© 2024. Published by The Company of Biologists Ltd | Journal of Experimental Biology (2024) 227, jeb247618. doi:10.1242/jeb.247618

RESEARCH ARTICLE

Implications of chronic hypoxia during development in red drum

Benjamin Negrete, Jr[1,2,*], Kerri Lynn Ackerly[1] and Andrew J. Esbaugh[1]

ABSTRACT

Respiratory plasticity is a beneficial response to chronic hypoxia in fish. Red drum, a teleost that commonly experiences hypoxia in the Gulf of Mexico, have shown respiratory plasticity following sublethal hypoxia exposure as juveniles, but implications of hypoxia exposure during development are unknown. We exposed red drum embryos to hypoxia (40% air saturation) or normoxia (100% air saturation) for 3 days post fertilization (dpf). This time frame encompasses hatch and exogenous feeding. At 3 dpf, there was no difference in survival or changes in size. After the 3-day hypoxia exposure, all larvae were moved and reared in common normoxic conditions. Fish were reared for ∼3 months and effects of the developmental hypoxia exposure on swim performance and whole-animal aerobic metabolism were measured. We used a cross design wherein fish from normoxia (N=24) were exercised in swim tunnels in both hypoxia (40%, n=12) and normoxia (100%, n=12) conditions, and likewise for hypoxia-exposed fish (n=10 in each group). Oxygen consumption, critical swim speed (U_{crit}), critical oxygen threshold (P_{crit}) and mitochondrial respiration were measured. Hypoxia-exposed fish had higher aerobic scope, maximum metabolic rate, and higher liver mitochondrial efficiency relative to control fish in normoxia. Interestingly, hypoxia-exposed fish showed increased hypoxia sensitivity (higher P_{crit}) and recruited burst swimming at lower swim speeds relative to control fish. These data provide evidence that early hypoxia exposure leads to a complex response in later life.

KEY WORDS: Swim performance, Metabolic rate, Phenotypic plasticity, Respiratory physiology, Oxygen

INTRODUCTION

Hypoxia, or low oxygen (O_2), is an environmental stressor impacting marine systems and has many implications for living organisms (Breitburg et al., 2018; Sampaio et al., 2021). Generally, hypoxia is defined as ≤2 mg O_2 l^{-1} (∼30% air saturation) but can also be described at species-specific levels based on observed physiological impairment. Hypoxia imposes a significant stressor on biological processes because O_2 is the final electron acceptor in the mitochondrial electron transport chain that generates ATP. From a whole-organism perspective hypoxia constrains the ability of the respiratory system to take up oxygen from the environment and deliver it to the tissues, which generally manifests as a reduction in maximum metabolic rate (MMR; Fry, 1971). This can have a suite of downstream effects – such as reduced swim performance (Johnston et al., 2013) or digestive function (Vanderplancke et al., 2015; Jordan and Steffensen, 2007) – and has been hypothesized to contribute to species biogeographical patterns (Deutsch et al., 2015, 2020). Hypoxia can also be particularly consequential in early life as embryonic and larval fish maximize ATP generation to meet the high energy demand to support development and growth (Applebaum et al., 2014; Weltzien et al., 1999). The wide-ranging effects of hypoxia on aquatic organisms reinforces the importance of understanding the implications of hypoxic events – which are increasing in time and space (Breitburg et al., 2018) – on marine life.

Fish are often capable of making physiological adjustments to mitigate the impacts of environmental stress; a strategy known as phenotypic plasticity (Burggren, 2019; Vagner et al., 2019). One particular type of phenotypic plasticity is developmental plasticity, which is classically described as phenotypic changes in response to environmental conditions during ontogeny that become inflexible to environmental variability as juveniles or adults; although, a recent review expands this definition and provides evidence that some responses may be reversible in later life (Burggren, 2019). Vagner et al. (2019) reviewed developmental plasticity in response to environmental change and reported that ∼29% of studies between 2003 and 2019 tested hypoxic responses in developmental or transgenerational plasticity, the latter describing responses carried over across generations. Half of those studies focused on model or freshwater species, with few marine examples (Cadiz et al., 2017; Ishibashi et al., 2007; Vanderplancke et al., 2015; Zambonino-Infante et al., 2017).

Developmental plasticity in response to hypoxia can cause negative and positive responses in larvae that manifest as changes in morphology (Bagowski et al., 2011; Lofeu et al., 2021) or physiology (Bianchini and Wright, 2013; Robertson et al., 2014). Limited O_2 availability during development in fish larvae causes lower hatch success (Del Rio et al., 2019; Hassell et al., 2008; Vanderplancke et al., 2015), malformation (Hassell et al., 2008), hatch or developmental delay (Del Rio et al., 2019; Johnston et al., 2013; Polymeropoulos et al., 2016) and impacts in later life such as lowered growth rate or mortality (Cadiz et al., 2018a; Vanderplancke et al., 2015). Much like adults, developing fish combat hypoxia-induced constraints in oxygen supply by increasing ventilation (Pan et al., 2019) and altering blood flow patterns (Hughes and Perry, 2021). Furthermore, some larval fish have been shown to upregulate and maintain the expression of embryonic haemoglobins (Bianchini and Wright, 2013; Cadiz et al., 2018a). Embryonic haemoglobins generally have increased O_2 binding affinity, which presumably acts to counteract environmental O_2 limitation (Bianchini and Wright, 2013). In adults, high affinity haemoglobins have also been correlated with lower critical O_2 tension (P_{crit}; Mandic et al., 2009; Pan et al., 2017; Speers-Roesch et al., 2012) – a measure of hypoxia vulnerability. While these ventilatory and gene expression responses are likely to benefit animal performance, hypoxia has also been shown to disrupt heart development and function in developing rainbow trout (*Onchorhyncus mykiss*), which was linked to impaired later life swim performance (Johnston et al., 2013).

[1]Marine Science Institute, The University of Texas at Austin, Port Aransas, TX 78373, USA. [2]Department of Zoology, The University of British Columbia, Vancouver, BC, Canada V6T 1Z4.

*Author for correspondence (bnegrete@zoology.ubc.ca)

B.N.J., 0000-0001-6030-6122; K.L.A., 0000-0002-3111-724X; A.J.E., 0000-0002-7262-4408

Received 28 February 2024; Accepted 17 July 2024

Journal of Experimental Biology (2024) 227, jeb247618. doi:10.1242/jeb.247618

List of symbols and abbreviations

AS	aerobic scope
BL	body length
CI	complex I
CII	complex II
COT	cost of transport
COT_{crit}	cost of transport at U^{crit}
COT_{opt}	cost of transport at U^{opt}
CR	coupling ratio, the ratio of LEAK to OXPHOS
DO	dissolved oxygen
dpf	days post-fertilization
ETS	electron transport system
hpf	hours post-fertilization
LEAK	leak respiration
MMR	maximum metabolic rate
$\dot{M}_{O_2}$	mass-specific oxygen consumption rate
OXPHOS	oxidative phosphorylation
P_{crit}	critical oxygen tension for SMR
ROX	residual background O^2 respiration
SL	standard length
SMR	standard metabolic rate
TL	total length
U_{burst}	critical burst speed
U_{crit}	critical swim speed
U_{opt}	optimal swim speed
α	oxygen supply capacity
αP_{crit}	critical oxygen tension for SMR using oxygen supply capacity
ΔU	difference between U^{crit} and U^{burst}

The red drum, *Sciaenops ocellatus*, is an estuarine fish commonly found in the Gulf of Mexico and along the East coast of the USA. These fish are often found in hypoxic zones, and their spawning is associated with estuarine habitats that experience diel oxygen fluctuations. Red drum development has been well characterized, whereby they hatch at ∼1 day post fertilization (dpf), begin exogenously feeding at 3 dpf and complete flexion at ∼14 dpf (Holt et al., 1981; Holt, 1993). At 3 dpf, red drum have a reasonably developed cardiovascular system with a developed heart, heartbeat and blood circulation (our observations), which are major components of the O_2 supply cascade. Importantly, juvenile life stages have previously been shown to exhibit respiratory plasticity that reduces hypoxia vulnerability, and improves aerobic scope and swimming performance (Dichiera et al., 2022; Negrete et al., 2022; Pan et al., 2017). This occurs, in part, through differences in haemoglobin gene expression and increased haemoglobin O_2 affinity (Negrete et al., 2022; Pan et al., 2017). We predicted a similar response in developing red drum. Specifically, that red drum exposed to hypoxia as larvae would show improved respiratory performance and reduced hypoxia vulnerability in later life (i.e. lower P_{crit}). To test this, we exposed embryonic red drum to hypoxia or normoxia from ∼8 h post-fertilization (hpf) to 3 dpf, which covers two critical developmental time points: hatching (1 dpf) and first feeding (3 dpf). Animals from both treatments were then reared in normoxia for 65–70 days, after which individuals were tested for whole-animal metabolic traits (SMR, MMR and P_{crit}), swim performance and mitochondrial respiration.

MATERIALS AND METHODS

Protocols and procedures were carried out in accordance with the UT Institutional Animal Care and Use Committee (AUP-2018-00231 and AUP-2021-00204).

Embryos

Eggs from red drum [*Sciaenops ocellatus* (Linnaeus 1766)] were obtained from two sources based on availability. Eggs were collected from Texas Parks and Wildlife CCA Marine Development Center in Corpus Christi, Texas ∼8-10 hpf and transported to the University of Texas at Austin Marine Science Institute (UTMSI) in Port Aransas, TX (<40 min transport time) in a tank with constant aeration. Additionally, drum eggs were collected from red drum brood stock at the Fisheries and Mariculture lab at UTMSI in Port Aransas, TX at ∼8–10 hpf. All eggs were washed in 1% formalin seawater for 1 h. After the formalin wash, the eggs were rinsed with autoclaved seawater. The eggs were allowed to settle and floating fertilized eggs were collected.

Eggs (n=20) were randomly collected under a dissecting scope and transferred to baskets in either normoxic control or hypoxic treatment systems with three replicates per O_2 treatment (N=60 per O_2 treatment). Each system consisted of a tray, a seawater and gas mixing column, pumps, heater, and floating baskets. Autoclaved seawater (36.0±1.65 ppt, mean±s.e.m.; 28°C) was circulated from the tray to the gas mixing column that maintained either normoxia control [103±0.94% dissolved O_2 (DO), air saturation] or hypoxia treatment (43.7±8.55% DO) conditions. This was repeated for three different spawns for parental diversity. There were no statistical or qualitative differences between spawns from Texas Parks and Wildlife or UTMSI (data not shown). Water quality and O_2 was checked twice daily using a YSI probe (Yellow Springs, OH, USA). Hypoxia was induced and maintained by gently bubbling N_2 through an automated gas controller Oxy-Reg (Loligo Systems, Viborg, Denmark) which was set to the desired O_2 level. Trays were covered with a sheet of glass over the floating baskets to reduce surface O_2 mixing.

At 3 dpf, prior to first feeding, baskets were transferred to a bowl containing respective treatment water dosed with an anaesthetic of buffered MS-222 (250 mg l^{-1}; 500 mg l^{-1} $NaHCO_3$). Larvae were collected using glass pipettes and transferred to a Petri dish containing 3% methyl cellulose dissolved in isotonic saline (300 mOsm l^{-1} NaCl). Larvae were counted from each basket for survival and photographed using Nikon software. Images of surviving larvae were blinded and analysed for measurements of standard length (SL, the length from head to tip of the body). Relative SL was calculated wherein all fish were compared with the average SL of control fish.

A second group of embryos were collected from 10 spawns from UTMSI and exposed to either normoxia control or hypoxia treatment. Eggs were set in floating baskets in cone tanks (120 l) filled with seawater filtered through a 25 µm filter and UV light. All tanks contained a heater and standpipe. Control tanks contained an air-line connected to a stand pipe for maintaining normoxia (100% DO) and vertical mixing. Hypoxic cone tanks (47±1.5% DO) contained two air-lines controlled by an Oxy-Reg gas controller (Loligo Systems, Viborg, Denmark). One line was connected to an air bubbler and a second air-line connected to an N_2 tank. These hypoxic tanks contained two additional pumps: one that passed water over a DO probe attached to the Oxy-Reg to maintain hypoxia, and the other pump to the standpipe for vertical water mixing. All tanks were covered with a layer of plastic that did not lay on the water surface, but over the tank to prevent O_2 mixing.

Cone tanks were maintained at normoxia or hypoxia until 3 dpf. Water temperature (28°C), salinity (35 ppt) and DO were checked twice daily. At 3 dpf, all tanks were uncovered and in hypoxic tanks the bubbler was turned on continuously to achieve normoxia, and the gas controller turned off. All tanks were then maintained under

Journal of Experimental Biology (2024) 227, jeb247618. doi:10.1242/jeb.247618

normoxic conditions for the duration of the experiment until swim trials. All larvae at 3 dpf were fed a diet of rotifers (enriched with Algamac, Aquafauna Bio-Marine, Inc., Hawthorne, California), until 10 dpf where they transitioned to a diet of artemia, until 16 dpf then they transitioned to dry food (Otohime, Tokyo, Japan). Food was withheld for 24 h prior to swim trials.

Swim trials

Fish were raised to ∼2 g (∼65–70 dpf) to undergo swim trials. Fish of both treatments went through trials in 170 ml swim tunnel respirometers (Loligo Systems, Viborg, Denmark). We used a cross design wherein fish from both treatments were swam in either normoxic (100% DO) or hypoxic (40% DO) water. This resulted in the following sample sizes: control-reared swam in 100% (n=12, 74.7±3.11 dpf, mass: 1.98±0.12 g, total length, TL: 6.35±0.13 cm; means±s.e.m.); control-reared swam in 40% (n=12, 72.3±2.69 dpf, mass: 2.05±0.14 g, TL: 6.39±0.17); hypoxia-exposed swam in 100% (n=10, 65.2±2.42 dpf, mass: 2.39±0.15 g, TL: 6.47±0.15); and hypoxia-exposed swam in 40% (n=10, 67.1±1.86 dpf, mass: 2.32±0.13 g, TL: 6.57±0.12). Two swim tunnels were used and alternated for each trial between 40% and 100% trials to account for tunnel biases. Fish were moved from the cone tanks into the swim tunnels where they rested overnight in normoxia at 0.5 body lengths per second (BL s^{-1}). Normoxia was maintained by bubbling air, and hypoxia was maintained using the Oxy-Reg gas controller (Loligo Systems, Viborg, Denmark).

Mass-specific O_2 consumption ($\dot{M}_{O_2}$, mg O_2 kg^{-1} h^{-1}) was measured during critical swim speed (U_{crit}) trials in the swim tunnel respirometer (working section 2.64 cm diameter×10 cm length) submerged in a 20 l water bath. Fish were weighed to the nearest mg and measures of TL, SL, width and body depth in cm were taken using ImageJ. Blocking effects of the fish were calculated and corrected using the fish's cross-sectional area and the working area of the chamber according to Bell and Terhune (1970). The fractional error for fish area ranged from 1.85% to 5.21% (3.35±0.11%, mean±s.e.m.). Fish were raised to ∼2 g to minimize the fractional error at larger sizes.

Flow velocity was calibrated using green fluorescent PE microspheres (Loligo Systems, Viborg, Denmark) and ImageJ to track bead flow in increasing speeds. Swim tunnels contained an air stone and an Eheim pump controlled by a DAQ-M pump controller (Loligo Systems, Viborg, Denmark) to flush the working area with surrounding seawater (35 ppt) that was temperature controlled using a titanium heater and temperature controller (28°C). A 200 mm O_2 probe was placed in the back honeycomb of the tunnel and connected to an Oxy-4 Mini oxygen sensor (PreSens, Regensburg, Germany). Tunnels used for hypoxia trials contained an additional N_2 air stone and compact Eheim pump which flushed water over an additional O_2 probe attached to an Oxy-Reg gas controller. Hypoxia was induced gradually over 1 h before swim trials began, and maintained during swimming and recovery. Background measurements were taken prior to fish introduction into the chamber, and after the fish was removed from the chamber with motors running at 0.5 BL s^{-1} for recirculation. During background, swimming, and recovery the measurement periods were 90 s flush, 30 s wait, and 180 s measure (5 min total). During closed P_{crit} trials there was no flush or wait period and the measurement period was changed to 120 s.

Fish swam in increasing increments of 0.5 BL s^{-1} every 20 min. This resulted in 4 O_2 consumption ($\dot{M}_{O_2}$) measurements per speed interval (r^2≥0.95). Trials ended when fish could no longer maintain position in the tunnel, or fish spent more than 3 s on the back honeycomb with a bent caudal fin. The swim speed at which fish performed at least 4 burst events in a single wait/measure period was recorded. After trials ended the motor was reduced slowly to 0.5 BL s^{-1} and fish recovered for ∼1 h. During this time, most fish reached $\dot{M}_{O_2}$ levels within 20% of pre-swim trial values, with no difference between any treatment groups. This threshold was determined based on previous measures of swim trial recovery in red drum that show resting $\dot{M}_{O_2}$ varies ∼20% at a constant speed (Johansen and Esbaugh, 2017). Thus, we deduced these fish were adequately recovered from the swim trial before P_{crit} measurements. Two fish did not reach these levels and were excluded from all P_{crit} comparisons. Immediately after recovery, the chambers were closed for P_{crit} trials whereby the fish consumed the O_2 in the chamber until 5% air sat., or the fish experienced loss of equilibrium. All P_{crit} $\dot{M}_{O_2}$ measures had an oxygen decline with r^2≥0.95. Fish were removed and euthanized with an overdose of buffered MS-222 (500 mg l^{-1}; 500 mg l^{-1} $NaHCO_3$) followed by spinal transection. Hearts were dissected and weighed to the nearest mg.

Swim trial $\dot{M}_{O_2}$ were analysed starting at 1.0 BL s^{-1}, which was the speed at which fish started swimming in the chamber. The first interval at each new swim speed was disregarded for all calculations and analyses as often the fish reacted to the change in swim speed before adjustment and physical correction. This resulted in three $\dot{M}_{O_2}$ measures per swim interval. The critical swim speed, U_{crit}, was calculated using the following equation:

$$U_{crit} = U_f + dU\left(\frac{T_f}{t}\right),$$

where U_f is the penultimate swim interval the fish completed, dU is the speed interval (0.5 BL), T_f is the 5 min period the fish last completed, and t is the time interval (20 min). This same equation was used to also calculate U_{burst}, the speed at which fish begin to rapidly incorporate burst swimming. U_{burst} was calculated with the parameter U_f modified as the final speed at which a fish completed an interval with <4 burst events. The difference in U_{crit} and U_{burst} was calculated to find ΔU, or the swim interval a fish used predominantly burst swimming. Cost of transport (COT), or the energy used to move during swimming, was calculated by dividing $\dot{M}_{O_2}$ by swim speed and plotting COT against swim speed to generate a J-shaped curve. The optimal swim speed, or the speed at which COT is lowest, $U_{opt,}$ was calculated as the swim speed at the lowest point of the J-curve. The COT at U_{opt} was derived as the asymptote of the J-shaped COT curve (COT_{opt}) and the COT at U_{crit} was calculated using maximum metabolic rate, MMR, MMR/U_{crit} (COT_{crit}).

The relationship between $\dot{M}_{O_2}$ and swim speed is analysed using either exponential regression or log-transformed linear regression (Stieglitz et al., 2016). For the current study, we opted to use linear regression as it resulted in models that better fit the data (i.e. higher r^2 values). Linear regression of $\log_{10}$ transformed $\dot{M}_{O_2}$ at each swim speed was used to find metabolic rates (r^2≥0.68 for all trials; Mager et al., 2014). The $\dot{M}_{O_2}$ at U_{crit} was derived as the maximum metabolic rate (MMR), and the y-intercept (i.e. the $\dot{M}_{O_2}$ at swim speed of 0 BL s^{-1}) derived as the standard metabolic rate (SMR). Aerobic scope (AS) was calculated as the difference between MMR and SMR. Hypoxia sensitivity was assessed using the critical O_2 tension (P_{crit}), or the P_{O_2} where fish switch from oxyregulation to oxyconformation and AS is zero. The $\dot{M}_{O_2}$ from P_{crit} trials were plotted with a horizontal line to designate SMR and a linear regression was done where $\dot{M}_{O_2}$ declined linearly (r^2≥0.95) with P_{O_2}. The P_{O_2} where this regression was equal to SMR was

designated as P_{crit} (Negrete and Esbaugh, 2019). Since SMR was calculated in these trials and this method is dependent on SMR, we used an additional calculation for P_{crit} according to Seibel et al. (2021). Here, P_{crit} is defined as the P_{O_2} where physiological O_2 supply mechanisms operate at maximum capacity. Each $\dot{M}_{O_2}$ during the P_{crit} trial was taken as a function of P_{O_2} ($\dot{M}_{O_2}/P_{O_2}$) to find O_2 supply capacity (α, mg O_2 kg^{-1} h^{-1}% O_2^{-1}) which increases until it reaches a peak at P_{crit}. The three highest consecutive α were averaged, and the average P_{O_2} at α was calculated as αP_{crit}. A sample visual representation of these P_{crit} calculations is shown in Fig. 4A,B. The average $\dot{M}_{O_2}$ versus P_{O_2} data for all fish, as well as individual traces using α during P_{crit} trials for these calculations are presented in Fig. S1.

Mitochondrial respiration

Fish from control and hypoxia-exposed spawns were raised to ∼4–6 g (∼90–100 dpf) for mitochondrial respiration in cardiac and liver tissue. We opted for a larger size of fish as it was required for collection of the minimum amount of tissue for mitochondrial respiration measurements. Fish were euthanized in an overdose of buffered MS-222 (500 mg l^{-1}; 500 mg l^{-1} $NaHCO_3$) followed by spinal transection. Individuals had their hearts (ventricle) and livers immediately sampled. Tissues were gently blotted with a Kimwipe™ and 6.49±0.56 mg of ventricle or 44.1±3.31 mg of liver immediately placed in 500 µl of ice-cold respiration solution (0.5 mmol l^{-1} EGTA, 3 mmol l^{-1} $MgCl_2$, 60 mmol l^{-1} lactobionic acid, 20 mmol l^{-1} taurine, 10 mmol l^{-1} KH_2PO_4, 20 mmol l^{-1} HEPES, 110 mmol l^{-1} D-sucrose, and buffered to pH 7.0 using 5 mol l^{-1} KOH) and gently homogenized using a 7 ml glass Dounce homogenizer (Wheaton, USA). These homogenates were moved to an Oroboros Oxygraph-2k respirometer system (Oroboros Instruments, Innsbruck, Austria) in a test chamber containing respiration solution (final volume: 2.2 ml). Mass-specific mitochondrial O_2 consumption (pmol O_2 s^{-1} mg^{-1} tissue) was measured in real time at 28°C using DatLab (v.7; Oroboros Instruments, Innsbruck, Austria).

A modified substrate–uncoupler–inhibition–titration (SUIT) protocol was used similar to previous experiments with red drum (Johansen and Esbaugh, 2017). Briefly, the titration sequence was as follows: (1) 280 U ml^{-1} catalase and 3 µl 3% H_2O_2 to increase chamber O_2 to 200% air saturation to prevent O_2 limitations on mitochondria; (2) 2 mmol l^{-1} malate, 5 mmol l^{-1} pyruvate, and 10 mmol l^{-1} glutamate for complex I (CI) without ADP; (3) 1 mmol l^{-1} ADP in steps until saturation for CI oxidative phosphorylation (CI-OXPHOS); (4) 10 µmol l^{-1} cytochrome *c* to assess mitochondrial membrane integrity; (5) 10 mmol l^{-1} succinate for complex II (CII) and 1 mmol l^{-1} ADP to induce maximal respiration (OXPHOS; state 3) (Chance and Williams, 1955); (6) 5 mmol l^{-1} oligomycin to inhibit ATP synthase to measure LEAK (state 4) (Chance and Williams, 1955); (7) 0.25 µmol l^{-1} carbonyl cyanide p-(trifluoro-methoxy) phenyl-hydrazone (FCCP) in steps to measure uncoupled electron transport system (ETS) capacity; (8) 0.5 µmol l^{-1} rotenone to inhibit CI and assess contribution of CII to ETS (state 3) (Chance and Williams, 1955); (9) 2.5 µmol l^{-1} antimycin a to inhibit OXPHOS and assess residual O_2 consumption (ROX).

When trials concluded, the chambers were washed and cleaned with a 5 min DI water rinse, 20 min rinse with proofed yeast, three 5 min rinses with DI water, and finally three 5 min rinses with 70% ethanol. Mitochondrial performance measures reported here are ROX-corrected respiration values shown as mass-specific O_2 consumption, capacity measures, and control ratios. To assess aspects of oxidative phosphorylation in addition to raw respiration rates, these data were used to calculate the following measures: respiratory control ratio (OXPHOS/LEAK; to assess OXPHOS relative to LEAK); OXPHOS control efficiency [(OXPHOS–LEAK)/OXPHOS; to assess efficiency relative to LEAK]; coupling control ratio (CR) (LEAK/OXPHOS; the ratio of LEAK to OXPHOS); complex I coupled CR (CI/OXPHOS; the ratio of OXPHOS driven by CI); and OXPHOS capacity (OXPHOS–LEAK; an assessment of coupled respiration above LEAK respiration). Additionally, mitochondrial membrane integrity was assessed by calculating the percentage change in respiration between CI–OXPHOS and addition of cytochrome *c*. There was no significant change (on average <5%) in respiration rates seen in either cardiac or liver tissue.

Statistical analysis

Changes in survival to 3 dpf, relative ventricular mass and mitochondrial performance were tested using a two-tailed Student's *t*-test between control and hypoxia-exposed fish. Relative ventricle mass to body mass were pooled by treatment between swimming and mitochondrial fish. Relative standard length at 3 dpf was tested using a Mann–Whitney non-parametric test. Cohen's *d* effect sizes were calculated for the pair-wise tests where 0.2 indicates small, 0.5 indicates medium and 0.8 indicates large effect sizes.

All data were tested for normality using a Shapiro–Wilk test, and homogeneity of variance using a Levene's test in R (r-project.org/). Data were transformed as needed prior to statistics. Outliers that were greater or less than two standard deviations from the mean in each group were removed prior to analyses. Analyses for all metabolic rate and swim performance measurements were carried out using a two-way ANOVA with larval treatment (hypoxia versus control) and swim tunnel DO (normoxia versus hypoxia) as main effects. ANOVA effect sizes (η^2) were calculated as small (0.01), medium (0.06) and large (0.14). If an interaction was detected between the two, we performed pair-wise Tukey's *post hoc* test. Statistical threshold for significance was $P \leq 0.05$, and all numbers and data are presented as mean±s.e.m.

RESULTS

Detailed statistical outputs are presented in Table S1. We did not observe any difference in hatch success, or any obvious morphological abnormalities in response to hypoxia (data not shown). There was no difference in survival or standard length at 3 dpf between controls and hypoxia-exposed larvae (Fig. 1, P=0.34 and P=0.17, respectively). At ∼70 dpf, when the fish underwent swim trials, there was no difference in relative ventricular mass (ratio of heart mass in mg to body mass in g) between normoxia-exposed (0.96±0.05) or hypoxia-exposed fish (0.86±0.03; P=0.14).

Fish from both developmental O_2 regimes reached U_{crit} and U_{burst} at lower speeds in hypoxic swim trials (Fig. 2A,B; P<0.01 and P<0.01, respectively). The speed between U_{crit} and U_{burst} (ΔU, or the speeds over which fish used bursting to supplement swimming) was impacted by developmental O_2 where hypoxia-exposed fish had higher ΔU than controls in all swim trials, indicating increased burst swimming overall (Fig. 2C; P=0.02). U_{opt}, COT_{opt}, and COT_{crit} had no effects of developmental O_2 or swim tunnel DO (Table 1, P>0.05).

Although fish were size- and age-matched, there was a small but significant discrepancy between hypoxia-exposed and control fish mass prior to swim trials (pooled by treatment: 2.35±0.10 g versus 2.02±0.09 g, respectively, P=0.02). Thus,

Journal of Experimental Biology (2024) 227, jeb247618. doi:10.1242/jeb.247618

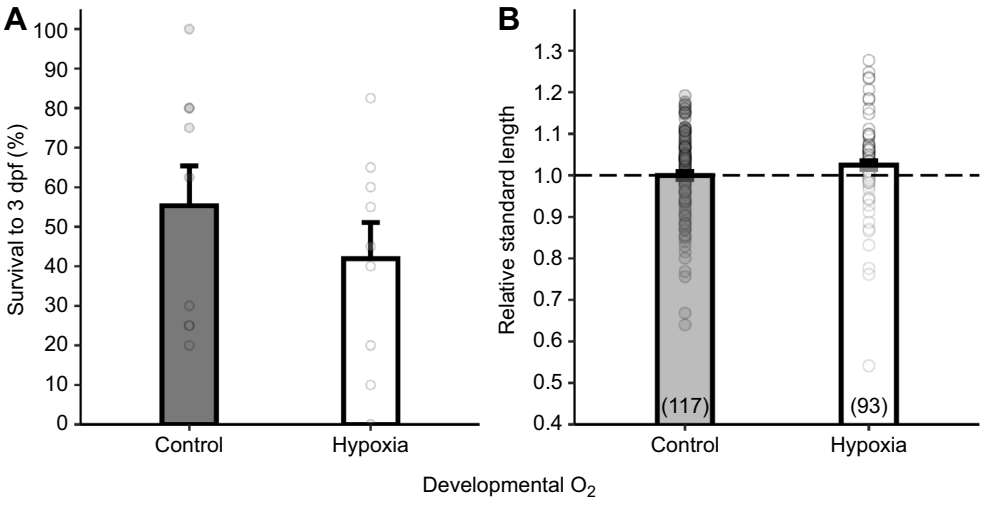

Fig. 1. Survival and standard length of fish exposed to normoxia or hypoxia over 3 days post-fertilization. (A) Survival and (B) relative standard length. The dotted horizontal line in B represents the normalized control standard length at 1. Data are mean±s.e.m., and *n*=9 for each treatment in A and noted in parentheses in B. There were no significant impacts of hypoxia on survival or length at 3 dpf (Student's *t*-test, *P*>0.05).

we size corrected metabolic measures (SMR and MMR) for statistical analyses using established scaling equations for red drum (Ackerly and Esbaugh, 2020; Pan et al., 2016) and an average common mass of 2.17 g. There was no difference in the statistics between base- and size-corrected metabolic rates, so we present the original, uncorrected values and statistics. The mean standard length was similar across both developmental treatments (Table 1, *P*=0.12).

Hypoxia-exposed fish had higher MMR than control fish when they swam in normoxia (Fig. 3A, *P*=0.05), but both developmental treatments were inhibited to similar MMR levels in hypoxic trials. SMR was similar between experimental groups in normoxic swim trials (Fig. 3B). Control fish showed a significant decrease in SMR from normoxia to hypoxia (*P*=0.04), whereas hypoxia-exposed fish did not decrease SMR as a result of hypoxic swim trials (Fig. 3B). Hypoxia-exposed fish had a higher AS compared with controls in

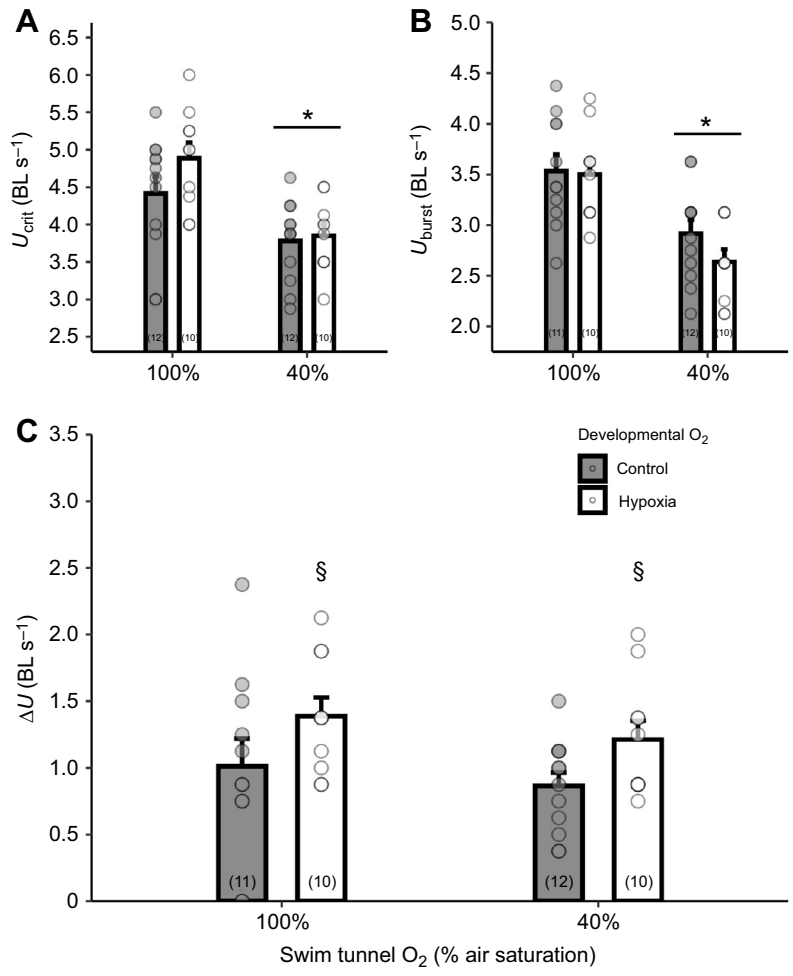

Fig. 2. Critical swim speed, burst speed and the difference for fish exposed to early developmental normoxia or hypoxia and swam in 100% or 40% air saturation. (A) Critical swim speed (U_{crit}), (B) burst speed (U_{burst}) and (C) the difference between the two (ΔU). U_{crit} and U_{burst} were significantly decreased in hypoxia swim trials for both developmental treatments (*P<0.05, two-way ANOVA). ΔU, indicating how long fish used burst swimming to reach U_{crit}, was elevated in fish exposed to hypoxia during development (§). Data are means±s.e.m. and sample sizes are indicated in parentheses.

Journal of Experimental Biology (2024) 227, jeb247618. doi:10.1242/jeb.247618

Table 1. Swim metrics of fish exposed to normoxia or hypoxia during early life and swam in 100% or 40% dissolved oxygen (DO)

	Swim DO	Mass[§] (g)	Standard length (cm)	U_{opt} (BL s^{-1})	COT_{opt} (mg O_2 kg^{-1} h^{-1})	COT_{crit} (mg O_2 kg^{-1} h^{-1})
Control	100%	1.98±0.12 (12)	5.25±0.11 (12)	3.28±0.16 (12)	245±14.3 (12)	271±9.13 (11)
	40%	2.05±0.14 (12)	5.31±0.14 (12)	3.08±0.20 (12)	225±22.3 (12)	278±5.92 (9)
Hypoxia	100%	2.39±0.15 (10)	5.42±0.12 (10)	3.49±0.25 (10)	236±10.7 (10)	266±23.4 (12)
	40%	2.32±0.13 (10)	5.53±0.11 (10)	3.54±0.14 (10)	228±19.9 (9)	235±13.7 (9)

Measures were tested using a two-way ANOVA. [§]Main effects of developmental treatment when $P \leq 0.05$. Data are means±s.e.m. and numbers in parentheses indicate sample size after removal of outliers. Optimal swim speed, U_{opt}; cost of transport at U_{opt}, COT_{opt} and at U_{crit}, COT_{crit}.

normoxia ($P<0.01$), which was significantly diminished in hypoxic swim trials (Fig. 3C, $P<0.01$). Conversely, control fish had similar AS in both normoxia and hypoxia.

Hypoxia sensitivity, as measured by P_{crit}, was reduced in hypoxic trials (i.e. lower P_{crit}; Fig. 4C, $P<0.01$) and hypoxia-exposed fish had higher P_{crit} values overall (i.e. they were more sensitive to hypoxia; Fig. 4C, $P=0.03$). This pattern was consistent when P_{crit} was calculated using the O_2 supply capacity (Fig. 4D, $P<0.01$ for both developmental treatment and swim tunnel oxygen).

There was no difference in cardiac mitochondrial respiration between normoxia- or hypoxia-exposed fish (Table 2). Liver mitochondria of control and hypoxia-exposed fish did not differ in OXPHOS or LEAK respiration (Fig. 5A,B). In contrast, the liver mitochondria in hypoxia-exposed fish had significantly higher OXPHOS capacity and lower coupling ratios (Fig. 5C,D), as well as higher control efficiencies and respiratory control ratios (Table 2, $P<0.05$).

DISCUSSION

Our goal was to investigate the capacity for respiratory plasticity in developing red drum exposed to sublethal hypoxia during an important developmental window in early life. Hypoxia-exposed red drum showed improvements to later life respiratory performance, as denoted by increased MMR and AS, and adjustments to liver mitochondrial respiration that increase OXPHOS capacity and the efficiency of ATP production. However, the early life hypoxia exposure caused fish to be more vulnerable to hypoxia in later life, as evidenced by the elevated P_{crit}.

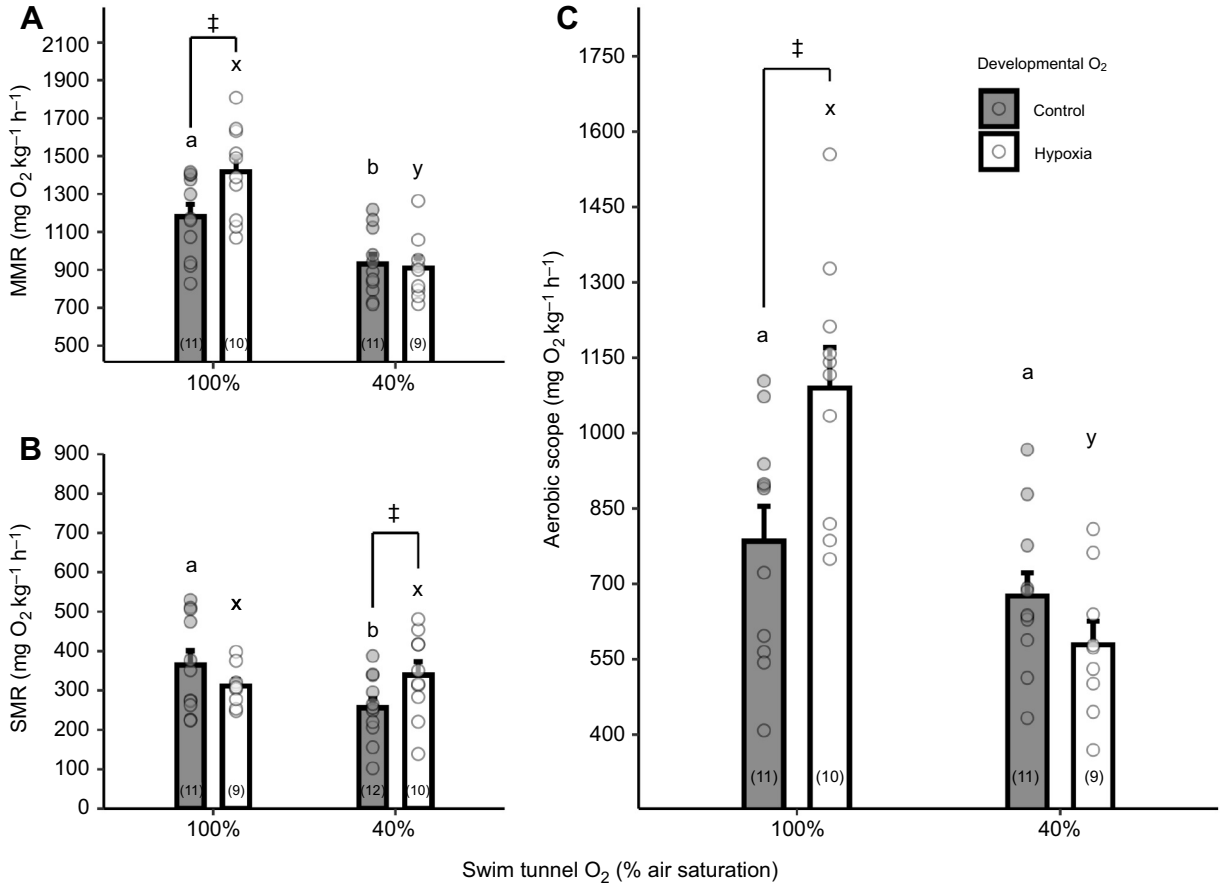

Fig. 3. Maximum metabolic rate, standard metabolic rate and aerobic scope for fish exposed to normoxia or hypoxia during development and exercised in 100% or 40% air saturation. (A) Maximum metabolic rate (MMR), (B) standard metabolic rate (SMR) and (C) aerobic scope (AS). MMR and AS were higher in 100% air saturation swim trials (two-way ANOVA, $P<0.05$). Data are means±s.e.m. and sample sizes are noted in parentheses. All measures show interactions between developmental O_2 and swim tunnel O_2. Dissimilar letters indicate pair-wise differences of developmental O_2 across swim tunnel DO (a,b: control; x,y: hypoxia) and ‡ indicates pair-wise differences between developmental history within a swim trial (Tukey's *post hoc*, $P<0.05$).

Journal of Experimental Biology (2024) 227, jeb247618. doi:10.1242/jeb.247618

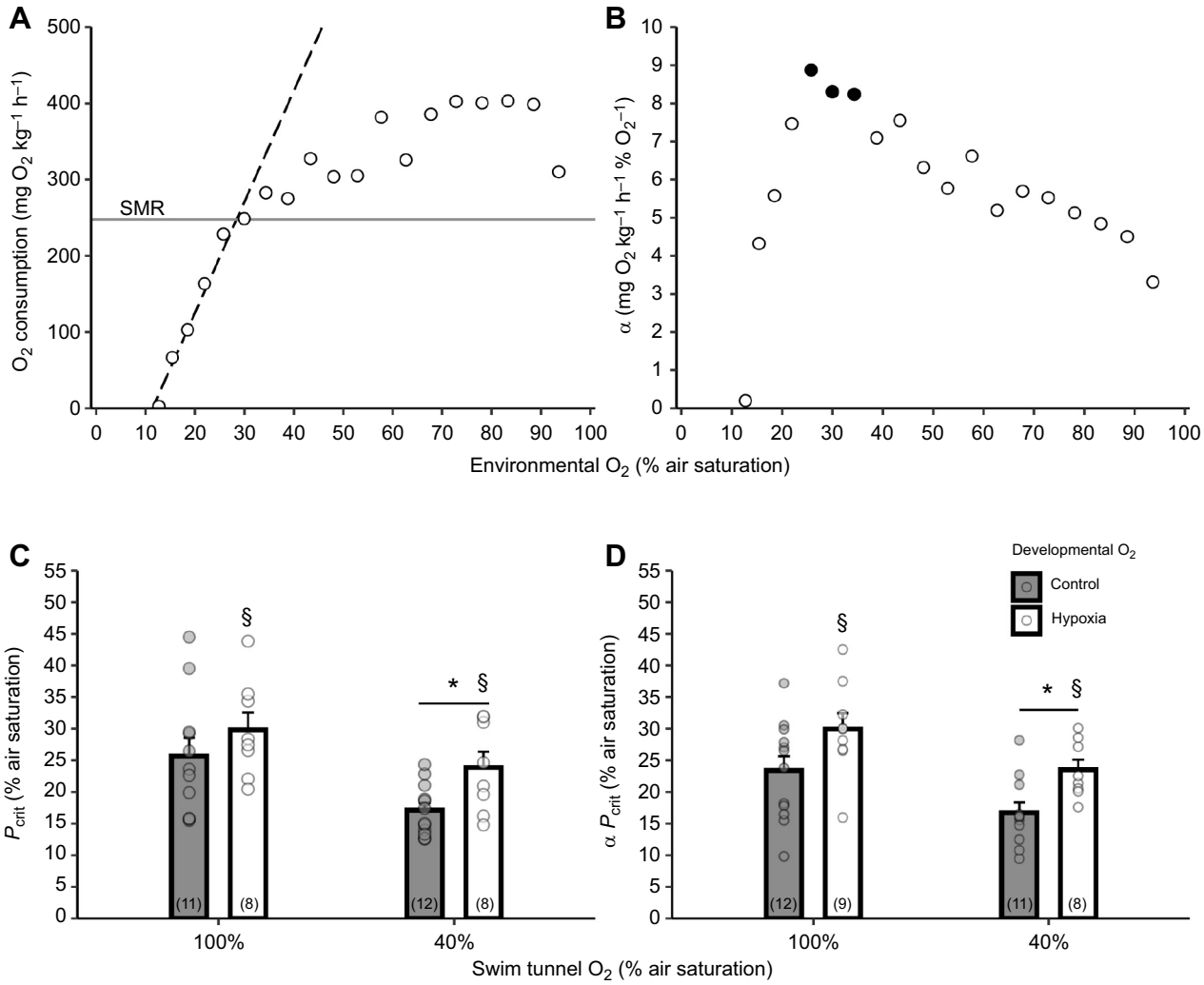

Fig. 4. Critical oxygen tension (P_{crit}) determination methods. P_{crit} determination using (A) standard metabolic rate (SMR) and (B) O_2 supply capacity (α). Results of P_{crit} using (C) SMR and (D) α. The horizontal line in A represents SMR, and the dotted line is the linear regression for declining O_2 consumption with environmental O_2. The intersection of these lines is P_{crit}. The filled circles in B represent the three highest consecutive α. These three are averaged and the O_2 saturation at that average is the P_{crit}. Differences in P_{crit} were measured using a two-way ANOVA. Main effects of treatment (§) and swim O_2 (*) are noted when $P \leq 0.05$. Data are means±s.e.m. and sample sizes are noted in parentheses. Fish that did not recover, i.e. reach pre-swim trial $\dot{M}_{O_2}$ levels were excluded from all P_{crit} analyses.

Additionally, early life exposure caused treated fish to increase instances of anaerobic burst swimming to maintain U_{crit}. Importantly, there was no difference in larval survival or size at hatch between developmental exposures. This, along with the fact that multiple spawns of variable parental origin were used to generate the dataset suggest that the changes noted here were not due to genetic selection or maternal bias.

To our knowledge, this is the first study that demonstrated beneficial impacts of developmental hypoxia exposure on normoxic MMR in fish. In contrast, Atlantic salmon (*Salmo salar*) exposed to hypoxia for 100 days and subsequently reared in normoxia for 15 months showed no effects on MMR or AS (Wood et al., 2017). Zebrafish exposed to varying 4 h periods of hypoxia within the first 36 hpf also showed no differences in routine metabolic rate (RMR) when tested as adults (Robertson et al., 2014). However, it is important to note that RMR, the O_2 consumption to support routine activity and movement, and MMR, the O_2 consumed when the respiratory system is pushed to its maximum limit, are very different measures that should not be compared. The discrepancies between findings can have a multitude of explanations; but it seems likely that the differences in exposure scenarios, life history and the life stage of testing may play an important role. For example, fish in the current study were tested for metabolic traits at ∼70 days post hatch, which is akin to an early fry stage that is still undergoing exponential growth. In contrast, Atlantic salmon were tested well into the smolt life stage, and zebrafish were tested as adults. One hypothesis from this combination of findings is that the differences in metabolic traits observed in red drum may not be life long, as is often characteristic of rigid developmental plasticity. This is somewhat intuitive because MMR can also be impacted by a number of other factors and developmental effects might be expected to be most obvious in post-settlement life stages where individuals are competing fiercely for food and habitat while prioritizing growth, which decreases over time as fish become reproductive adults.

Surprisingly, the U_{crit} of red drum exposed to early life hypoxia was not different from control fish when tested in normoxia, despite the fact that both MMR and AS were higher in hypoxia-exposed fish. With respect to U_{crit}, our findings generally corroborate

Journal of Experimental Biology (2024) 227, jeb247618. doi:10.1242/jeb.247618

Table 2. Mitochondrial respiration for fish exposed to normoxia or hypoxia during early life

	Heart		Liver	
Parameter	Control	Hypoxia	Control	Hypoxia
Complex I	46.8±16.8	48.6±6.71	8.22±0.68	11.2±1.68
OXPHOS	67.2±25.2	79.1±8.87	10.1±0.93	14.9±2.78
OXPHOS+ADP	68.4±25.2	82.3±8.54	10.1±0.98	15.3±2.86
LEAK	29.1±11.6	30.9±5.97	3.59±0.71	3.14±0.59
Complex I inhibited	11.8±4.07	11.4±3.49	1.63±1.02	2.31±1.01
Coupling (LEAK/OXPHOS)	0.44±0.06	0.40±0.08	0.35±0.05	0.21±0.01§
CI/OXPHOS	0.84±0.13	0.58±0.04	0.82±0.03	0.77±0.04
OXPHOS capacity (OXPHOS–LEAK)	39.3±14.6	51.4±10.4	6.56±0.75	12.1±2.30§
Respiratory control (OXPHOS/LEAK)	2.57±0.43	3.29±0.81	3.34±0.56	4.91±0.34§
Control efficiency [(OXPHOS–LEAK)/OXPHOS]	0.56±0.06	0.60±0.08	0.65±0.05	0.79±0.01§

Data are means±s.e.m. and n=7 per treatment. §Differences based on developmental exposure within tissue type (Student's t-test, P<0.05).

previous work on rainbow trout (Johnston et al., 2013) and zebrafish (Widmer et al., 2006) that suggested U_{crit} was insensitive to early life hypoxia – although it should be noted that transient changes in rainbow trout were noted throughout development. Typically, a higher MMR is accompanied by an increase in aerobic swim performance (i.e. U_{crit}). Surprisingly, the increase in MMR observed in hypoxia-exposed red drum did not result in any improvements to normoxic U_{crit}. While seemingly at odds, it is not uncommon to find differential responses in MMR or U_{crit}. It is important to note the differences in what these metrics tell us about a fish's aerobic performance. MMR is a measure of the maximum capacity of the respiratory system to take up and transport oxygen. U_{crit} is a metric reliant on the sum of the aerobic, anaerobic and physical capabilities of the fish (i.e. muscle power generation;

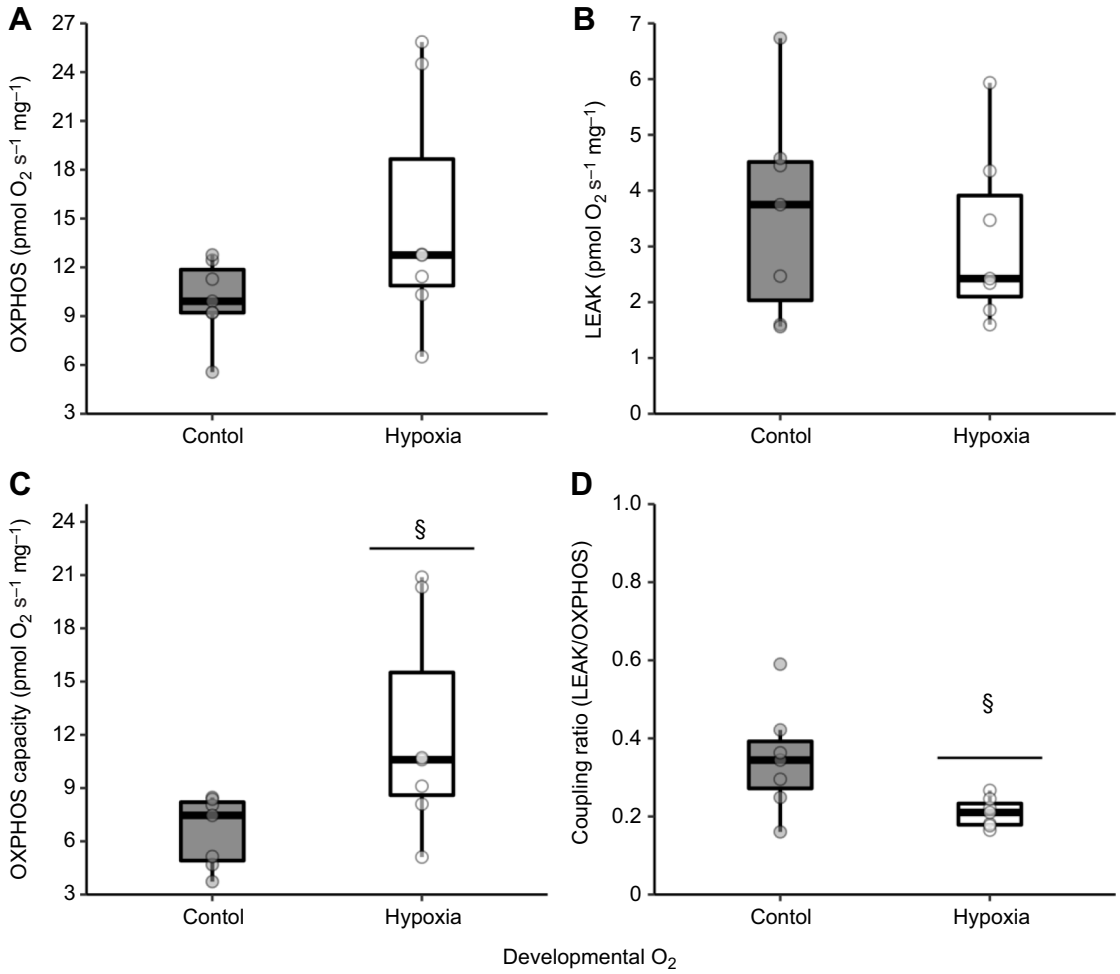

Fig. 5. Measurement of mitochondrial respiration in the liver of fish exposed to control or hypoxia in early life. (A) Oxidative phosphorylation (OXPHOS), (B) proton leak (LEAK), (C) OXPHOS capacity and (D) coupling ratio. OXPHOS capacity is calculated as OXPHOS–LEAK, and the coupling ratio as LEAK/OXPHOS. Whiskers represent data within 1.5 standard deviations of the mean, the edges of the box depict the interquartile range, and the solid line is the median. Differences were tested using a Student's t-test between control and hypoxia exposure (§P<0.05) and data are n=7 for each treatment.

Lurman et al., 2007). For example, a physiological investigation into U_{crit} found that $\dot{M}_{O_2}$ increases until 100% U_{crit}, but anaerobic metabolism and anaerobically driven body functions, such as tail-beat frequency, increase and dominate past 80% of U_{crit} (Lurman et al., 2007). In fact, our data demonstrate that while both treatment fish had similar U_{crit} speeds, the contribution of U_{crit} that derived from anaerobic swimming – as defined by a transition to burst-and-coast swimming (i.e. ΔU) – was increased in hypoxia-exposed fish, as evident from the fact that hypoxia-exposed fish burst for ∼0.5 BL s^{-1} longer than control fish before failing in swim trials (Fig. 2C).

The changing reliance on anaerobic swimming observed in hypoxia-exposed red drum did not result in any changes to swimming efficiency metrics, such as U_{opt} or COT (Table 1), nor were any absolute changes in U_{burst} observed (Fig. 2B). Nonetheless, these results suggest a change in the dynamics of red and white muscle function for swimming performance as a consequence of hypoxia exposure in early life. Previous work has demonstrated the capacity of fish species to adjust anaerobic contributions to U_{crit} (Tudorache et al., 2008), probably by shifting the abundance of energy stores available for glycolytic muscle activity (Vanderplancke et al., 2015). Cadiz et al. (2018a) reported that European sea bass (*Dicentrarchus labrax*) showed changes in anaerobic fuel stores, notably an increase in liver glycogen, when exposed to hypoxia in early life. Interestingly, Dichiera et al. (2022) also reported an increase in U_{crit} for hypoxia-acclimated juvenile red drum that was driven by anaerobically powered burst swimming, despite the fact that the hypoxia exposure was well above P_{crit}. This reinforces the premise that while anaerobic and aerobic processes are often discussed independently – particularly when contextualized against P_{crit}, U_{crit} and U_{burst} – fish probably regulate these processes in tandem in response to environmental stressors.

Contrary to expectations, and to prior work on hypoxia acclimation in juvenile red drum (Pan et al., 2017), fish exposed to early life hypoxia did not exhibit improvements in hypoxia vulnerability (i.e. P_{crit}). In fact, P_{crit} was significantly elevated in fishes exposed to hypoxia early in life, which appears contradictory to previous findings related to developmental hypoxia. For example, zebrafish exposed as embryos were able to maintain higher O_2 consumption at lower P_{O_2} than control fish at 3 days post exposure (Robertson et al., 2014) and in later life stages (Barrionuevo et al., 2010). Similarly, Chinook salmon (*Onchorhyncus tshawytscha*) showed increased time to loss of equilibrium (Del Rio et al., 2019). Meanwhile, Atlantic salmon (Wood et al., 2017) and European sea bass (Cadiz et al., 2018b) lost equilibrium at higher P_{O_2}. However, it is noteworthy that none of these studies actually measured P_{crit}, and time to loss of equilibrium tolerance endpoints are likely impacted by anaerobic capacity and substrate availability, which, as noted above, may shift in tandem with aerobic processes. It is also interesting to note that in the current study P_{crit} was lower in animals that had undergone swimming tests and recovery in hypoxia (i.e. the 40% O_2 trial), the effect of which was independent of hypoxia exposure early in life. This is likely the result of short-term acclimation processes that have previously been shown to impact P_{crit} estimates (Regan and Richards, 2017). In fact, these findings may be partly due to hypoxia-induced metabolic suppression, as evidenced by the reduced SMR that was observed in the control fish. Metabolic suppression is a common response in fish exposed to acute hypoxia (Chippari-Gomes et al., 2005; Richards, 2010; Wood et al., 2017). However, the effects of early hypoxia treatment were robust regardless of the short-term hypoxia fish experienced during the metabolic trait determination protocols. Thus, these data reflect a combination of the acute and early life exposure effects. It should also be noted that our P_{crit} estimates were performed in the post-exercise recovery state, and while we ensured that routine $\dot{M}_{O_2}$ had returned to within range of SMR prior to initiating the experiments, it is impossible to say whether residual impacts from exercise could impact our raw P_{crit} estimates. However, the same procedure was performed on both acclimation treatment groups, and thus it seems very unlikely that this could be the source of the counterintuitive effects of early life hypoxia on P_{crit}.

The results of the current study present an intriguing picture whereby red drum exposed to hypoxia in early life undertake physiological adjustments that change the trajectory of their respiratory development, which ultimately improves MMR and AS while sacrificing P_{crit}. This pattern contradicts theories that P_{crit} is simply a by-product of the evolutionary pressures on MMR (Seibel and Deutsch, 2020), and supports the notion that MMR and P_{crit} may be under separate selective pressures (Esbaugh et al., 2021). Unfortunately, our work cannot directly inform on the mechanistic underpinnings that may explain the observed data. However, we can offer several hypotheses that should be explored through future study. Since mechanisms underlying differences in MMR are generally attributed to changes in O_2 delivery, we hypothesize that hypoxia-exposed fish develop greater maximal cardiac output. While we found no difference in the absolute size of the ventricle, or relative size of the ventricle to body mass between treatments, it is important to note that our ability to detect mass differences is limited by the accuracy of the balance owing to the very small size of the hearts. It is also noteworthy that changes in heart mass do not necessarily translate to changes in swim performance and oxygen delivery (Gallaugher et al., 2001). Developmental hypoxia exposure can affect the adrenergic and cholinergic mechanisms that regulate heart rate (Miller et al., 2011), and by extension cardiac output. Similarly, by impacting adrenergic and cholinergic responses, developmental hypoxia could also modulate the exercise-based tachycardia or hypoxia-induced bradycardia common to teleost fishes (Esbaugh et al., 2021). These changes may not be evident in resting fish, but manifest when exhaustive exercise or hypoxia are encountered in later life. A recent study in American alligators (*Alligator mississippiensis*) exposed to hypoxia in early development showed marked chamber-specific size increases, and cardiac function was thus different when the alligators were re-exposed to acute hypoxia in later life (Crossley et al., 2022). It seems possible that a similar response may have occurred in red drum exposed to hypoxia early in life.

A second complementary hypothesis is that red drum may be altering the patterns of haemoglobin gene expression as a consequence of hypoxia exposure during development. Specifically, we hypothesize that red drum are improving tissue oxygen extraction efficiency by upregulating haemoglobin isoforms with larger Root effects (an increase in the pH sensitivity of haemoglobin). Many teleosts have been shown to increase tissue oxygen extraction through a Root effect-mediated process involving red blood cell and plasma-accessible carbonic anhydrase 4 (CA4; Dichiera and Esbaugh, 2020; Rummer and Brauner, 2011). This process has been linked to oxygen extraction in several tissues, including the heart (Alderman et al., 2016), red muscle (Rummer et al., 2013), retina (Damsgaard et al., 2020) and intestine (Cooper et al., 2014). Note that juvenile red drum have been shown to exhibit plasma-accessible CA4 in both red muscle and heart tissues (Dichiera et al., 2022, 2023) and to express Root-effect haemoglobin isoforms that are dynamically regulated in response to hypoxia (Negrete et al., 2022). Interestingly, juvenile red drum appear to prioritize improving affinity processes when acclimated to

hypoxia, which reduced P_{crit} and improved MMR only when tested under hypoxic conditions (Dichiera et al., 2022; Negrete et al., 2022; Pan et al., 2017). The upregulation of high affinity (non-Root effect) haemoglobin genes occurred after one exposure to chronic hypoxia. But it is important to note that in the current study, the changes in metabolic traits were not only in response to hypoxia, but are the result of a combination of a period of hypoxia exposure in early development followed by 70 days of rearing in full normoxia. The changes in MMR and P_{crit} here suggest that hypoxia-exposed fish may have upregulated respiratory genes (i.e. CA4 and haemoglobin isoforms) that are involved in oxygen delivery at the tissue, instead of oxygen affinity at the gill.

As a final assessment of the metabolic phenotype of red drum exposed to hypoxia in early life, we sought to assess whether hypoxia exposure stimulated improvements in mitochondrial efficiency. We observed significant improvements in efficiency in hypoxia-exposed fish, as described by the coupling and respiratory control ratios, as well as improved OXPHOS capacity and OXPHOS control efficiency in the liver. In short, liver mitochondria of fish exposed to hypoxia in early life were significantly more efficient and had greater net OXPHOS rates (i.e. OXPHOS capacity; OXPHOS–LEAK). Interestingly, this is similar to changes in mitochondrial characteristics of juvenile red drum red muscle, following hypoxia acclimation (Ackerly et al., 2023), and the liver, after warming acclimation (Zambie et al., 2024). Recently, differences in liver mitochondrial proton leak respiration and efficiency control ratios have also been linked to inter-individual variation in SMR (Salin et al., 2016) and growth rates (Salin et al., 2019; Dawson et al., 2022). These studies show that fish with higher liver mitochondria efficiency, that is more ATP produced per O_2 molecule consumed, had significantly elevated growth rates under similar feeding regimes. Furthermore, decreases in mitochondria efficiency have been directly linked to decreases in growth and development in tadpoles (*Rana temporaria*), underscoring a key role in early life (Salin, et al., 2012). Growth rate was not specifically studied in the current study, but there was an interesting difference in mass in our fish. While we controlled for similar age (in dpf) there was a slight, yet statistically significant, difference in mass wherein hypoxia-exposed fish used for swimming were slightly larger than control fish. If we take this final mass and divide by dpf for fish used for swimming, we find a lifetime growth rate of 27.1±1.05 mg day^{-1} for control fish and lifetime growth rate of 35.0±1.52 mg day^{-1} for hypoxia-exposed fish. We saw a similar pattern in fish used for mitochondrial respiration (control rate 46.0±5.38 mg day^{-1}, hypoxia-exposed rate 70.2±6.07 mg day^{-1}), which was associated with an increase in liver mitochondria efficiency. It is important to note that these lifetime growth rate metrics are crude, and assume linear growth and similar feed intake, so should be treated with caution. Nonetheless, these data may suggest that hypoxia exposure in early development not only results in higher MMR and AS, but also leads to a more efficient utilization of oxygen that can significantly improve growth rates. Indeed, there are studies that report increased growth rates after developmental hypoxia exposure (Cadiz et al., 2018b). However, growth rate can vary with developmental time, and compensatory growth can occur when hypoxia is removed as a stressor. Salmonids have shown differences in growth rate and condition factor, depending on developmental stage (Del Rio et al., 2019; Johnston et al., 2013). Other studies report lower growth rates (Zambonino-Infante et al., 2017) or no change in body mass (Cadiz et al., 2018b; Widmer et al., 2006; Wood et al., 2017) with respect to hypoxia exposure.

Conclusions

We have found evidence of developmental plasticity of a marine teleost when exposed to sub-lethal hypoxia during a critical period in early life that led to an altered metabolic phenotype as early juveniles. This phenotype was characterized by increased MMR and AS, but increased hypoxia sensitivity in later life (i.e. higher P_{crit}). Furthermore, hypoxia-exposed individuals also exhibited evidence of improved efficiency of mitochondrial oxygen utilization, specifically in the liver. The mechanisms underlying the observed changes are still to be established, and it is unclear whether the changes are transient or would persist into adulthood. Nonetheless, the increased aerobic performance and mitochondrial efficiency could carry a number of ecophysiological benefits for animals during a critical life stage where individuals are subject to severe predation pressure and are competing for food and habitat while recruiting to nursery habitat. Future work is needed to understand the mechanisms by which the observed phenotypic changes occurred, as well as to explore the ecophysiological trade-offs related to hypoxia vulnerability and later life hypoxia exposure.

Acknowledgements

We thank the team at Texas Parks and Wildlife who provided eggs from red drum spawns. We also thank Leigh Walsh and Rene Lopez for caring for the brood stock at the UT Marine Science Institute and supplying red drum eggs. We thank Cynthia Faulk and Dr Lee Fuiman for expertise in larval red drum and providing facilities for raising multiple spawns. Finally, we would like to thank two anonymous reviewers for their helpful insights and suggestions to improve the manuscript.

Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: B.N.J., K.L.A., A.J,E.; Methodology: B.N.J., K.L.A.; Software: A.J,E.; Validation: B.N.J., K.L.A., A.J,E.; Formal analysis: B.N.J.; Investigation: B.N.J., K.L.A.; Resources: A.J,E.; Data curation: B.N.J.; Writing - original draft: B.N.J.; Writing - review & editing: B.N.J., K.L.A., A.J,E.; Visualization: B.N.J., K.L.A.; Supervision: A.J,E.; Project administration: A.J,E.; Funding acquisition: B.N.J., A.J.E.

Funding

This material is based upon work supported by the National Science Foundation Graduate Research Fellowship Program (grant #1610403), the University of Texas at Austin Graduate School Continuing Fellowship, and University of Texas at Austin Marine Science Institute Lund Fellowship for B.N.J. and National Science Foundation (grant #2002549) for A.J.E. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation.

Data availability

All relevant data can be found within the article and its supplementary information.

ECR Spotlight

This article has an associated ECR Spotlight interview with Benjamin Negrete.

References

Ackerly, K. L. and Esbaugh, A. J. (2020). The additive effects of oil exposure and hypoxia on aerobic performance in red drum (*Sciaenops ocellatus*). *Sci. Total Environ.* **737**, 140174. doi:10.1016/j.scitotenv.2020.140174

Ackerly, K. L., Negrete, B., Jr, Dichiera, A. M. and Esbaugh, A. J. (2023). Hypoxia acclimation improves mitochondrial efficiency in the aerobic swimming muscle of red drum (*Sciaenops ocellatus*). *Comp. Biochem. Physiol. A. Mol. Integr. Physiol.* **282**, 111443. doi:10.1016/j.cbpa.2023.111443

Alderman, S. L., Harter, T. S., Wilson, J. M., Supuran, C. T., Farrell, A. P. and Brauner, C. J. (2016). Evidence for a plasma-accessible carbonic anhydrase in the lumen of salmon heart that may enhance oxygen delivery to the myocardium. *J. Exp. Biol.* **219**, 719-724. doi:10.1242/jeb.130443

Applebaum, S. L., Pan, T.-C. F., Hedgecock, D. and Manahan, D. T. (2014). Separating the nature and nurture of the allocation of energy in response to global change. *Integr. Comp. Biol.* **54**, 284-295. doi:10.1093/icb/icu062

Bagowski, C. P., Bertola, L. D., Schoonheere, E., Wilms, I., Kabli, S., Alia, A. and de Groot, H. J. M. (2011). Effects of hypoxia on buoyancy control and the

development of lordosis in physostomous and physoclistus fish species. *Res. Reviews Biosci.* **5**, 1-7.

Barrionuevo, W. R., Fernandes, M. N. and Rocha, O. (2010). Aerobic and anaerobic metabolism for the zebrafish, Danio rerio, reared under normoxic and hypoxic conditions and exposed to acute hypoxia during development. *Braz. J. Biol.* **70**, 425-434. doi:10.1590/S1519-69842010000200027

Bell, W. H. and Terhune, L. D. B. (1970). Water tunnel design for fisheries research. *J. Fish Res. Board Can. Tech. Rep.* **195**, 1-69.

Bianchini, K. and Wright, P. A. (2013). Hypoxia delays hematopoiesis: retention of embryonic hemoglobin and erythrocytes in larval rainbow trout, *Oncorhynchus mykiss*, during chronic hypoxia exposure. *J. Exp. Biol.* **216**, 4415-4425. doi:10.1242/jeb.083337

Breitburg, D., Levin, L. A., Oschlies, A., Grégoire, M., Chavez, F. P., Conley, D. J., Garçon, V., Gilbert, D., Gutiérrez, D., Isensee, K. et al. (2018). Declining oxygen in the global ocean and coastal waters. *Science* **359**, 46. doi:10.1126/science.aam7240

Burggren, W. W. (2019). Phenotypic switching resulting from developmental plasticity: fixed or reversible? *Front. Physiol.* **10**, 1634. doi:10.3389/fphys.2019.01634

Cadiz, L., Servili, A., Quazuguel, P., Madec, L., Zambonino-Infante, J.-L. and Mazurais, D. (2017). Early exposure to chronic hypoxia induces short- and long-term regulation of hemoglobin gene expression in European sea bass (*Dicentrarchus labrax*). *J. Exp. Biol.* **220**, 3119-3126. doi:10.1242/jeb.160713

Cadiz, L., Zambonino-Infante, J.-L., Quazuguel, P., Madec, L., Le Delliou, H. and Mazurais, D. (2018a). Metabolic response to hypoxia in European sea bass (*Dicentrarchus labrax*) displays developmental plasticity. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **215**, 1-9. doi:10.1016/j.cbpb.2017.09.005

Cadiz, L., Ernande, B., Quazuguel, P., Servili, A., Zambonino-Infante, J.-L. and Mazurais, D. (2018b). Moderate hypoxia but not warming conditions at larval stage induces adverse carry-over effects on hypoxia tolerance of European sea bass (*Dicentrarchus labrax*) juveniles. *Mar. Environ. Res.* **138**, 28-35. doi:10.1016/j.marenvres.2018.03.011

Chance, B. and Williams, G. R. (1955). Respiratory enzymes in oxidative phosphorylation: III. The steady state. *J. Biol. Chem.,* **217**, 409-427. doi:10.1016/S0021-9258(19)57191-5

Chippari-Gomes, A. R., Gomes, L. C., Lopes, N. P., Val, A. L. and Almeida-Val, V. M. F. (2005). Metabolic adjustments in two Amazonian cichlids exposed to hypoxia and anoxia. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **141**, 347-355. doi:10.1016/j.cbpc.2005.04.006

Cooper, C. A., Regan, M. D., Brauner, C. J., De Bastos, E. S. R. and Wilson, R. W. (2014). Osmoregulatory bicarbonate secretion exploits H(+)-sensitive haemoglobins to autoregulate intestinal O2 delivery in euryhaline teleosts. *J. Comp. Physiol. B* **184**, 865-876. doi:10.1007/s00360-014-0844-x

Crossley, J. L., Lawrence, T., Tull, M., Elsey, R. M., Wang, T. and Crossley, D. A., II. (2022). Development oxygen preadapts ventricular function of Juvenile American alligators, *Alligator mississippiensis*. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **323**, R739-R748. doi:10.1152/ajpregu.00059.2022

Damsgaard, C., Lauridsen, H., Harter, T. S., Kwan, G. T., Thomsen, J. S., Funder, A. M. D., Supuran, C. T., Tresguerres, M., Matthews, P. G. D. and Brauner, C. J. (2020). A novel acidification mechanism for greatly enhanced oxygen supply to the fish retina. *eLife* **9**, e58995. doi:10.7554/eLife.58995

Dawson, N. J., Millet, C., Selman, C. and Metcalfe, N. B. (2022). Inter-individual variation in mitochondrial phosphorylation efficiency predicts growth rates in ectotherms at high temperatures. *FASEB J.* **36**, e22333. doi:10.1096/fj.202101806RR

Del Rio, A. M., Davis, B. E., Fangue, N. A. and Todgham, A. E. (2019). Combined effects of warming and hypoxia on early life stage Chinook salmon physiology and development. *Conserv. Physiol.* **7**, coy078. doi:10.1093/conphys/coy078

Deutsch, C., Ferrel, A., Seibel, B., Pörtner, H.-O. and Huey, R. B. (2015). Ecophysiology. Climate change tightens a metabolic constraint on marine habitats. *Science* **348**, 1132-1135. doi:10.1126/science.aaa1605

Deutsch, C., Penn, J. L. and Seibel, B. (2020). Metabolic trait diversity shapes marine biogeography. *Nature* **585**, 557-562. doi:10.1038/s41586-020-2721-y

Dichiera, A. M. and Esbaugh, A. J. (2020). Red blood cell carbonic anhydrase mediates oxygen delivery via the Root effect in red drum. *J. Exp. Biol.* **223**, jeb232991. doi:10.1242/jeb.232991

Dichiera, A. M., Negrete, B., Jr, Ackerly, K. L. and Esbaugh, A. J. (2022). The role of carbonic anhydrase-mediated tissue oxygen extraction in a marine teleost acclimated to hypoxia. *J. Exp. Biol.* **225**, jeb244474. doi:10.1242/jeb.244474

Dichiera, A. M., De Anda, V., Gilmour, K. M., Baker, B. J. and Esbaugh, A. J. (2023). Functional divergence of teleost carbonic anhydrase 4. *Comp. Biochem. Physiol. A. Mol. Integr. Physiol.* **277**, 111368. doi:10.1016/j.cbpa.2023.111368

Esbaugh, A. J., Ackerly, K. L., Dichiera, A. M. and Negrete, B. (2021). Is hypoxia vulnerability in fishes a by-product of maximum metabolic rate? *J. Exp. Biol.* **224**, jeb232520. doi:10.1242/jeb.232520

Fry, F. E. J. (1971). The effect of environmental factors on the physiology of fish. In *Fish Physiol.*, Vol. VI (ed. W. S. Hoar and D. J. Randall), pp. 1-98. London: Academic Press.

Gallaugher, P. E., Thorarensen, H., Kiessling, A. and Farrell, A. P. (2001). Effects of high intensity exercise training on cardiovascular function, oxygen uptake, internal oxygen transport and osmotic balance in chinook salmon (*Oncorhynchus tshawytscha*) during critical speed swimming. *J. Exp. Biol.* **204**, 2861-2872. doi:10.1242/jeb.204.16.2861

Hassell, K. L., Coutin, P. C. and Nugegoda, D. (2008). Hypoxia impairs embryo development and survival in black bream (*Acanthopagrus butcheri*). *Mar. Pollut. Bull.* **57**, 302-306. doi:10.1016/j.marpolbul.2008.02.045

Holt, G. J. (1993). Feeding larval red drum on microparticulate diets in a closed recirculating water system. *J. World Aqua. Soc.* **24**, 225-230. doi:10.1111/j.1749-7345.1993.tb00011.x

Holt, J., Johnson, A. G., Arnold, C. R., Fable, W. A., Jr and Williams, T. A. (1981). Description of eggs and larvae of laboratory reared red drum, *Sciaenops ocellata*. *Copeia* **4**, 751-756. doi:10.2307/1444174

Hughes, M. C. and Perry, S. F. (2021). Does blood flow limit acute hypoxia performance in larval zebrafish (*Danio rerio*)? *J. Comp. Physiol. B* **191**, 469-478. doi:10.1007/s00360-020-01331-z

Ishibashi, Y., Kotaki, T., Yamada, Y. and Ohta, H. (2007). Ontogenic changes in tolerance to hypoxia and energy metabolism of larval and juvenile Japanese flounder *Paralichthys olivaceus*. *J. Exp. Mar. Bio. Ecol.* **352**, 42-49. doi:10.1016/j.jembe.2007.06.036

Johansen, J. L. and Esbaugh, A. J. (2017). Sustained impairment of respiratory function and swim performance following acute oil exposure in a coastal marine fish. *Aquat. Toxicol.* **187**, 82-89. doi:10.1016/j.aquatox.2017.04.002

Johnston, E. F., Alderman, S. L. and Gillis, T. E. (2013). Chronic hypoxia exposure of trout embryos alters swimming performance and cardiac gene expression in larvae. *Physiol. Biochem. Zool.* **86**, 567-575. doi:10.1086/672012

Jordan, A. D. and Steffensen, J. F. (2007). Effects of ration size and hypoxia on specific dynamic action in the cod. *Physiol. Biochem. Zool.* **80**, 178-185. doi:10.1086/510565

Lofeu, L., Anelli, V., Straker, L. C. and Kohlsdorf, T. (2021). Developmental plasticity reveals hidden fish phenotypes and enables morphospace diversification. *Evolution* **75**, 1170-1188. doi:10.1111/evo.14221

Lurman, G. J., Bock, C. H. and Pörtner, H.-O. (2007). An examination of the metabolic processes underpinning critical swimming in Atlantic cod (*Gadus morhua* L.) using *in vivo* 31P-NMR spectroscopy. *J. Exp. Biol.* **210**, 3749-3756. doi:10.1242/jeb.008763

Mager, E. M., Esbaugh, A. J., Stieglitz, J. D., Hoenig, R., Bodinier, C., Incardona, J. P., Scholz, N. L., Benetti, D. D. and Grosell, M. (2014). Acute embryonic or juvenile exposure to Deepwater Horizon crude oil impairs the swimming performance of mahi-mahi (*Coryphaena hippurus*). *Environ. Sci. Technol.* **48**, 7053-7061. doi:10.1021/es501628k

Mandic, M., Todgham, A. E. and Richards, J. G. (2009). Mechanisms and evolution of hypoxia tolerance in fish. *Proc. R. Soc. B.* **276**, 735-744. doi:10.1098/rspb.2008.1235

Miller, S. C., Gillis, T. E. and Wright, P. A. (2011). The ontogeny of regulatory control of the rainbow trout (*Onchorhyncus mykiss*) heart and how this is influenced by chronic hypoxia exposure. *J. Exp. Biol.* **214**, 2065-2072. doi:10.1242/jeb.054825

Negrete, B., Jr and Esbaugh, A. J. (2019). A methodological evaluation of the determination of critical oxygen threshold in an estuarine teleost. *Biol. Open* **8**, bio045310. doi:10.1242/bio.045310

Negrete, B., Jr, Ackerly, K. L., Dichiera, A. M. and Esbaugh, A. J. (2022). Respiratory plasticity improves aerobic performance in hypoxia in a marine teleost. *Sci. Total Environ.* **849**, 157880. doi:10.1016/j.scitotenv.2022.157880

Pan, Y. K., Ern, R. and Esbaugh, A. J. (2016). Hypoxia tolerance decreases with body size in red drum *Sciaenops ocellatus*. *J. Fish Biol.* **89**, 1488-1493. doi:10.1111/jfb.13035

Pan, Y. K., Ern, R., Morrison, P. R., Brauner, C. J. and Esbaugh, A. J. (2017). Acclimation to prolonged hypoxia alters hemoglobin isoform expression and increases hemoglobin oxygen affinity and aerobic performance in a marine fish. *Sci. Rep.* **7**, 7834. doi:10.1038/s41598-017-07696-6

Pan, Y. K., Mandic, M., Zimmer, A. M. and Perry, S. F. (2019). Evaluating the physiological significance of hypoxic hyperventilation in larval zebrafish (*Danio rerio*). *J. Exp. Biol.* **222**, jeb204800. doi:10.1242/jeb.204800

Polymeropoulos, E. T., Elliott, N. G. and Frappell, P. B. (2016). The maternal effect of differences in egg size influence metabolic rate and hypoxia induced hatching in Atlantic salmon eggs: implications for respiratory gas exchange across the egg capsule. *Can. J. Fish. Aquat. Sci.* **73**, 1173-1181. doi:10.1139/cjfas-2015-0358

Regan, M. D. and Richards, J. G. (2017). Rates of hypoxia induction alter mechanisms of O2 uptake and the critical O2 tension of goldfish. *J. Exp. Biol.* **220**, 2536-2544. doi:10.1242/jeb.154948

Richards, J. G. (2010). Metabolic rate suppression as a mechanism for surviving environmental challenge in fish. *Prog. Mol. Subcell. Biol.* **49**, 113-139. doi:10.1007/978-3-642-02421-4_6

Robertson, C. E., Wright, P. A., Köblitz, L. and Bernier, N. J. (2014). Hypoxia-inducible factor-1 mediates adaptive developmental plasticity of hypoxia tolerance in zebrafish, *Danio rerio*. *Proc. Biol. Sci.* **281**, 20140637. doi:10.1098/rspb.2014.0637

Rummer, J. L. and Brauner, C. J. (2011). Plasma-accessible carbonic anhydrase at the tissue of a teleost fish may greatly enhance oxygen delivery: in vitro

evidence in rainbow trout, *Oncorhynchus mykiss*. *J. Exp. Biol.* **214**, 2319-2328. doi:10.1242/jeb.054049

Rummer, J. L., McKenzie, D. J., Innocenti, A., Supuran, C. T. and Brauner, C. J. (2013). Root effect hemoglobin may have evolved to enhance general tissue oxygen delivery. *Science* **340**, 1327-1329. doi:10.1126/science.1233692

Salin, K., Luquet, E., Rey, B., Roussel, D. and Voituron, Y. (2012). Alteration of mitochondrial efficiency affects oxidative balance, development and growth in frog (*Rana temporaria*) tadpoles. *J. Exp. Biol.* **215**, 863-869. doi:10.1242/jeb.062745

Salin, K., Auer, S. K., Rudolf, A. M., Anderson, G. J., Selman, C. and Metcalfe, N. B. (2016). Variation in metabolic rate among individuals is related to tissue-specific differences in mitochondrial leak respiration. *Physiol. Biochem. Zool.* **89**, 511-523. doi:10.1086/688769

Salin, K., Villasevil, E. M., Anderson, G. J., Lamarre, S. G., Melanson, C. A., McCarthy, I., Selman, C. and Metcalfe, N. B. (2019). Differences in mitochondrial efficiency explain individual variation in growth performance. *Proc. R. Soc. B* **286**, 20191466. doi:10.1098/rspb.2019.1466

Sampaio, E., Santos, C., Rosa, I. C., Ferreira, V., Pörtner, H.-O., Duarte, C. M., Levin, L. A. and Rosa, R. (2021). Impacts of hypoxic events surpass those of future ocean warming and acidification. *Nat. Ecol. Evol.* **5**, 311-321. doi:10.1038/s41559-020-01370-3

Seibel, B. A. and Deutsch, C. (2020). Oxygen supply capacity in animals evolves to meet maximum demand at the current oxygen partial pressure regardless of size or temperature. *J. Exp. Biol.* **223**, jeb210492. doi:10.1242/jeb.210492

Seibel, B. A., Andres, A., Birk, M. A., Burns, A. L., Shaw, C. T., Timpe, A. W. and Welsh, C. J. (2021). Oxygen supply capacity breathes new life into critical oxygen partial pressure (Pcrit). *J. Exp. Biol.* **224**, jeb242210. doi:10.1242/jeb.242210

Speers-Roesch, B., Richards, J. G., Brauner, C. J., Farrell, A. P., Hickey, A. J. R., Wang, Y. S. and Renshaw, G. M. C. (2012). Hypoxia tolerance in elasmobranchs. I. Critical oxygen tension as a measure of blood oxygen transport during hypoxia exposure. *J. Exp. Biol.* **215**, 93-102. doi:10.1242/jeb.059642

Stieglitz, J. D., Mager, E. M., Hoenig, R. H., Benetti, D. D. and Grosell, M. (2016). Impacts of *Deepwater Horizon* crude oil exposure on adult mahi-mahi (*Coryphaena hippurus*) swim performance. *Environ. Toxicol. Chem.* **35**, 2613-2622. doi:10.1002/etc.3436

Tudorache, C., Viaene, P., Blust, R., Vereecken, H. and De Boeck, G. (2008). A comparison of swimming capacity and energy use in seven European freshwater fish species. *Ecol. Freshw. Fish* **17**, 284-291. doi:10.1111/j.1600-0633.2007.00280.x

Vagner, M., Zambonino-Infante, J.-L. and Mazurais, D. (2019). Fish facing global change: are early stages the lifeline? *Mar. Environ. Res.* **147**, 159-178. doi:10.1016/j.marenvres.2019.04.005

Vanderplancke, G., Claireaux, G., Quazuguel, P., Madec, L., Ferraresso, S., Sévère, A., Zambonino-Infante, J.-L. and Mazurais, D. (2015). Hypoxic episode during the larval period has long-term effects on European sea bass juveniles (*Dicentrarchus labrax*). *Mar. Biol.* **162**, 367-376. doi:10.1007/s00227-014-2601-9

Weltzien, F.-A., Døving, K. B. and Carr, W. E. S. (1999). Avoidance reaction of yolk-sac larvae of the inland silverside *Menidia beryllina* (Atherinidae) to hypoxia. *J. Exp. Biol.* **202**, 2869-2876. doi:10.1242/jeb.202.20.2869

Widmer, S., Moore, F. B.-G. and Bagatto, B. (2006). The effects of chronic developmental hypoxia on swimming performance in zebrafish. *J. Fish Biol.* **69**, 1885-1891. doi:10.1111/j.1095-8649.2006.01242.x

Wood, A. T., Clark, T. D., Andrewartha, S. J., Elliott, N. G. and Frappell, P. B. (2017). Developmental hypoxia has negligible effects on long-term hypoxia tolerance and aerobic metabolism of atlantic salmon (*Salmo salar*). *Physiol. Biochem. Zool.* **90**, 494-501. doi:10.1086/692250

Zambie, A. D., Ackerly, K. L., Negrete, B., Jr and Esbaugh, A. J. (2024). Warming-induced “plastic floors” improve hypoxia vulnerability, not aerobic scope, in red drum (*Sciaenops ocellatus*). *Sci. Total Environ.* **922**, 171057. doi:10.1016/j.scitotenv.2024.171057

Zambonino-Infante, J. L., Mazurais, D., Dubuc, A., Quéau, P., Vanderplancke, G., Servili, A., Cahu, C., Le Bayon, N., Huelvan, C. and Claireaux, G. (2017). An early life hypoxia event has a long-term impact on protein digestion and growth in juvenile European sea bass. *J. Exp. Biol.* **220**, 1846-1851. doi:10.1242/jeb.154922