RMR was similarly 15% lower in the H-N test group over the 2-day quiescent period when compared with N-N fish (117.3 ± 3.7 vs. 138.1 ± 4.4 mg O_2 h^{-1} kg^{-1}, $F_{2, 53} = 3.6$, $p = 0.033$; Fig. 2d). Furthermore, a sustained metabolic depression of the hypoxia-acclimated group was quite

328 evident throughout the quiescent period after inspection of both individual (Fig. 2a) as well as
329 grouped mean $\dot{M}O_2$ traces (Fig. 2b). Such a sustained reduction in RMR was likely not due to a
330 lower spontaneous activity because the individual variance for RMR, our index of spontaneous
331 activity, was similar for the H-N and N-N test groups ($F_{2, 53} = 1.7$, $p = 0.98$, power = 0.334; Fig.
332 2e).

333 Despite a reduction of SMR in the hypoxia-acclimated phenotype, both $\dot{M}O_{2max}$ and AAS
334 were maintained; they were similar for the H-N and N-N tests groups ($F_{2, 53} \leq 7.2$, $p \geq 0.53$,
335 power ≥ 0.98; Fig. 2f, g). With maximal aerobic capacity unchanged and SMR lowered, FAS
336 was significantly higher for the H-N test group compared with the N-N test group ($F_{2, 53} = 7.1$, p
337 $= 0.035$; Fig. 2h).

338 The phenotype after hypoxic acclimation also had an improved hypoxia tolerance.
339 Specifically, three indicators were significantly lower for the H-N test group than for the N-N
340 test group: P_{crit} by 28% (2.69 ± 0.10 vs. 3.75 ± 0.14 kPa, $F_{2, 53} = 18.1$; $p < 0.001$), ILOP by 22%
341 (1.02 ± 0.084 vs. 1.31 ± 0.063 kPa, $F_{2, 52} = 4.2$; $p = 0.03$) and SOD by 34% (1.65 ± 0.09 vs. 2.39
342 ± 0.14 % sat., $F_{2, 52} = 7.7$, $p = 0.001$) (Fig. 4).

343 Nonetheless, the hypoxic ambient environment clearly constrained peak respiratory
344 performance, as revealed when the hypoxic phenotype was tested under the ambient hypoxic
345 condition (*i.e.,* the H-H test group). For example, $\dot{M}O_{2max}$, AAS and FAS of the H-H test group
346 were all significantly reduced, almost halved ($F_{3, 69} \geqslant 14.6$, $p \leqslant 0.0007$) when compared with
347 the H-N test group. However, SMR and RMR were the same as the H-N test group ($F_{3, 69} \leqslant 7.9$,
348 $p \geqslant 0.29$; Fig. S4).

Given the improved aerobic performance and hypoxia tolerance of the hypoxic phenotype, the original normoxic phenotype was compared with the hypoxic phenotype using a hypoxic performance curve (HPC). Their $\dot{M}O_{2peak}$ values in normoxia were statistically indistinguishable (365.1 ± 15.3 vs. 334.6 ± 13.3 O_2 h^{-1} kg^{-1}, respectively; t-test: $t = 1.4$, $p = 0.17$, power = 0.296). The hypoxic phenotype, however, had a significantly left-shifted HPC compared with the normoxic phenotype. This shift produced a 21% lower P_{AAS-50} [7.92 vs. 10.0 kPa (38 vs. 48 % sat.); $t = 1.6$, $p = 0.0031$; Fig. 5]. As quality assurance for the HPC of the hypoxic phenotype, the interpolated $\dot{M}O_{2peak}$ at 10.4 kPa (50% sat.) was compared to and was similar to that measured as $\dot{M}O_{2max}$ at a similar level of ambient hypoxia [*i.e.*, H-H; 10.4 kPa (50% sat.)].

Discussion

As an extension of the cellular metabolic signalling pathways proposed for hypoxic phenotypes (Hochachka, 1986), we considered the lesser studied whole-animal respiratory phenotype. We demonstrated that the hypoxia-acclimated phenotype of sea bass had an enhanced O_2 uptake in hypoxia, a lower P_{crit}, and a left-shifted HPC (a higher $\dot{M}O_{2peak}$ under moderate hypoxia). While the lower P_{crit} is clearly influenced by a lower SMR, the $\dot{M}O_{2peak}$ under moderate hypoxia is not. Moreover, the re-acclimation of hypoxia-acclimated sea bass to normoxia produced a new normoxic phenotype. This new normoxic phenotype also had an enhanced $\dot{M}O_{2max}$ and AAS, as well as a reduced P_{crit} like the hypoxic phenotype, but not the reduced SMR. Thus, it seems probable, but not definitely demonstrated, that certain (but not all) respiratory enhancements shown for the hypoxic phenotype were retained after 4-weeks of re-acclimation to normoxia. Such potential and sustained (many weeks) effects on the current performance of an animal's previous experience are more generally termed a carryover effect (O'Connor *et al*., 2014), but

we cannot be certain that our demonstration for sea bass is necessarily a carryover effect. Nonetheless, and regardless of the exact mechanism, phenotypic plasticity following a hypoxic acclimation can clearly benefit whole-animal aerobic performance and O_2 handling in normoxia as well as hypoxia. Moreover, European sea bass could be a 'fence-sitter' when exploiting hypoxic habitats, taking advantage of both a reduced maintenance metabolic demand and an improved aerobic performance during the hypoxic experience, two processes that are typically thought of as mutually exclusive strategies for fish living in an oxygen-limiting environment.

(a) Sustained effect of improved aerobic performance

A hypoxia-acclimated fish returned to their original normoxic condition can have three general outcomes (Fig. 1): i) Restoring their original normoxic phenotype, *i.e.*, status quo, ii) Suffering a compromised normoxic performance, a negative consequence of hypoxic acclimation, or iii) Acquiring enhanced normoxic performance, a beneficial prolonged consequence of hypoxic acclimation. We observed increased aerobic performance and capacity ($\dot{M}O_{2max}$, AAS & FAS) after hypoxia-acclimated sea bass were re-acclimated to and tested in normoxia. These whole animal changes align with the observed increase in CS activity in their red muscles. This new normoxic phenotype may have maintained some of the same physiological improvements for at least 4 weeks after hypoxia-acclimated sea bass were returned to normoxia. An enhanced aerobic capacity, for example, reduced the need for glycolytic capacity (Farrell, 2016; Zhang *et al*., 2018) because EPOC was reduced in the normoxia re-acclimated group when compared to the normoxia group (Fig. 4).

A novel discovery was a left-shifted HPC after an acclimation of sea bass to hypoxia. This means that hypoxia-acclimated sea bass had improved O_2 handling for $\dot{M}O_{2peak}$, as indicated

by their 21% lower P_{AAS-50} compared with hypoxia-acclimated fish. Likewise, a lower P_{crit} has been correlated with a higher AAS among eight populations of four fish species (Zhang *et al*., 2018; Zhang *et al*., 2021). However, in terms of hypoxic acclimation of sea bass, SMR and P_{crit} were not reduced when they were tested in hypoxia, but were reduced when tested in normoxia. Therefore, the observed suppression of SMR is likely not a contributing factor to the lower P_{AAS-50} because the HPC and IRAP testing was performed at a similar 50% hypoxia. Other beneficial hypoxia acclimation mechanisms include remodeling of gill secondary lamellae (Brauner and Rombough, 2012; Anttila *et al*., 2015; Yang *et al*., 2021), which would reduce the O_2 diffusion distance (Randall, 1982), could improve hypoxia tolerance and could defend $\dot{M}O_{2peak}$ under ambient hypoxia. Improved cardiovascular O_2 delivery and O_2 utilization by mitochondria are also possible. For example, hemoglobin-O_2 binding affinity could increase (Weber and Jensen, 1988; Montgomery *et al*., 2019; Wells *et al*., 1989), venous blood stores could be better mobilized by increasing venous tone, and capillarity could increase in cardiac (Gillis and Johnston, 2017) and swimming muscles (McKenzie *et al*., 2004).

Future experiments should test whether or not the beneficial P_{AAS-50} is retained on re-acclimation to normoxia because logistical constraints prevented us from doing so in the present study. Indeed, and more generally, the malleability of the O_2 transport cascade system of sea bass could be a useful model system to study how a fish might benefit from prior hypoxic exposures. Beyond hypoxia, other environmental stressors are also known to have sustained effects, increasing subsequent tolerance to that stressor (Kawabata *et al*., 1998; Gamperl *et al*., 2001). Another uncertainty generated by the present study is the exact time course for developing new phenotypes and for how long the benefits of a hypoxic exposure might persist. Our acclimation periods (4 weeks for normoxia and 6 weeks for hypoxia) were based on many previous studies

and did not consider any potential modulating effects of seasonality. We do know, however, that IRAP metrics in normoxic fish can be stable for up to 18 weeks (Zhang *et al*., 2019; Polinski *et al*., 2021; Zhang, 2021), but time-series studies with time-matched controls over different acclimation periods and developmental stages will be needed to resolve this unknown.

(b) Reduced SMR as a mechanism for hypoxic acclimation in active marine fish

While a maintained aerobic scope of hypoxia-acclimated sea bass is consistent with previous hypoxia acclimation studies for rainbow trout (Bushnell *et al*., 1984), Atlantic cod (*Gadus morhua*) (Petersen and Gamperl, 2010; 2011) and silver seabream (*Pagrus auratus* Forster, 1801) (Cook *et al*., 2013), all of which maintained $\dot{M}O_{2max}$ and aerobic scope when they were tested in normoxia, none of these previous studies measured SMR. Therefore, a reduced metabolism (a lower SMR and RMR without any apparent change in locomotory activity, differs from the inactivity in overwintering fishes, Reeve *et al*., 2022) in active marine fish after acclimation to a moderate hypoxic condition is, to the best of our knowledge, a novel finding and adds to previous reports a much larger suppression seen typically in anoxia for a limited group of extremely anoxia-tolerant fish species (*see* review by Stecyk, 2017). Nonetheless, a 19% SMR reduction in sea bass was not nearly as extreme as the up to 90% reduction seen for anoxia-tolerant fishes in very severe hypoxia (Vornanen *et al*., 2009; Thoral *et al*., 2022). While P_{crit} was not improved when measured in hypoxia, this index of hypoxic tolerance was improved after an acute transfer to (and IRAP testing) in normoxia where SMR reduction was manifested, and was subsequently retained after 4 weeks in normoxia. Yet, the reduced SMR remains as a meaningful energy saving in hypoxia because it cascaded through to a 15% reduction in RMR. Notably, body condition of hypoxia-acclimated sea bass was unaffected by a 50% reduction in oxygen

availability in the water, along with a similar liver mass (Fig. S3d) and activity of citrate synthase in both red and white skeletal muscle (Fig. 3; Fig S7) compared to the normoxia-acclimated group.

Unanswered, however, is why SMR of the hypoxia-acclimated fish tested in hypoxia (H-H) was similar to that for normoxia-acclimated fish tested in normoxia (N-N) (Fig. S4 & S5) and the metabolic suppression was only revealed by testing hypoxia-acclimated sea bass after an acute transfer to normoxia (H-N). A possible explanation, one that would be worth testing, relates to the fact that in hypoxia O_2 availability was reduced by 50%, and so the hypoxic phenotype would have had to compensate by increasing ventilation volume, which would present an increased O_2 cost of ventilation. Improvements to O_2 extraction at the gills through expansion of gill blood vessels, an increase in cardiac output and gill blood flow, lamellar recruitment, increased barrier permeability and greater Hb-O_2 affinity are all possible contributing mechanisms to improve O_2 transfer at the gills, but ventilation volume would still have to increase for the halving of water O_2 content given the efficiency of oxygen exchange in normoxia. A review of the ventilatory response of 34 teleost species to acute hypoxia found that approximately halving the ambient water O_2 content, as in the present study, produced at least a 100% compensatory increase in ventilation volume for the majority of species (*see* Table S2; Perry *et al*., 2009). Even the exceptions (*i.e.,* tuna species, dourado and plaice) had a 45–75% compensatory increase in ventilation volume (Table S2). Consequently, if ventilation costs 10–15% of RMR for normoxic, resting rainbow trout (Farrell and Steffensen, 1987), a doubling of this for the hypoxic sea bass phenotype in hypoxia might double the energy cost of ventilation. In this case, the observed reduction in SMR of the hypoxic phenotype would largely offset this increase in routine energy expenditure. While this quantitative matching could be an association

rather than a causation, we did not observe the well-documented increase in restlessness associated with an acute hypoxic exposure (*e.g.* Steffensen *et al*., 1982; van Raaij *et al*., 1996) because neither RMR nor its variability increased in hypoxia-acclimated sea bass tested in hypoxia.

How widespread a modest reduction in SMR is a strategy used by active marine fishes to acclimate to a challenging hypoxic environment is unclear until we have a better understanding of the specific mechanisms (*see* review by Hochachka *et al*., 1996). For example, we know that SMR is stable for up to 18 weeks under normoxic conditions (Zhang *et al*., 2019; Polinski *et al*., 2021; Zhang, 2021), we cannot exclude the possibility of the seasonal effects on SMR. Furthermore, if suppression of protein turnover was the mechanism to reduce SMR, protein turnover would need to be halved to quantitatively account for our observed 19% decrease in SMR given that protein turnover accounts for about 30–40% of SMR (Houlihan *et al.*, 1988; Houlihan *et al*., 1992; Carter *et al*., 1993). While suppression of protein synthesis occurs with acute hypoxia exposure in cichlids (*Astronotus ocellatus* Agassiz, 1831) (Cassidy *et al*., 2018), Arctic char (*Salvelinus alpinus* Linnaeus, 1758) (Cassidy and Lamarre; 2019), and jumbo squid (*Dosidicus gigas* d'Orbigny, 1835) (Seibel *et al*., 2014), which are all hypoxia-sensitive organisms, no acclimation studies besides the present study have shown a similar response. What is also clear from the present study is that a reduction of SMR, while a key response to hypoxic acclimation in sea bass, was not carried over on their return to normoxia.

Conclusion

Prolonged encounters with environmental stressors such as hypoxia can substantially change the respiratory phenotype of fish. Indeed, we characterized how European sea bass, an athletic

marine fish that naturally exploits both hypoxic and normoxic habitats, remodeled its respiratory phenotype during hypoxia acclimation (a reduced minimum maintenance metabolism, better hypoxia performance, enhanced aerobic performance and capacity). Moreover, after hypoxia-acclimated sea bass were returned to normoxia, a different normoxic phenotype was still evident after 4 weeks in normoxia, one that displayed a better hypoxia performance and enhanced aerobic performance and scope compared with the original normoxic phenotype. Given these findings for sea bass, greater attention should be given the cyclic nature of the ambient environment (both short-term and long-term), especially in view of the scarcity of studies on phenotype reversibility (*see* review by Burggren, 2020).

Ethics. Fish holding and all experimental procedures were in compliance with the guidelines of current animal care rules and regulations in France (Apafis 2018040916374437).

Acknowledgements. We appreciate the staff in Ifremer (PFOM-LARN), for access to the rearing and laboratory facilities and for husbandry supports.

Author contribution. Y.Z., A.P.F., G.C. conceived and designed the experiments. Y.Z. conducted the respirometry experiment, data analysis and drafted the manuscript. F.M. assisted with the respirometry experiments and respirometry data acquisition. L.P., K.A. analyzed molecular samples. H.O., F.L., FM. greatly contribute to all aspects of the project. A.P.F., G.C., and K.A. collaborated in editing the manuscript. All authors read, contributed to, and approved the final manuscript.

Competing Interest. The authors declare that they have no competing interests.

Funding. This work was funded by an NSERC Discovery Grant held by A.P.F., who also holds a Canada Research Chair Tier I. Y.Z. held Elizabeth R Howland Fellowship, Pei-Huang Tung and Tan-Wen Tung Graduate Fellowship during the course of the study and currently holds NSERC Postdoctoral Fellowship. Logistic costs of Y.Z. were supported by Travelling Fellowships from the Company of Biologists and Mobility Grant for International Doctoral Student from Université de Bretagne Occidentale. Kone Foundation supported L.P., K.A. and Collegium of Science and Medicine supported K.A..

Data accessibility. All data generated or analysed during this study are included in this published article (and its supplementary information files).

References

523 Anttila, K., Lewis, M., Prokkola, J. M., Kanerva, M., Seppänen, E., Kolari, I., & Nikinmaa, M.
524 (2015). Warm acclimation and oxygen depletion induce species-specific responses in
525 salmonids. *Journal of Experimental Biology*, *218*(10), 1471–1477.
526 https://doi.org/10.1242/jeb.119115
527 Blanchard, T. S., Whitehead, A., Dong, Y. W., & Wright, P. A. (2019). Phenotypic flexibility in
528 respiratory traits is associated with improved aerial respiration in an amphibious fish out
529 of water. *Journal of Experimental Biology*, *222*(2), jeb186486.
530 https://doi.org/10.1242/jeb.186486
531 Brauner, C. J., & Rombough, P. J. (2012). Ontogeny and paleophysiology of the gill: New
532 insights from larval and air-breathing fish. *Respiratory Physiology & Neurobiology*,
533 *184*(3), 293–300. https://doi.org/10.1016/j.resp.2012.07.011
534 Burggren, W. W. (2020). Phenotypic Switching Resulting From Developmental Plasticity: Fixed
535 or Reversible? *Frontiers in Physiology*, *10*. https://doi.org/10.3389/fphys.2019.01634
536 Bushnell, P. G., Steffensen, J. F., & Johansen, K. (1984). Oxygen consumption and swimming
537 performance in hypoxia-acclimated rainbow trout *salmo gairdneri*. *Journal of*
538 *Experimental Biology*, *113*(1), 225–235.
539 Carnevale, C., Syme, D. A., & Gamperl, A. K. (2021). Effects of hypoxic acclimation, muscle
540 strain, and contraction frequency on nitric oxide-mediated myocardial performance in
541 steelhead trout (*Oncorhynchus mykiss*). *American Journal of Physiology-Regulatory,*
542 *Integrative and Comparative Physiology*, *320*(5), R588–R610.
543 https://doi.org/10.1152/ajpregu.00014.2020
544 Carter, C. G., Houlihan, D. F., Brechin, J., & McCarthy, I. D. (1993). The relationships between
545 protein intake and protein accretion, synthesis, and retention efficiency for individual

546 grass carp, Ctenopharyngodon idella (*Valenciennes*). *Canadian Journal of Zoology*,
547 *71*(2), 392–400. https://doi.org/10.1139/z93-055
548 Cassidy, A. A., Driedzic, W. R., Campos, D., Heinrichs-Caldas, W., Almeida-Val, V. M. F., Val,
549 A. L., & Lamarre, S. G. (2018). Protein synthesis is lowered by 4EBP1 and eIF2-α
550 signaling while protein degradation may be maintained in fasting, hypoxic Amazonian
551 cichlids *Astronotus ocellatus*. *Journal of Experimental Biology*, *221*(2).
552 https://doi.org/10.1242/jeb.167601
553 Cassidy, A. A., & Lamarre, S. G. (2019). Activation of oxygen-responsive pathways is
554 associated with altered protein metabolism in Arctic char exposed to hypoxia. *Journal of*
555 *Experimental Biology*, *222*(22). https://doi.org/10.1242/jeb.203901
556 Childress, J. J., & Somero, G. N. (1979). Depth-related enzymic activities in muscle, brain and
557 heart of deep-living pelagic marine teleosts. *Marine Biology*, *52*(3), 273–283.
558 https://doi.org/10.1007/BF00398141
559 Clack, J. A. (2007). Devonian climate change, breathing, and the origin of the tetrapod stem
560 group. *Integrative and Comparative Biology*, *47*(4), 510–523.
561 https://doi.org/10.1093/icb/icm055
562 Claireaux, G., & Lagardère, J.-P. (1999). Influence of temperature, oxygen and salinity on the
563 metabolism of the European sea bass. *Journal of Sea Research*, *42*(2), 157–168.
564 https://doi.org/10.1016/S1385-1101(99)00019-2
565 Claireaux, G., Théron, M., Prineau, M., Dussauze, M., Merlin, F.-X., & Le Floch, S. (2013).
566 Effects of oil exposure and dispersant use upon environmental adaptation performance
567 and fitness in the European sea bass, *Dicentrarchus labrax*. *Aquatic Toxicology*

(Amsterdam, Netherlands), *130–131*, 160–170. https://doi.org/10.1016/j.aquatox.2013.01.004

Claireaux, G., Webber, D. M., Lagardère, J.-P., & Kerr, S. R. (2000). Influence of water temperature and oxygenation on the aerobic metabolic scope of Atlantic cod (*Gadus morhua*). *Journal of Sea Research*, *44*(3), 257–265. https://doi.org/10.1016/S1385-1101(00)00053-8

Clark, T. D., Eliason, E. J., Sandblom, E., Hinch, S. G., & Farrell, A. P. (2008). Calibration of a hand-held haemoglobin analyser for use on fish blood. *Journal of Fish Biology*, *73*(10), 2587–2595. https://doi.org/10.1111/j.1095-8649.2008.02109.x

Cook, D. G., Iftikar, F. I., Baker, D. W., Hickey, A. J. R., & Herbert, N. A. (2013). Low-O2 acclimation shifts the hypoxia avoidance behaviour of snapper (*Pagrus auratus*) with only subtle changes in aerobic and anaerobic function. *Journal of Experimental Biology*, *216*(3), 369–378. https://doi.org/10.1242/jeb.073023

Dalziel, A. C., Ou, M., & Schulte, P. M. (2012). Mechanisms underlying parallel reductions in aerobic capacity in non-migratory threespine stickleback (*Gasterosteus aculeatus*) populations. *Journal of Experimental Biology*, *215*(5), 746–759. https://doi.org/10.1242/jeb.065425

Deutsch, C., Ferrel, A., Seibel, B., Pörtner, H.-O., & Huey, R. B. (2015). Ecophysiology. Climate change tightens a metabolic constraint on marine habitats. *Science (New York, N.Y.)*, *348*(6239), 1132–1135. https://doi.org/10.1126/science.aaa1605

Dong, Y., Blanchard, T. S., Noll, A., Vasquez, P., Schmitz, J., Kelly, S. P., Wright, P. A., & Whitehead, A. (2021). Genomic and physiological mechanisms underlying skin plasticity

590 during water to air transition in an amphibious fish. *Journal of Experimental Biology*,
591 *224*(2), jeb235515. https://doi.org/10.1242/jeb.235515
592 Douglas, E. L., Friedl, W. A., & Pickwell, G. V. (1976). Fishes in oxygen-minimum zones:
593 blood oxygenation characteristics. *Science*, *191*(4230), 957–959.
594 https://doi.org/10.1126/science.1251208
595 Drinkwater, K. F., Belgrano, A., Borja, A., Conversi, A., Edwards, M., Greene, C. H., Ottersen,
596 G., Pershing, A. J., & Walker, H. (2003). The Response of marine ecosystems to climate
597 variability associated with the north atlantic oscillation. In *The North Atlantic*
598 *Oscillation: Climatic Significance and Environmental Impact* (pp. 211–234). American
599 Geophysical Union (AGU). https://doi.org/10.1029/134GM10
600 Fangue, N. A., Mandic, M., Richards, J. G., & Schulte, P. M. (2008). Swimming performance
601 and energetics as a function of temperature in killifish *Fundulus heteroclitus.*
602 *Physiological and Biochemical Zoology: Ecological and Evolutionary Approaches*,
603 *81*(4), 389–401. https://doi.org/10.1086/589109
604 Fangue, N. A., Podrabsky, J. E., Crawshaw, L. I., & Schulte, P. M. (2009). Countergradient
605 variation in temperature preference in populations of killifish *Fundulus heteroclitus*.
606 *Physiological and Biochemical Zoology: Ecological and Evolutionary Approaches*,
607 *82*(6), 776–786. https://doi.org/10.1086/606030
608 Farrell, A. P. (2007). Tribute to P. L. Lutz: A message from the heart--why hypoxic bradycardia
609 in fishes? *The Journal of Experimental Biology*, *210*(Pt 10), 1715–1725.
610 https://doi.org/10.1242/jeb.02781
611 Farrell, A. P. (2016). Pragmatic perspective on aerobic scope: Peaking, plummeting, pejus and
612 apportioning. *Journal of Fish Biology*, *88*(1), 322–343. https://doi.org/10.1111/jfb.12789

Farrell, A. P., & Steffensen, J. F. (1987). An analysis of the energetic cost of the branchial and cardiac pumps during sustained swimming in trout. *Fish Physiology and Biochemistry*, *4*(2), 73–79. https://doi.org/10.1007/BF02044316

Fry, F. E. J. (1971). The effect of environmental factors on the physiology of fish. *Fish Physiology*, 1–98.

Gamperl, A. K., & Driedzic, W. R. (2009). Chapter 7 Cardiovascular function and cardiac metabolism. In J. G. Richards, A. P. Farrell, & C. J. Brauner (Eds.), *Fish Physiology* (Vol. 27, pp. 301–360). Academic Press. https://doi.org/10.1016/S1546-5098(08)00007-1

Gamperl, A. K., Todgham, A. E., Parkhouse, W. S., Dill, R., & Farrell, A. P. (2001a). Recovery of trout myocardial function following anoxia: Preconditioning in a non-mammalian model. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *281*(6), R1755–R1763. https://doi.org/10.1152/ajpregu.2001.281.6.R1755

Gamperl, A. K., Todgham, A. E., Parkhouse, W. S., Dill, R., & Farrell, A. P. (2001b). Recovery of trout myocardial function following anoxia: Preconditioning in a non-mammalian model. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*, *281*(6), R1755-1763. https://doi.org/10.1152/ajpregu.2001.281.6.R1755

Gilbert, M. J. H., Middleton, E. K., Kanayok, K., Harris, L. N., Moore, J.-S., Farrell, A. P., & Speers-Roesch, B. (2022). Rapid cardiac thermal acclimation in wild anadromous Arctic char (*Salvelinus alpinus*). *Journal of Experimental Biology*, jeb.244055. https://doi.org/10.1242/jeb.244055

Gillis, T. E., & Johnston, E. F. (2017). 3—Cardiac preconditioning, remodeling and regeneration. In A. K. Gamperl, T. E. Gillis, A. P. Farrell, & C. J. Brauner (Eds.), *Fish*

635 *Physiology* (Vol. 36, pp. 185–233). Academic Press.
636 https://doi.org/10.1016/bs.fp.2017.09.004
637 GjØsaeter, J. (1984). Mesopelagic fish, a large potential resource in the Arabian Sea. *Deep Sea*
638 *Research Part A. Oceanographic Research Papers*, *31*(6), 1019–1035.
639 https://doi.org/10.1016/0198-0149(84)90054-2
640 Graham, J. B., Aguilar, N. M., Dudley, R., & Gans, C. (1995). Implications of the late
641 Palaeozoic oxygen pulse for physiology and evolution. *Nature*, *375*(6527), Article 6527.
642 https://doi.org/10.1038/375117a0
643 Hochachka, P. W. (1986). Defense strategies against hypoxia and hypothermia. *Science*,
644 *231*(4735), 234–241. https://doi.org/10.1126/science.2417316
645 Hochachka, P. W., Buck, L. T., Doll, C. J., & Land, S. C. (1996). Unifying theory of hypoxia
646 tolerance: Molecular/metabolic defense and rescue mechanisms for surviving oxygen
647 lack. *Proceedings of the National Academy of Sciences of the United States of America*,
648 *93*(18), 9493–9498.
649 Houlihan, D. F., Hall, S. J., Gray, C., & Noble, B. S. (1988). Growth rates and protein turnover
650 in Atlantic cod, *Gadus morhua*. *Canadian Journal of Fisheries and Aquatic Sciences*,
651 *45*(6), 951–964. https://doi.org/10.1139/f88-117
652 Houlihan, D. F., Wieser, W., Foster, A., & Brechin, J. (1992). In vivo protein synthesis rates in
653 larval nase (*Chondrostoma nasus* L.). *Canadian Journal of Zoology*, *70*(12), 2436–2440.
654 https://doi.org/10.1139/z92-327
655 Kawabata, K., Osada, M., Netticadan, T., & Dhalla, N. S. (1998). Beneficial effect of ischemic
656 preconditioning on Ca2+ paradox in the rat heart. *Life Sciences*, *63*(8), 685–692.
657 https://doi.org/10.1016/s0024-3205(98)00319-1

Lefrancois, C., & Claireaux, G. (2003). Influence of ambient oxygenation and temperature on metabolic scope and scope for heart rate in the common sole *Solea solea*. *Marine Ecology Progress Series*, *259*, 273–284. https://doi.org/10.3354/meps259273

MacKenzie, B. R., & Mariani, P. (2012). Spawning of bluefin tuna in the black sea: historical evidence, environmental constraints and population plasticity. *PLOS ONE*, *7*(7), e39998. https://doi.org/10.1371/journal.pone.0039998

McKean, T., & Mendenhall, W. (1996). Comparison of the responses to hypoxia, ischaemia and ischaemic preconditioning in wild marmot and laboratory rabbit hearts. *Journal of Experimental Biology*, *199*(3), 693–697.

McKenzie, D. J., Wong, S., Randall, D. J., Egginton, S., Taylor, E. W., & Farrell, A. P. (2004). The effects of sustained exercise and hypoxia upon oxygen tensions in the red muscle of rainbow trout. *The Journal of Experimental Biology*, *207*(Pt 21), 3629–3637. https://doi.org/10.1242/jeb.01199

Milligan, C. L. (1996). Metabolic recovery from exhaustive exercise in rainbow trout. *Comparative Biochemistry and Physiology Part A: Physiology*, *113*(1), 51–60. https://doi.org/10.1016/0300-9629(95)02060-8

Montgomery, D. W., Simpson, S. D., Engelhard, G. H., Birchenough, S. N. R., & Wilson, R. W. (2019). Rising CO2 enhances hypoxia tolerance in a marine fish. *Scientific Reports*, *9*(1), 15152. https://doi.org/10.1038/s41598-019-51572-4

Morash, A. J., Neufeld, C., MacCormack, T. J., & Currie, S. (2018). The importance of incorporating natural thermal variation when evaluating physiological performance in wild species. *Journal of Experimental Biology*, *221*(14). https://doi.org/10.1242/jeb.164673

Mueller, C. A., & Seymour, R. S. (2011). The regulation index: A new method for assessing the relationship between oxygen consumption and environmental oxygen. *Physiological and Biochemical Zoology*, *84*(5), 522–532. https://doi.org/10.1086/661953

O'Connor, C. M., Norris, D. R., Crossin, G. T., & Cooke, S. J. (2014). Biological carryover effects: Linking common concepts and mechanisms in ecology and evolution. *Ecosphere*, *5*(3), art28. https://doi.org/10.1890/ES13-00388.1

Perry, S. F., Jonz, M. G., & Gilmour, K. M. (2009). Chapter 5 Oxygen Sensing And The Hypoxic Ventilatory Response. In J. G. Richards, A. P. Farrell, & C. J. Brauner (Eds.), *Fish Physiology* (Vol. 27, pp. 193–253). Academic Press. https://doi.org/10.1016/S1546-5098(08)00005-8

Petersen, L. H., & Gamperl, A. K. (2010a). Effect of acute and chronic hypoxia on the swimming performance, metabolic capacity and cardiac function of Atlantic cod (*Gadus morhua*). *Journal of Experimental Biology*, *213*(5), 808–819. https://doi.org/10.1242/jeb.033746

Petersen, L. H., & Gamperl, A. K. (2010b). Effect of acute and chronic hypoxia on the swimming performance, metabolic capacity and cardiac function of Atlantic cod (*Gadus morhua*). *Journal of Experimental Biology*, *213*(5), 808–819. https://doi.org/10.1242/jeb.033746

Petersen, L. H., & Gamperl, A. K. (2011a). Cod (*Gadus morhua*) Cardiorespiratory physiology and hypoxia tolerance following acclimation to low-oxygen conditions. *Physiological and Biochemical Zoology*, *84*(1), 18–31. https://doi.org/10.1086/657286

Petersen, L. H., & Gamperl, A. K. (2011b). Cod (*Gadus morhua*) cardiorespiratory physiology and hypoxia tolerance following acclimation to low-oxygen conditions. *Physiological and Biochemical Zoology: PBZ*, *84*(1), 18–31. https://doi.org/10.1086/657286

Pichavant, K., Maxime, V., Soulier, P., Boeuf, G., & Nonnotte, G. (2003). A comparative study of blood oxygen transport in turbot and sea bass: Effect of chronic hypoxia. *Journal of Fish Biology*, *62*(4), 928–937. https://doi.org/10.1046/j.1095-8649.2003.00089.x

Pickett, G. D. (1994). *Sea bass: Biology, exploitation, and conservation /* (1st ed.). Chapman & Hall,.

Polinski, M. P., Zhang, Y., Morrison, P. R., Marty, G. D., Brauner, C. J., Farrell, A. P., & Garver, K. A. (2021). Innate antiviral defense demonstrates high energetic efficiency in a bony fish. *BMC Biology*, *19*(1), 138. https://doi.org/10.1186/s12915-021-01069-2

Randall, D. (1982). The control of respiration and circulation in fish during exercise and hypoxia. *Journal of Experimental Biology*, *100*(1), 275–288.

Randall, D. J., & Shelton, G. (1963). The effects of changes in environmental gas concentrations on the breathing and heart rate of a teleost fish. *Comparative Biochemistry and Physiology*, *9*(3), 229–239. https://doi.org/10.1016/0010-406X(63)90046-X

Reeve, C., Rowsey, L. E., & Speers-Roesch, B. (2022). Inactivity and the passive slowing effect of cold on resting metabolism as the primary drivers of energy savings in overwintering fishes. *Journal of Experimental Biology*, *225*(8), jeb243407. https://doi.org/10.1242/jeb.243407

Richards, J. G. (2009). Chapter 10 Metabolic and molecular responses of fish to hypoxia. In J. G. Richards, A. P. Farrell, & C. J. Brauner (Eds.), *Fish Physiology* (Vol. 27, pp. 443–485). Academic Press. https://doi.org/10.1016/S1546-5098(08)00010-1

Seibel, B. A., Andres, A., Birk, M. A., Burns, A. L., Shaw, C. T., Timpe, A. W., & Welsh, C. J. (2021). Oxygen supply capacity breathes new life into critical oxygen partial pressure (Pcrit). *Journal of Experimental Biology*, *224*(8). https://doi.org/10.1242/jeb.242210

Seibel, B. A., & Deutsch, C. (2020). Oxygen supply capacity in animals evolves to meet maximum demand at the current oxygen partial pressure regardless of size or temperature. *Journal of Experimental Biology*, *223*(12). https://doi.org/10.1242/jeb.210492

Seibel, B. A., Häfker, N. S., Trübenbach, K., Zhang, J., Tessier, S. N., Pörtner, H.-O., Rosa, R., & Storey, K. B. (2014). Metabolic suppression during protracted exposure to hypoxia in the jumbo squid, Dosidicus gigas, living in an oxygen minimum zone. *Journal of Experimental Biology*, *217*(14), 2555–2568. https://doi.org/10.1242/jeb.100487

Sollid, J., Angelis, P. D., Gundersen, K., & Nilsson, G. E. (2003). Hypoxia induces adaptive and reversible gross morphological changes in crucian carp gills. *Journal of Experimental Biology*, *206*(20), 3667–3673. https://doi.org/10.1242/jeb.00594

Somero, G. N. (2002). Thermal physiology and vertical zonation of intertidal animals: optima, limits, and costs of living. *Integrative and Comparative Biology*, *42*(4), 780–789. https://doi.org/10.1093/icb/42.4.780

Stecyk, J. A. W. (2017). 5—Cardiovascular responses to limiting oxygen levels. In A. K. Gamperl, T. E. Gillis, A. P. Farrell, & C. J. Brauner (Eds.), *Fish Physiology* (Vol. 36, pp. 299–371). Academic Press. https://doi.org/10.1016/bs.fp.2017.09.005

Steffensen, J. F. (1989). Some errors in respirometry of aquatic breathers: How to avoid and correct for them. *Fish Physiology and Biochemistry*, *6*(1), 49–59. https://doi.org/10.1007/BF02995809

748 Sutcliffe, R. L., Li, S., Gilbert, M. J. H., Schulte, P. M., Miller, K. M., & Farrell, A. P. (2020). A
749 rapid intrinsic heart rate resetting response with thermal acclimation in rainbow trout,
750 Oncorhynchus mykiss. *Journal of Experimental Biology*, *223*(12), jeb215210.
751 https://doi.org/10.1242/jeb.215210
752 Svendsen, M. B. S., Bushnell, P. G., & Steffensen, J. F. (2016). Design and setup of intermittent-
753 flow respirometry system for aquatic organisms. *Journal of Fish Biology*, *88*(1), 26–50.
754 https://doi.org/10.1111/jfb.12797
755 Svendsen, M. B. S., Bushnell, P., & Steffensen, J. F. (2019). AquaResp 3. Zenodo.
756 https://doi.org/10.5281/zenodo.2584015
757 Thetmeyer, H., Waller, U., Black, K. D., Inselmann, S., & Rosenthal, H. (1999). Growth of
758 European sea bass (*Dicentrarchus labrax* L.) under hypoxic and oscillating oxygen
759 conditions. *Aquaculture*, *174*(3), 355–367. https://doi.org/10.1016/S0044-
760 8486(99)00028-9
761 Thoral, E., Farhat, E., Roussel, D., Cheng, H., Guillard, L., Pamenter, M. E., Weber, J.-M., &
762 Teulier, L. (2022). Different patterns of chronic hypoxia lead to hierarchical adaptive
763 mechanisms in goldfish metabolism. *Journal of Experimental Biology*, *225*(1),
764 jeb243194. https://doi.org/10.1242/jeb.243194
765 Trübenbach, K., Pegado, M. R., Seibel, B. A., & Rosa, R. (2013). Ventilation rates and activity
766 levels of juvenile jumbo squid under metabolic suppression in the oxygen minimum
767 zone. *Journal of Experimental Biology*, *216*(3), 359–368.
768 https://doi.org/10.1242/jeb.072587
769 Vornanen, M., Stecyk, J. A. W., & Nilsson, G. E. (2009). Chapter 9 The Anoxia-Tolerant
770 Crucian Carp (*Carassius Carassius* L.). In J. G. Richards, A. P. Farrell, & C. J. Brauner

(Eds.), *Fish Physiology* (Vol. 27, pp. 397–441). Academic Press. https://doi.org/10.1016/S1546-5098(08)00009-5

Wang, T., Lefevre, S., Thanh Huong, D. T., Cong, N. van, & Bayley, M. (2009). Chapter 8 The effects of hypoxia on growth and digestion. In J. G. Richards, A. P. Farrell, & C. J. Brauner (Eds.), *Fish Physiology* (Vol. 27, pp. 361–396). Academic Press. https://doi.org/10.1016/S1546-5098(08)00008-3

Weber, R. E., & Jensen, F. B. (1988). Functional adaptations in hemoglobins from ectothermic vertebrates. *Annual Review of Physiology*, *50*(1), 161–179. https://doi.org/10.1146/annurev.ph.50.030188.001113

Weber, R. E., & Lykkeboe, G. (1978). Respiratory adaptations in carp blood influences of hypoxia, red cell organic phosphates, divalent cations and CO2 on hemoglobin-oxygen affinity. *Journal of Comparative Physiology*, *128*(2), 127–137. https://doi.org/10.1007/BF00689476

Weber, R. E., Wood, S. C., & Lomholt, J. P. (1976). Temperature acclimation and oxygen-binding properties of blood and multiple haemoglobins of rainbow trout. *Journal of Experimental Biology*, *65*(2), 333–345.

Yang, Y., Wang, Z., Wang, J., Lyu, F., Xu, K., & Mu, W. (2021). Histopathological, hematological, and biochemical changes in high-latitude fish *Phoxinus lagowskii* exposed to hypoxia. *Fish Physiology and Biochemistry*, *47*(4), 919–938. https://doi.org/10.1007/s10695-021-00947-4

Zhang, Y. (2021). Interpreting species, intraspecific and intra-individual variability by comprehensively characterizing a fish's respiratory phenotype with valid measures of oxygen uptake [University of British Columbia]. https://doi.org/10.14288/1.0396683

794 Zhang, Y., Claireaux, G., Takle, H., Jørgensen, S. M., & Farrell, A. P. (2018). A three-phase
795 excess post-exercise oxygen consumption in Atlantic salmon *Salmo salar* and its
796 response to exercise training. *Journal of Fish Biology*. https://doi.org/10.1111/jfb.13593
797 Zhang, Y., & Gilbert, M. J. H. (2017). Comment on 'Measurement and relevance of maximum
798 metabolic rate in fishes by Norin & Clark (2016).' *Journal of Fish Biology*, *91*(2), 397–
799 402. https://doi.org/10.1111/jfb.13291
800 Zhang, Y., Gilbert, M. J. H., & Farrell, A. P. (2019). Finding the peak of dynamic oxygen uptake
801 during fatiguing exercise in fish. *Journal of Experimental Biology*, jeb.196568.
802 https://doi.org/10.1242/jeb.196568
803 Zhang, Y., Gilbert, M. J. H., & Farrell, A. P. (2020). Measuring maximum oxygen uptake with
804 an incremental swimming test and by chasing rainbow trout to exhaustion inside a
805 respirometry chamber yields the same results. *Journal of Fish Biology*, *97*(1), 28–38.
806 https://doi.org/10.1111/jfb.14311
807 Zhang, Y., Healy, T. M., Vandersteen, W., Schulte, P. M., & Farrell, A. P. (2018). A rainbow
808 trout *Oncorhynchus mykiss* strain with higher aerobic scope in normoxia also has superior
809 tolerance of hypoxia. *Journal of Fish Biology*, *92*(2), 487–503.
810 https://doi.org/10.1111/jfb.13530
811 Zhang, Y., Mauduit, F., Farrell, A. P., Chabot, D., Ollivier, H., Rio-Cabello, A., Le Floch, S., &
812 Claireaux, G. (2017). Exposure of European sea bass (*Dicentrarchus labrax*) to
813 chemically dispersed oil has a chronic residual effect on hypoxia tolerance but not
814 aerobic scope. *Aquatic Toxicology (Amsterdam, Netherlands)*, *191*, 95–104.
815 https://doi.org/10.1016/j.aquatox.2017.07.020

816 Zhang, Y., Montgomery, D. W., White, C. F., Richards, J. G., Brauner, C. J., & Farrell, A. P.
817 (2022). Characterizing the hypoxic performance of a fish using a new metric: PAAS-50.
818 *Journal of Experimental Biology*, jeb.244239. https://doi.org/10.1242/jeb.244239
819 Zhang, Y., Polinski, M., Morrison, P., Brauner, C., Farrell, A., & Garver, K. (2019a). High-load
820 reovirus infections do not imply physiological impairment in salmon. *Frontiers in*
821 *Physiology*, *10*. https://doi.org/10.3389/fphys.2019.00114
822 Zhang, Y., Polinski, M. P., Morrison, P. R., Brauner, C. J., Farrell, A. P., & Garver, K. A.
823 (2019b). High-load reovirus infections do not imply physiological impairment in salmon.
824 *Frontiers in Physiology*, *0*. https://doi.org/10.3389/fphys.2019.00114
825 Zhang, Y., So, B. E., & Farrell, A. P. (2021). Hypoxia performance curve: assess a whole-
826 organism metabolic shift from a maximum aerobic capacity towards a glycolytic capacity
827 in fish. *Metabolites*, *11*(7), Article 7. https://doi.org/10.3390/metabo11070447
828 Zhang, Y., Timmerhaus, G., Anttila, K., Mauduit, F., Jørgensen, S. M., Kristensen, T., Claireaux,
829 G., Takle, H., & Farrell, A. P. (2016). Domestication compromises athleticism and
830 respiratory plasticity in response to aerobic exercise training in Atlantic salmon (*Salmo*
831 *salar*). *Aquaculture*, *463*(Supplement C), 79–88.
832 https://doi.org/10.1016/j.aquaculture.2016.05.015
833

Figure Captions

Fig. 1. A theoretical framework for phenotypic plasticity (*i.e.* within a generation) for hypoxic acclimation and a return to ambient normoxia, *i.e.*, environmental changes. (a) Even with acclimation, which may take some time, the performance of a hypoxic phenotype may be constrained by the limiting ambient environmental factor, *i.e.,* hypoxic water at 10.4 kPa (50 % air saturation) (panel b). Also, when the hypoxia-acclimated animal is returned to normoxia, the normoxic phenotype might revert to the original normoxic phenotype, or another normoxic phenotype might emerge with either a compromised or even an enhanced performance in normoxia. The present study investigated the respiratory plasticity of a marine fish species, the European sea bass (*Dicentrarchus labrax*).

Fig. 2. The respiratory phenotype in juvenile European sea bass (*Dicentrarchus labrax*) at 16 ˚C based on individual oxygen uptake ($\dot{M}O_2$) measurements. The three test groups were normoxia-acclimated fish tested in normoxia (N-N; grey), hypoxia-acclimated fish tested in normoxia (H-N; orange), and hypoxia-acclimated fish re-acclimated to and tested in normoxia (HN-N; green). (a) Continuous $\dot{M}O_2$ traces from representative individuals for the three treatment groups over the first 40 h of IRAP. The individual's standard metabolic rate (SMR; solid horizontal lines) and maximum oxygen uptake ($\dot{M}O_{2max}$; dotted horizontal lines) are provided for reference. White-&-grey segments indicate average summer diel cycles in western France (~15 L:9 D). (b) Continuous mean $\dot{M}O_2$ traces (solid line) ± s.e.m (shaded area) for all individuals in each of the three test groups over the first 40 h of IRAP. Panels (c) to (h) summarize mean values for five key aerobic respiratory indices derived from $\dot{M}O_2$: (c) SMR, (d) routine metabolic rate (RMR), (e) the variance of RMR, (f) $\dot{M}O_{2max}$, and (g) absolute aerobic scope (AAS = $\dot{M}O_{2max}$ – SMR). Phenotypic plasticity associated with hypoxic acclimation is indicated by statistically significant differences between N-N (grey) and H-N (orange) test groups. Comparison of N-N (grey) and HN-N (green) reveals the new normoxic phenotype that results from a prior hypoxic acclimation. The boxplots indicate the bar as the 25-75 percentile, the whiskers as the 10-90 percentile, the line as the median and

'+' as the mean (n = 13–28). Different letters denote a statistical significance (ANCOVA with Holm-Šídák *post-hoc* tests, $\alpha < 0.05$). No mathematical or statistical transformations are applied to the data presented.

Fig. 3. Effects of a 6-week hypoxic acclimation on the maximal activity of citrate synthase (CS) in red and white muscles of juvenile European sea bass (*Dicentrarchus labrax*) at 16 ˚C. Phenotypic plasticity associated with hypoxic acclimation is indicated by statistically significant differences between normoxia-acclimated fish (N; grey) with hypoxia-acclimated (H; orange) test groups. A comparison of the normoxia-acclimated (grey) and the hypoxia-acclimated re-acclimated to normoxia (HN; green) reveals the new normoxic phenotype that results from a prior hypoxic acclimation. The boxplots indicate the bar as the 25-75 percentile, the whiskers as the 10-90 percentile, the line as the median and '+' as the mean (n = 13–23). Different letters denote a statistical significance (one-way ANOVA with Tukey-Kramer *post-hoc* tests, $\alpha < 0.05$). No mathematical or statistical transformations are applied to the data presented.

Fig. 4. The respiratory phenotype in juvenile European sea bass (*Dicentrarchus labrax*) at 16 ˚C based on individual oxygen uptake ($\dot{M}O_2$) measurements. Panel (a, critical oxygen partial pressure, P_{crit}) is the PO_2 level at which SMR could no longer be maintained, (b) incipient lethal oxygen partial pressure (ILOP). Panels (c) & (d) summarize mean values for four key indices of glycolytic capacity derived from $\dot{M}O_2$ [(c) scope for oxygen deficit (SOD) and (d) excess post-exercise oxygen consumption (EPOC)]. The three test groups were normoxia-acclimated fish tested in normoxia (N-N; grey), hypoxia-acclimated fish tested in normoxia (H-N; orange), and hypoxia-acclimated fish re-acclimated to and tested in normoxia (HN-N; green). Phenotypic plasticity associated with hypoxic acclimation is indicated by statistically significant differences between N-N (grey) and H-N (orange) test groups. A comparison of N-N (grey) and HN-N (green) reveals the new normoxic phenotype that results from a prior hypoxic acclimation. The boxplots indicate the bar as the 25-75 percentile, the whiskers as the 10-90 percentile, the line as the median and '+' as the mean (n = 13–28). Different letters denote statistical significance (one-way ANCOVA with

887 Holm-Šídák *post-hoc* tests, $\alpha < 0.05$). No mathematical or statistical transformations are applied to the
888 data presented.
889
890
891 Fig. 5. Hypoxic performance curves of normalized oxygen uptake ($\dot{M}O_2$) for normoxia- and hypoxia-
892 acclimated juvenile European sea bass (*Dicentrarchus labrax*) at 16 ˚C. The data were taken from
893 individual-based hypoxic performance curves where $\dot{M}O_2$ were normalized as a percentage of each
894 individual's absolute aerobic scope (% AAS). (a) Mean % AAS (dots) ± s.e.m (error bars) across a range
895 of partial pressure of O_2 (PO_2, kPa) were modeled using one-phase association equations [normoxic
896 phenotype: $y = -39.5 + (130.0 + 39.5) \times [1-e^{(-0.005 \times x)}]$; $R^2 = 0.88$, AIC=339.8; hypoxic phenotype: $y = -$
897 $85.8 + (88.3 + 85.8) \times [1-e^{(-0.057 \times x)}]$; $R^2 = 0.81$, AIC=412.6]. The solid curves are one-phase association
898 regression models, and the shaded areas are the 95% confidence intervals of these curves. Blue dash lines
899 graphically illustrate the comparison of mean values for the minimum O_2 partial pressure that supports
900 50% of AAS (P_{AAS-50}). (b) A statistic comparison of the interpolated P_{AAS-50} values for the hypoxic and
901 normoxic phenotype. The mean values were based on the P_{AAS-50} values interpolated from each individual
902 hypoxic performance curve. The boxplots indicate the bar as the 25-75 percentile, the whiskers as the 10-
903 90 percentile, the line as the median and '+' as the mean (n = 6–7). Different letters denote statistical
904 significance (independent sample t-test, $\alpha < 0.05$). No mathematical or statistical transformations are
905 applied to the data presented.

906