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RESEARCH ARTICLE

Hypoxia tolerance is unrelated to swimming metabolism of wild, juvenile striped bass (*Morone saxatilis*)

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ABSTRACT

Juvenile striped bass residing in Chesapeake Bay are likely to encounter hypoxia that could affect their metabolism and performance. The ecological success of this economically valuable species may depend on their ability to tolerate hypoxia and perform fitness-dependent activities in hypoxic waters. We tested whether there is a link between hypoxia tolerance (HT) and oxygen consumption rate ($\dot{M}_{O_2}$) of juvenile striped bass measured while swimming in normoxic and hypoxic water, and to identify the interindividual variation and repeatability of these measurements. HT (loss of equilibrium) of fish (N=18) was measured twice collectively, 11 weeks apart, between which $\dot{M}_{O_2}$ was measured individually for each fish while swimming in low flow (10.2 cm s^{-1}) and high flow (~67% of critical swimming speed, U_{crit}) under normoxia and hypoxia. Both HT and $\dot{M}_{O_2}$ varied substantially among individuals. HT increased across 11 weeks while the rank order of individual HT was significantly repeatable. Similarly, $\dot{M}_{O_2}$ increased in fish swimming at high flow in a repeatable fashion, but only within a given level of oxygenation. $\dot{M}_{O_2}$ was significantly lower when fish were swimming against high flow under hypoxia. There were no clear relationships between HT and $\dot{M}_{O_2}$ while fish were swimming under any conditions. Only the magnitude of increase in HT over 11 weeks and an individual's $\dot{M}_{O_2}$ under low flow were correlated. The results suggest that responses to the interacting stressors of hypoxia and exercise vary among individuals, and that HT and change in HT are not simple functions of aerobic metabolic rate.

KEY WORDS: Metabolic rate, Exercise, Oxygen consumption rate

INTRODUCTION

In Chesapeake Bay, USA, bottom hypoxic or anoxic (dead) zones are expanding on an annual basis, challenging the ability of aerobic animals to find suitable habitat (Kraus et al., 2015). Weather and currents can drive these severely hypoxic bottom waters into the usually oxygenated surface regions to which the commercially and recreationally valuable species striped bass, *Morone saxatilis* (Walbaum 1972), has become restricted (Breitburg, 1992; Kemp et al., 2005; Pihl et al., 1991; Scully, 2016a; Scully, 2016b). As these fish require oxygen to accomplish energy-intensive and fitness-dependent tasks such as foraging, predator evasion, digestion and growth, the performance of these tasks might be diminished under hypoxia to a level that compromises their Darwinian fitness (e.g. Deutsch et al., 2015; Holt and Jørgensen, 2015). Therefore, if we are to assess future prospects for this fish, we need to study the ability of individuals to perform fitness-dependent tasks under commonly observed levels of hypoxia and the energetic costs of these tasks.

Striped bass exposed to rapid environmental O_2 depletion sometimes die (Rice et al., 2013). More likely, they will avoid encroaching hypoxia by swimming towards more oxygenated locations (Kraus et al., 2015). However, it is not uncommon for striped bass juveniles to be exposed to O_2 levels near 20% air saturation or even lower (<2 mg O_2 l^{-1}; Breitburg, 1992; Testa et al., 2017). Physiological responses to hypoxia while swimming can be quite different from those at rest and can vary substantially among individuals (McKenzie et al., 2007; Nelson and Lipkey, 2015), but could be more ecologically relevant for a species like striped bass that is continuously active. The effects of hypoxia on sustained swimming physiology, locomotion and behaviour has been studied in fish (Chapman and Mckenzie, 2009; Claireaux and Chabot, 2016; Domenici et al., 2013; Weber et al., 2016), but the interrelationship between hypoxia tolerance (HT) and physiological performance under hypoxia is poorly known and directly addressed here.

Oxygen consumption rate ($\dot{M}_{O_2}$) represents a proxy for aerobic metabolic rate in fish (Nelson and Chabot, 2011) and is used to gauge performance efficiency (Fry, 1971; Secor, 2011). All energy-requiring activities, including swimming, must trade-off within an animal's available aerobic energy budget (Holt and Jørgensen, 2015; Pörtner and Knust, 2007) to avoid unsustainable anaerobic metabolism. So, maintenance of $\dot{M}_{O_2}$ while swimming in hypoxic waters may be critical for the success of striped bass in Chesapeake Bay. Thus, both absolute hypoxia tolerance and metabolic responses to hypoxia while swimming could be informative and possibly predictive of striped bass success during hypoxic events.

HT, $\dot{M}_{O_2}$ under hypoxia and swimming performance in hypoxic water are potentially linked through the various steps of the oxygen cascade, e.g. oxygen uptake at the gill, blood oxygen transport, cardiac function, tissue oxygen extraction from blood and mitochondrial oxygen demand (Farrell, 2009; Hochachka et al., 1996). All the steps of the oxygen cascade have the partial pressure of oxygen (P_{O_2}) as a driving force (Weibel et al., 1991) – a force that is liable to be diminished under hypoxia. Whilst these steps are functionally linked in an animal, differential function at any given level may be manifest in different ways at the whole-animal level. For instance, improved gas exchange at the respiratory surface, more efficient convective transport of blood and more efficient uptake and utilization of oxygen by mitochondria are all going to improve both HT (e.g. Chapman et al., 2002; Crans et al., 2015; Nilsson, 2007; reviewed by Mandic and Regan, 2018) and aerobic swimming performance (Petersen and Gamperl, 2010), yet possibly trade-off with other physiological functions. However, increasing haemoglobin (Hb) oxygen affinity (decrease in P_{50}, the P_{O_2} at which

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Hb is 50% saturated with O_2; Mandic et al., 2008) or blood [Hb] or haematocrit might improve hypoxia tolerance (Nelson et al., 2019) but could diminish swimming performance through decreased oxygen unloading at the tissues or increases in blood viscosity. In contrast, differential allocation of available blood to tissues critical to a swimming effort could improve performance at the time (Axelsson and Fritsche, 1991), but could affect the health of tissues deprived of blood, thereby impacting future hypoxia tolerance.

Swimming performance under hypoxia, HT and oxygen demand are also plastic traits in many fish and can change in response to the environment (Domenici et al., 2013; Fu et al., 2011; Fu et al., 2014; Killen et al., 2013; Killen et al., 2016; Norin et al., 2016). These acclimation responses are likely to be as important as physiological capacity when coping with episodic hypoxia and may involve substantial allocation of metabolic resources. Thus, we hypothesized that plasticity of HT could also trade-off with an individual's aerobic swimming capacity, and investigated how well striped bass can maintain $\dot{M}_{O_2}$ while swimming in hypoxic waters and how this relates to an individual's HT and ability to acclimate to hypoxia.

We posed three main questions: (1) is the substantial variation in HT (Nelson and Lipkey, 2015; Nelson et al., 2019) among individual striped bass of similar size a function of their $\dot{M}_{O_2}$ during routine low speed or vigorous swimming?; (2) as HT while swimming appears to be unrelated to HT at rest (Nelson and Lipkey, 2015), do individuals change their $\dot{M}_{O_2}$ uniformly in response to changing metabolic demand when swimming in either normoxic or hypoxic conditions?; and (3) are HT and the increase in HT with repetitive hypoxia exposure (Nelson and Lipkey, 2015; Nelson et al., 2019) related to a fish's oxygen consumption rate under either routine or vigorous swimming conditions? To answer these questions, we measured HT twice, collectively, in a cohort of striped bass captured at the same time and place; between these two tests, we measured oxygen consumption of each individual at two different swimming speeds under both hypoxic and normoxic conditions. As variation provides raw material for natural selection and repeatability can set the upper limit to heritability (Dohm, 2002), we also analysed the repeatability of each metric. Our results will help identify phenotypes that are best able to cope with future hypoxic events and whether there are relationships or trade-offs between hypoxia tolerance and metabolic performance.

MATERIALS AND METHODS

Fish collection and maintenance

Fish (N=18, mixed sex; for fish size, see Table 1) were collected by beach seine from the Potomac River near its confluence with Chesapeake Bay and transported in river water (temperature 26°C, salinity 6 ppt) to Towson University. Fish were acclimated to lab conditions by lowering the temperature by 2.5°C per day, gradually switched over to a mixture of Baltimore city tap water and commercial sea salts and kept in three 285 l tanks (N=6 per tank) at a temperature of 18.7±0.7°C (mean±s.d.) and a salinity of 10–11 ppt with a photoperiod cycle of 12 h light:12 h dark. Water quality was maintained with weekly water exchanges (30–40% total volume water) and water quality was monitored regularly ($[NH_3/NH_4^+]$=0 ppt, $[NO_2^-]$=0.28±1.7 ppt and $[NO_3^-]$=62.9±33.62 ppt; means±s.d.). Fish were fed once daily with commercial food (Hikari® tropical food sticks), at least 6 times a week. After 10 weeks in the laboratory, all individuals were anaesthetized with MS-222 (100 mg l^{-1}, buffered 1:1 with $NaHCO_3$), weighed (g), measured (cm; total length and fork length) and tagged with a passive integrated transponder (PIT-tag; Biomark® Inc.) for identification purposes. Experimentation began 2 months after tagging. Fish size at each test and the time between tests are summarized in Table 1.

Table 1. Size parameters of the fish as they progressed through the experiment

	N	Mass (g)	Total length (mm)	Relative time of the test (days)
HCT1	18	23.18	132.6	0
		15.6–31.3	119–145	
HCT2	18	37.34	156.7	83
		21.2–57.9	134–179	
Swim test	17[a]	29.56	135.4	51
		18.2–46.6	127–157	31–72

HCT, hypoxia challenge test. Data are means and range.
[a]One individual did not complete the swim test but was used in both HCT tests.

Hypoxia challenge tests

Group hypoxia challenge tests (HCT) were performed twice, at the beginning of the experiment, ∼4 weeks before the first individual respirometry trial (see 'Respirometry', below: HCT1), and 10 days after the last respirometry trial (HCT2). Thus, these two hypoxia challenges were conducted 11 weeks apart (Table 1, Fig. 1). The methods described below pertain to both HCTs. All individuals (N=18) were transferred to the experimental tank (190 l) without air exposure and were allowed to habituate for 24 h. Feeding was discontinued 24 h before transfer and while in the experimental tank (temperature: HCT1 18.8°C, HCT2 18.0°C; salinity: HCT1 and HCT2 10 ppt). Oxygen levels at the start of HCT1 and HCT2 were 92% air saturation (AS), which was reduced to an ecologically relevant 10±2% AS over 1 h by bubbling nitrogen gas directly into the tank (Fabrizio et al., 2017). This rate of oxygen decrease reflects the most rapid hypoxia incursions that have been measured in Chesapeake Bay neritic waters (Breitburg, 1992). Two calibrated oxygen-sensing optodes (PreSens®) were used to monitor oxygen concentration during experimentation; % AS was recorded using a WITROX 4 (Loligo® Systems) interfaced to a computer running WitroxCTRL v.1 (Loligo® Systems) software. Oxygen concentration was maintained at 10.82±0.74% AS and 10.23±0.30% AS (means±s.d.) for 4 h during HCT1 and HCT2, respectively. No individuals lost equilibrium by this time, so oxygen concentration was further lowered at an average rate of 1.03% AS h^{-1} during HCT1, and 0.86% AS h^{-1} during HCT2 until they did. At the point of loss of equilibrium (LOE; when an individual fish could not maintain an upright position for ∼10 s), the fish was immediately removed from the experimental tank, identified, placed in fully oxygenated water, and the time of the LOE recorded to the closest minute. All individuals fully recovered from both HCTs and were also weighed and measured at this time. Recorded times were used to determine each individual's HT, expressed as cumulative oxygen deficit (D_{CO}) (Nelson and Lipkey, 2015). Briefly, to calculate the D_{CO}, when oxygen concentration (% AS) is plotted as a function of time, D_{CO} is the difference between the area under the hypothetical curve in normoxic water (initial % AS at the beginning of the experiment) and the actual % AS until LOE (Nelson and Lipkey, 2015). D_{CO} for each individual was calculated by summing integrated areas between every two time points (1 s) and is reported as per cent multiplied by time (% h).

Respirometry

Oxygen consumption was measured in a Brett-type swim tunnel-respirometer (Loligo® Systems) with a working area of 28×7.8×8.2 cm (l×w×d) and total volume of 5.25 l (measured empirically). The

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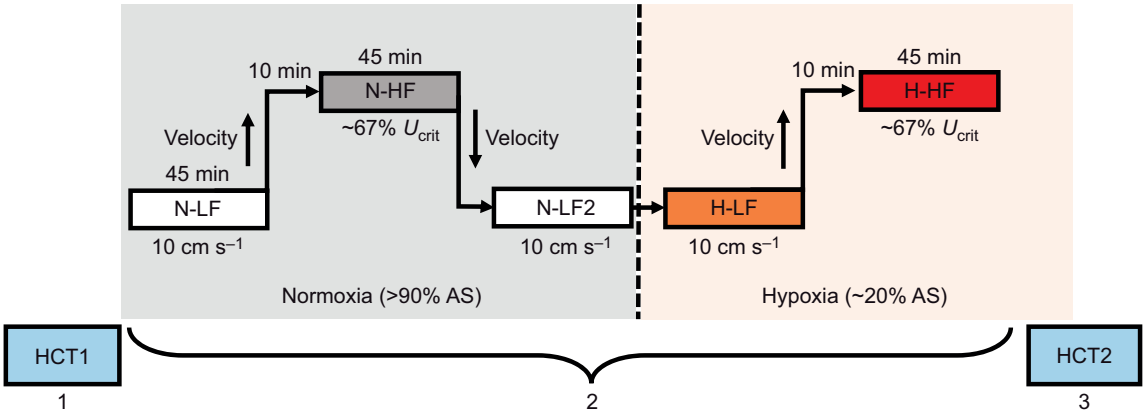

Fig. 1. Experimental timeline for the hypoxia challenge and swim tests. The first hypoxia challenge test (HCT1) (1) was performed 4 weeks prior to the first swim–oxygen consumption test (2), and the second hypoxia challenge test (HCT2) (3) was performed 10 days after the last fish was tested in a swim–oxygen consumption test. Between the two HCTs (blue boxes), randomly chosen fish were subjected to the swim test. An individual was first acclimated for 24 h at low flow (10.2 cm s^{-1}) and normoxia. All oxygen consumption periods consisted of three cycles of 10 min of measurement and a 5 min flush. Grey shaded areas indicate normoxic conditions, orange shaded areas indicate hypoxic conditions. Oxygen consumption ($\dot{M}_{O_2}$) was first measured at low flow (10.2 cm s^{-1}) in normoxia (normoxia-low flow, N-LF; white box), then the water velocity was increased to 67% critical swimming speed (U_{crit}) at a rate of 6 cm s^{-1} min^{-1} while reoxygenating the swim tunnel-respirometer. The fish was then held in the high-flow condition for 10 min before $\dot{M}_{O_2}$ was again measured 3 times (normoxia-high flow, N-HF; dark grey box). Water velocity was then decreased to 10.2 cm s^{-1} at a rate of 6 cm s^{-1} min^{-1} and $\dot{M}_{O_2}$ was re-measured (normoxia-low flow, N-LF2; white box). Finally, the oxygen level in the system was decreased to 20% air saturation (AS) over 1 h and the procedure was repeated (hypoxia-low flow, H-LF, orange box; hypoxia-high flow, H-HF, red box), except that there was no repeat low-flow swim test under hypoxia. Abbreviations and colour scheme are consistent across all figures.

swim tunnel-respirometer bath was supplied with UV-sterilized (Vecton®) and biologically filtered water of the same composition as the holding tanks. Water temperature was maintained at 19.8±1°C with a circulating water bath (Polytemp, Polyscience®).

An individual was randomly selected by drawing lots and then transferred to the swim tunnel-respirometer without air exposure and acclimated for 24 h at a current velocity of 10.2 cm s^{-1}. The working area containing the fish was darkened at all times to minimize stress. All respirometry tests were 45 min long and consisted of three cycles of a 10 min measuring period interspersed with a 5 min flush. After acclimation, the initial $\dot{M}_{O_2}$ (µmol O_2 min^{-1}) was measured in normoxic water at low flow (0.2 cm s^{-1}; normoxia-low flow, N-LF test; Fig. 1). Following the N-LF measurement period, the water velocity was increased at a rate of 6 cm s^{-1} min^{-1} to the high-flow (HF) regimen of 28.9±1.7 cm s^{-1} [velocity equivalent to 67±3.5% of an individual's estimated critical swimming speed (U_{crit}) as determined from a dataset on largemouth bass, *Micropterus salmoides*, of similar size swum at the same temperature; Beamish, 1970; Fig. 1]. The fish were allowed to adjust to the high-flow conditions for 10 min before $\dot{M}_{O_2}$ was again measured (normoxia-high flow, N-HF test; Fig. 1). Next, the current was returned to the LF velocity at the same rate (6 cm s^{-1} min^{-1}), and $\dot{M}_{O_2}$ was immediately measured to assess recovery from the HF exercise (N-LF2 test; Fig. 1). Following this measurement period, the oxygen concentration in the water was lowered to approximately 20% AS over a period of 1 h; N_2 gas was bubbled in the external water bath, keeping fish undisturbed, and oxygen was monitored in the swim tunnel-respirometer working area (oxygen-sensing optodes; PreSens®) as well as outside in the water bath (galvanic oxygen-sensing probe; OxyGuard Mini Probe, Loligo® Systems) to ensure a homogeneous O_2 environment throughout the system. The same experimental procedure as described above was repeated under hypoxic conditions (hypoxia-low flow, H-LF; hypoxia-high flow, H-HF), except that a second set of low-flow measurements under hypoxia was not made to avoid exposing the fish to excessive hypoxia. Fish were immediately removed from the working area after completion of the H-HF measurements. One fish was excluded from the swimming analysis because it refused to swim in the respirometer. Oxygen levels were maintained between 93.5% and 100% AS (mean: 97.7% AS) in the normoxia tests (N-LF, N-HF, N-LF2; Fig. 1), and between 17% and 26% AS (mean: 20.6% AS) in the hypoxia tests (H-LF, H-HF; Fig. 1). Before being returned to their holding tank, each individual was anaesthetized, measured and weighed as described above. Fig. 1 shows the experimental work flow and abbreviations for the experimental periods. Bacterial oxygen consumption was measured after each fish's swimming trials and each individual's $\dot{M}_{O_2}$ was corrected to account for this (see 'Data and statistical analysis', below).

Data and statistical analysis

All data and statistical analyses were done using R v3.5.1 (2018). $\dot{M}_{O_2}$ (µmol O_2 min^{-1}) was calculated as: $\dot{M}_{O_2}=(\Delta[O_2]_{fish}-\Delta[O_2]_{background})\times(V_T-M)$, where $\Delta[O_2]$ is the change in oxygen concentration in water over time (µmol l^{-1} min^{-1}), V_T is the volume of the swim tunnel-respirometer (5.25 l) and M is the mass of the fish (kg, assuming a fish density of 1 kg l^{-1}). First, the significance of correlations between mean $\dot{M}_{O_2}$ from each test and individual size (g) was determined using Pearson's correlation, and Shapiro–Wilk and Levene's tests were used to test for normality and heteroscedasticity of $\dot{M}_{O_2}$ measurements. To meet the parametric assumptions for the linear correlation between $\dot{M}_{O_2}$ and mass (g), data were $\log_{10}$ transformed. $\dot{M}_{O_2}$ was then size corrected to the mean fish size of our sample (29.3 g; range: 18.2–46.6 g) using a scaling coefficient of 0.76 for bentho-pelagic fish (Killen et al., 2010). Cost of transport (COT, µmol O_2 m^{-1}) was calculated as COT=$\dot{M}_{O_2}\times$speed^{-1}, which was used to estimate swimming costs across different conditions.

The rank order repeatability of hypoxia tolerance (HT; D_{CO}) across 11 weeks and 4 days was statistically determined using Spearman's rank order test. The non-parametric test was used to estimate the repeatability or stability in each individual's relative rank position in each HCT to account for changes in HT over time.

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The significance between mean HT over 11 weeks and three hypoxia exposures was tested with a paired *t*-test. We used Pearson's correlation to estimate the correlation between mean $\dot{M}_{O_2}$ of tests across the same flow conditions, and Spearman's rank order correlation between low- and high-flow tests. The correlation of three $\dot{M}_{O_2}$ values within each test was assessed with Pearson's correlation. Finally, the coefficient of variation [CV=(s.d./mean)×100] was calculated for all tests (HT and respirometry).

We examined the effects of flow and oxygen levels on individual $\dot{M}_{O_2}$ and COT using linear mixed effect models (lme4 package; Bates et al., 2015). The dependent variable was the average of three $\dot{M}_{O_2}$ measurements for each test. The independent variables were oxygen level (N, H) and flow (LF, HF) and were tested as independent predictors with and without interactions between them. Individual was included as a random effect to account for the repeated measures design; both slopes and intercepts were allowed to vary among individuals. Fits of the models were assessed visually by residual plots and candidate models were selected using Akaike's information criterion corrected for small sample size (MuMIn package; https://CRAN.R-project.org/package=MuMIn); only models with ΔAICc<2 were considered (Burnham and Anderson, 2002). Finally, we tested significance levels of fixed effects and their interaction using an ANOVA with Satterthwaite approximation for degrees of freedom (lmerTest package; Kuznetsova et al., 2017), and Tukey's HSD *post hoc* analyses (emmeans package; https://CRAN.R-project.org/package=emmeans). A significance level of α=0.05 was used for all tests. All methods were approved by Towson University's Institutional Animal Care and Use Committee (IACUC protocol # 1611000152).

RESULTS

Hypoxia tolerance

Mean HT, expressed as cumulative oxygen deficit (D_{CO}), of juvenile striped bass was 529.88% h (range: 348.53–642.31% h) at the beginning of the experiment and 657.33% h (445.05–841.81% h) at the end. The change in hypoxia tolerance (ΔHT) was defined as the change in each individual's HT over the 11 weeks between HCT1 and HCT2 with one intervening hypoxia exposure of lesser severity. The mean (±s.e.m.) ΔHT of each individual was 127.44±24.58% h, or a 24% increase. The average fish spent about two more hours under hypoxia in the second HCT before losing equilibrium (HT expressed in time to LOE: HCT1 range: 4 h 42 min to 8 h 10 min; HCT2 range: 5 h 55 min to 10 h 54 min). Sixteen of the 18 individuals were more hypoxia tolerant at the second HCT and ΔHT was significantly raised at the second HCT (paired *t*-test: t_{17}=−5.18, *P*<0.001; Fig. 2). The rank order of HT was significantly repeatable over 11 weeks (Spearman's ρ=0.59, *P*=0.012; Fig. 2), and it varied substantially between individuals (HCT1 CV=14.14; HCT2 CV=18.98). Neither HT in either test nor ΔHT significantly correlated with an individual's body mass or total length.

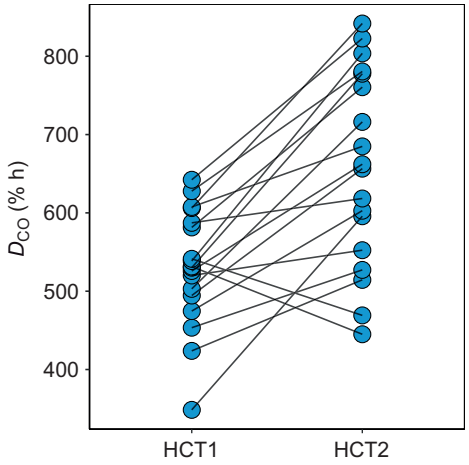

Fig. 2. Hypoxia tolerance of juvenile striped bass measured 11 weeks apart. Hypoxia tolerance (HT) was measured as the time to loss of equilibrium during the hypoxia challenge tests (HCT1 and HCT2), and expressed as cumulative oxygen deficit (D_{CO}, % h; Nelson and Lipkey, 2015). Lines connect the same individual (*N*=18). All but two individuals increased their hypoxia tolerance, and the relationship between HCT1 and HCT2 was significantly repeatable (Spearman's ρ=0.59, *P*=0.012).

$\dot{M}_{O_2}$ and swimming

Oxygen consumption was predictably influenced by flow, and how much an animal increased its oxygen consumption at high flow varied according to ambient oxygen level. This was manifested by a significant interaction term between flow and oxygen level (ANOVA: oxygen×flow, $F_{1,51}$=21.22, *P*<0.001; Fig. 3A,B). In addition to the significant difference between oxygen consumption at high and low flow for a given level of oxygenation, *post hoc* pairwise comparisons revealed a significantly lower oxygen uptake under hypoxia than under normoxia at high flow (Fig. 3B). Despite this reduced oxygen uptake, all but one of the fish was able to maintain position in the swim tunnel-respirometer for 50 min when swimming at an estimated 2/3 of U_{crit} and an [O_2] of 20% air saturation. $\dot{M}_{O_2}$ was also significantly repeatable between the two low-flow tests in normoxic water separated by the high-flow normoxia measurement (Pearson's *r*=0.67, *P*=0.003).

Considerable inter-individual variation in $\dot{M}_{O_2}$ was found for all respirometry tests (CV: 12.8–20.2). However, an individual's rank order of $\dot{M}_{O_2}$ was significantly repeatable between the low-flow and high-flow trials, but only within a given oxygen level (H-LF versus H-HF: Spearman's ρ=0.62, *P*=0.009; N-LF versus N-HF: ρ=0.58, *P*=0.017). This means that individuals with higher $\dot{M}_{O_2}$ in low flow also had higher $\dot{M}_{O_2}$ in high flow, suggesting a relatively constant metabolic response to increasing flow across the 17 individuals, but only when compared within a given level of water oxygenation. When correlating the $\dot{M}_{O_2}$ across the same flow condition but at different oxygen levels, there was no significant relationship, which shows that the rank order of oxygen consumption while swimming in these fish was shuffled as a result of their differential response to water with oxygen levels of 20% AS.

Both oxygen level and swimming speed were significant predictors of the metabolic cost to cover a fixed distance (COT; ANOVA: flow, $F_{1,51}$=400.15, *P*<0.001; oxygen, $F_{1,17.49}$=22.50, *P*<0.001). There was no interaction between oxygen level and swimming speed in predicting COT. The COT was lower if fish were swimming at ∼67% of their estimated U_{crit}, but higher under normoxia than under hypoxia (Fig. 3C,D).

Hypoxia tolerance, $\dot{M}_{O_2}$ and swimming

There was only a weak association between HT and any of the oxygen consumption measurements made (Fig. 4). The only significant relationships were between the increase in HT over 11 weeks (ΔHT) and $\dot{M}_{O_2}$ at low swimming speed (ΔHT versus $\dot{M}_{O_2}$ N-LF: *r*=−0.50, d.f.=15, *N*=17, *P*=0.042; and ΔHT versus pooled $\dot{M}_{O_2}$ N-LF and H-LF: *r*=−0.42, d.f.=32, *N*=34, *P*=0.013; Fig. 4C). The relationship between ΔHT and $\dot{M}_{O_2}$ at low swimming speed under hypoxia approached significance (*r*=−0.44, *P*=0.078; Fig. 4C). No other relationships between any HT measurement and any respirometry test were significant (Fig. 4A,B).

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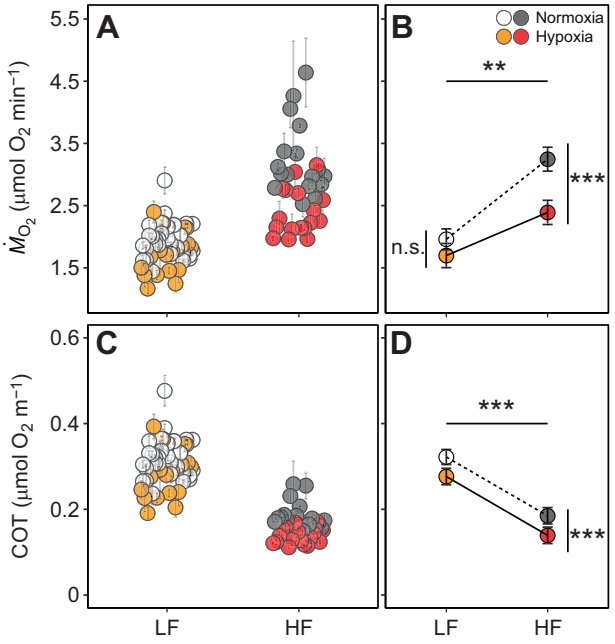

Fig. 3. Oxygen consumption ($\dot{M}_{O_2}$) and cost of transport (COT) under different environmental oxygen and flow conditions. The two flow conditions were low flow (LF, 10.2 cm s^{-1}) and high flow [HF; 67% of each individual's estimated U_{crit} (~29 cm s^{-1})]. (A) Mean $\dot{M}_{O_2}$ for each individual in each flow and oxygen condition (N=17 individuals; n=3 consecutive measurements of $\dot{M}_{O_2}$, except for N-LF, where n=6; see Materials and Methods; grey bars are s.e.m.). (B) Sample estimated mean for each swim test with 95% confidence level bounds and statistics. (C,D) COT of the same fish from the same swim tests (C) and sample estimated mean (D). Results from linear mixed models and *post hoc* tests: in B, flow and oxygenation level had a significant interactive effect on swimming $\dot{M}_{O_2}$ (linear mixed models: P<0.0001, pairwise *post hoc* comparisons indicated a significant difference in $\dot{M}_{O_2}$ across all levels (**P<0.01, ***P<0.001), except for $\dot{M}_{O_2}$ between N-LF and H-LF (ns, P=0.117); in D, COT significantly differed between flow and oxygenation levels (***P<0.001).

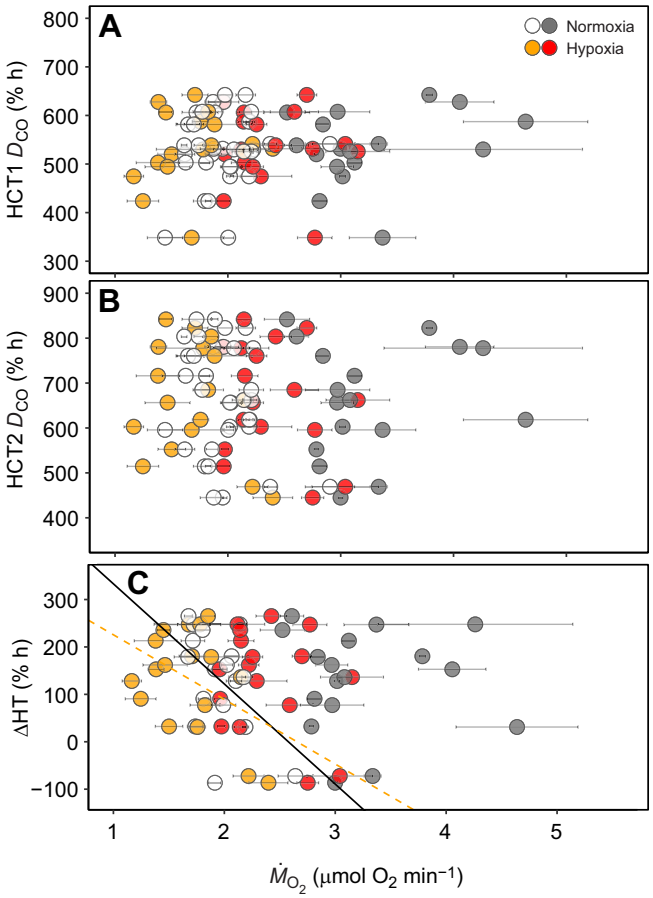

Fig. 4. Relationships between HT and $\dot{M}_{O_2}$ of juvenile striped bass. HT was measured twice (HT1 and HT2, 11 weeks later) as time to loss of equilibrium and is expressed as cumulative oxygen deficit (D_{CO}). (A–C) HT measured at the first (A) and at the second (B) hypoxia challenge test as a function of $\dot{M}_{O_2}$ during swimming, and (C) the change in hypoxia tolerance (ΔHT) of each individual and their $\dot{M}_{O_2}$ during swimming. Each circle is the mean of n=3 consecutive $\dot{M}_{O_2}$ measurements for an individual (A and B have 2 N-LF time points; see Fig. 1). In C, the white circles (N-LF) are the means of n=6 measurements (see Fig. 1 and Results for more details). The grey bars are s.e.m. in all panels. The solid line shows the significant correlation (r=−0.50, N=17, P=0.04) between $\dot{M}_{O_2}$ in N-LF and ΔHT; the dashed line represents a correlation (r=−0.44, N=17, P=0.08) between $\dot{M}_{O_2}$ in H-LF and ΔHT.

DISCUSSION

Environmental hypoxia will continue to be one of the most common stressors that fish have to deal with in the near future (Breitburg et al., 2018; Claireaux and Chabot, 2019; IPCC, 2014). Striped bass is a commercially and culturally valuable species that is currently being impacted by encroaching hypoxic zones in the primary nursery ground, Chesapeake Bay, and elsewhere. As anthropogenic hypoxic waters expand, hypoxia tolerance has become a frequent metric used to assess the ability of fishes to persist in a particular environment (e.g. Claireaux and Chabot, 2019; Joyce et al., 2016; Mandic et al., 2008; Rees and Matute, 2018; Roze et al., 2013). Yet, for active, pelagic fishes like striped bass, performance under hypoxia tolerance may be a more relevant metric (e.g. Kraskura and Nelson, 2018). HT also appears to be a highly plastic and context-dependent physiological trait in fishes and it varies substantially among individuals of the same size, age and species (Nelson et al., 2019; Nelson and Lipkey, 2015; Killen et al., 2016). Our previous work on striped bass determined that hypoxia is significantly repeatable among individuals, but increases with repetitive laboratory exposure to hypoxia (Nelson et al., 2019); we have also found that HT varies between individuals measured at rest versus that same animal measured while swimming (Nelson and Lipkey, 2015). The physiological trade-offs that underlie interindividual variability in hypoxia tolerance at different activity levels in fish and how they relate to other oxygen-dependent processes are unknown. Before embarking on studies of the mechanistic bases of differential HT and performance under hypoxia, it is necessary to first gauge the variability, repeatability and inter-relatedness of these measures themselves. Below, we address several measures of oxygen-related performance in the same individual, separately, and then link them.

Hypoxia tolerance

Although the active aerobic lifestyle of striped bass would suggest intolerance to hypoxia (Dixon et al., 2017), our work suggests a relatively high tolerance to hypoxia when compared with other active, pelagic species. For example, Atlantic cod, *Gadus morhua*, juveniles have 100% mortality at 10% AS (Plante et al., 1998) and Atlantic menhaden, *Brevoortia tyrannus*, had a 50% mortality rate at 10% AS (Burton et al., 2011). Juveniles of the co-familiar European sea bass, *Dicentrarchus labrax*, had similar HT to striped bass, losing equilibrium at AS between 9% and 3% when tested using techniques similar to those in this study (LOE; Claireaux et al., 2013; Zhang et al., 2017). As juvenile European sea bass and

striped bass both occupy estuaries that become seasonally hypoxic, their historic exposure to hypoxia possibly accounts for their exceptional tolerance to low oxygen when compared with other pelagic, marine fishes (Mandic and Regan, 2018).

HT varied substantially amongst this cohort of wild juvenile striped bass captured at the same time and place. Despite this variation and increase in HT with repetitive exposure (see below), the rank order of HT was repeatable when comparing HCT1 with HCT2 (11 weeks). The average CV of 16.6 was greater than that reported for farm-raised rainbow trout, *Oncorhynchus mykiss* (Roze et al., 2013), but lower than that of two other wild-caught striped bass cohorts (CV=34.3: Nelson and Lipkey, 2015; and CV=65.3: Nelson et al., 2019). Previously, significant repeatability of HT was found in *D. labrax* (Claireaux et al., 2013; Joyce et al., 2016), Gulf killifish, *Fundulus grandis* (Rees and Matute, 2018), and the Atlantic croaker, *Micropogonias undulates* (Pang et al., 2015). However, introduction of a potentially synergistic physiological challenge (e.g. oil exposure) has been shown to disrupt the repeatability of HT in *D. labrax* (Zhang et al., 2017). Repeatable inter-individual variance is increasingly recognized as a valuable trait in ecological and evolutionary physiology (Ballew et al., 2017; Killen et al., 2016; Norin and Malte, 2012; Rees and Matute, 2018) and may be considered a harbinger of heritability (Dohm, 2002; Killen et al., 2016). As we have here and elsewhere (Nelson and Lipkey, 2015; Nelson et al., 2019) measured substantial variability in HT that is repeatable across multiple hypoxia exposures, it is likely that juvenile striped bass are undergoing *in situ* acclimation when exposed to hypoxia in the wild. This can improve their chances of success with subsequent hypoxia exposures (but see Gamperl, 2004; Overgaard et al., 2004), which occur in high frequency and severity in summertime in Chesapeake Bay (Testa et al., 2017).

In the current study, HT in juvenile striped bass increased an average of 24% in a third hypoxia exposure after previous exposures to 10% and 20% AS. Our lab has previously reported a 600% increase in HT resulting from ∼4 exposures to 10% AS (Nelson et al., 2019). During acute hypoxia exposure, fish can engage in a variety of responses to defend their aerobic capacity (Mandic and Regan, 2018). These responses can be (i) behavioural: avoidance, agitation, surface respiration (Cook et al., 2013; Damsgård et al., 2019; Dixon et al., 2017; Domenici et al., 2000); (ii) physiological: increased ventilation rate, increased heart rate, lower cardiac output (Petersen and Gamperl, 2010) or release of red blood cells (Wang et al., 2017); (iii) biochemical: increased activity of metabolic enzymes (Borowiec et al., 2018; Crans et al., 2015; Omlin and Weber, 2010); or (iv) molecular: changes in gene expression and regulation (Hochachka et al., 1996; Wu, 2002). These responses vary with the length and severity of hypoxia exposure (Mandic and Regan, 2018). Changes to any or all of these could be responsible for the observed increase in HT here. While not measured in these fish, we did see a strong correlation between HT and blood oxygen carrying capacity of striped bass in a previous study (Nelson et al., 2019); other studies have also implicated increases in blood oxygen carrying capacity (Petersen and Gamperl, 2011; Val et al., 1990). In several fish species, increased gill surface area appears to be one of the key plastic traits that can improve HT (Fu et al., 2011; Nilsson, 2007; Timmerman and Chapman, 2003). Improved tolerance to hypoxia also appears to be associated with increased anaerobic enzyme capacity in the heart (Borowiec et al., 2016; Cook et al., 2013; Crocker et al., 2013), the brain (Mandic et al., 2013), muscle (Davies et al., 2011) and type I (red) skeletal muscle (Cook et al., 2013). Across these studies, the global mechanism behind this relatively uniform increase in HT with intermittent hypoxia exposure is still to be resolved in fish, but these are undoubtedly polygenic traits (Zhou and Haddad, 2013), the expression of which is modified by exposure to low oxygen levels. A clue from the present study is that the increase in each individual's hypoxia tolerance (ΔHT) was significantly and negatively correlated with $\dot{M}_{O_2}$ when swimming at low flow. This implies that in wild-caught striped bass, those that swim more efficiently (lower $\dot{M}_{O_2}$ at the same swimming speed) or are less stressed under laboratory conditions are able to allocate greater resources towards a plastic response to hypoxia exposure.

Swimming metabolism across flow regimes

Comparisons of our swimming $\dot{M}_{O_2}$ measurements made under normoxic conditions with literature values suggest that our fish were relatively relaxed and thus our conclusions are robust. The mean $\dot{M}_{O_2}$ of our fish swimming at 10 cm s^{-1} was 1.75 µmol O_2 min^{-1} which compares favourably with a standard $\dot{M}_{O_2}$ of 1.06 µmol O_2 min^{-1} and a routine $\dot{M}_{O_2}$ of 2.03 µmol O_2 min^{-1} reported by Brougher et al. (2005), and ∼4.4 µmol O_2 min^{-1} in similar-sized striped bass swimming at 10 cm s^{-1} and 20°C but uncorrected for microbial $\dot{M}_{O_2}$ (Kruger and Brocksen, 1978). Swimming oxygen consumption was significantly repeatable on a daily basis across high- and low-flow swimming speeds, suggesting that all fish responded in a similar way metabolically to changes in flow. Measurements of fish oxygen consumption date to the early 19th century (Nelson, 2016), but repeatability is still rarely reported, and appears to be context dependent in fish (Killen et al., 2016). Reidy et al. (2000) reported significant long-term repeatability of $\dot{M}_{O_2}$ in wild *G. morhua*, but only when swimming fast, not at rest or when swimming at lower speeds. Repeatability has also been shown to decline over time (Auer et al., 2018a; Norin and Malte, 2011). It is likely that laboratory residence, coupled with a variable metabolic response by the animals to human presence, may be blunting some measures of repeatability. As we found repeatable $\dot{M}_{O_2}$ in fish only within a single day, it may be fruitful to measure long-term repeatability of swimming metabolism, and compare that with individual HT.

We found a high inter-individual variability for $\dot{M}_{O_2}$ (CV: 13–20), which is not unusual for fish (Burton et al., 2011; Metcalfe et al., 2016; Norin and Gamperl, 2018; Norin and Malte, 2011). Metabolic rate is influenced by a multitude of biotic and abiotic environmental factors, physical and physiological activity, and lifestyle, and is both species and individual specific. Factors that have been found to influence intraspecific variation in $\dot{M}_{O_2}$ include, but are not limited to, shoaling (Domenici et al., 2017; Nadler et al., 2016), competition for food and intake (Auer et al., 2015; Norin and Malte, 2011), and thermal history (Auer et al., 2018b; Eliason and Farrell, 2016; Eliason et al., 2011; Killen et al., 2013). Substantial variation in $\dot{M}_{O_2}$ has been found even in clonal fishes (Plaut and Gordon, 1994) and full-sibs raised in identical environments (Burton et al., 2011), suggesting that genetics and environment both contribute to creating the large intraspecific variation in $\dot{M}_{O_2}$. Despite this variation in our fish, the strong repeatability signifies that the inter-individual variance in $\dot{M}_{O_2}$ under identical conditions significantly exceeds the variance among replicate trials of an individual over time.

Swimming metabolism across oxygen regimes

$\dot{M}_{O_2}$ in swimming striped bass was not repeatable across different oxygen levels, contrary to Maciak and Konarzewski (2010). This finding, coupled with the repeatable $\dot{M}_{O_2}$ across swimming speeds discussed above, indicates that individual striped bass do not

respond uniformly to the combined physiological challenges of swimming and hypoxia. Similarly, Nelson and Lipkey (2015) found juvenile striped bass to have different HT ranks depending upon whether they were tested while swimming or not. Hypoxia can clearly alter repeatability and inter-individual variability of metabolic performance in fish (Killen et al., 2016). Here, the lowest and highest variation in $\dot{M}_{O_2}$ was in fish swimming under hypoxia against high flow and low flow, respectively. Similarly, Norin et al. (2016) reported lower variation in maximum $\dot{M}_{O_2}$ compared with standard $\dot{M}_{O_2}$ in barramundi, *Lates calcarifer*, when both were measured under hypoxia. Two other studies, in spined loach, *Cobitis taenia* (Maciak and Konarzewski, 2010), and Gulf killifish, *F. grandis* (Virani and Rees, 2000), report reduced variation and repeatability of $\dot{M}_{O_2}$ when measured under hypoxic conditions. Repeatability of $\dot{M}_{O_2}$ can also decrease across thermal (Auer et al., 2018a) and food availability regimens (Auer et al., 2015; Norin and Malte, 2011). The results reported here are a likely consequence of metabolic constraints being imposed by hypoxia and the simultaneous demands of exercise and hypoxia. One possible mechanism is that blood is generally diverted from the gastrointestinal tract to swimming muscles of fish in exercise (Thorarensen and Farrell, 2006) and away from the gut during hypoxia (Axelsson and Fritsche, 1991; Axelsson et al., 2002), limiting the perfused tissues that are contributing to oxygen consumption.

COT

The oxygen consumed in covering a fixed distance was lower if fish were swimming at approximately 67% of their estimated U_{crit}. The decline in COT going from swimming at 10 cm s^{-1} (~23% U_{crit}) to swimming at approximately 29 cm s^{-1} (~67% U_{crit}) suggests that our selected high-flow test was closer to the fish's optimal swimming speed (U_{opt}) (Claireaux, 2006; Webb, 1998). Despite that we measure $\dot{M}_{O_2}$ only at two swimming speeds, the results support the commonly found J- or U-shaped COT curves in fish (Di Santo et al., 2017; Webb, 1998). The co-familiar *D. labrax* had a similar minimum COT closer to 67% of their U_{crit} than to 10 cm s^{-1} (Claireaux, 2006). All fish swam steadily and did not transition to burst-and-coast swimming, suggesting that these fish relied mostly on aerobic respiration at the selected speeds. Consistent and repeatable $\dot{M}_{O_2}$ in normoxic low-flow water measured before and immediately after the high-flow treatment shows that no significant oxygen debt accumulated at the high-flow treatment under normoxia, and is consistent with the animals being unperturbed by the high-flow treatment.

The significantly lower COT when the fish were swimming at both speeds under hypoxia most parsimoniously arose from them exploiting anaerobic metabolic pathways but could also have arisen from reduced metabolic activity of tissues not involved with swimming and the aforementioned re-distribution of blood away from those tissues. Hypoxia exposure itself can initiate anaerobic metabolism producing lactate in some fish (Weber et al., 2016), supporting the anaerobic metabolism idea. Whether our juvenile striped bass were approaching exhaustion from relying on anaerobic respiration during the H-HF swim was not apparent but is likely (Domenici et al., 2013). For example, U_{crit} can be significantly reduced when fish are swum under similar or even milder levels of hypoxia to those used here; an approximate 30% reduction in U_{crit} was reported for Atlantic cod, *G. morhua* (~40% AS: Dutil et al., 2007; Petersen and Gamperl, 2010), and a 20% reduction for rainbow trout, *O. mykiss* (~25% AS: Bushnell et al., 1984), black carp, *Mylopharyngodon piceus* (30% AS: Pang et al., 2015), coho salmon, *Oncorhynchus kisutch*, and largemouth bass, *M. salmoides* (12–27% AS: Dahlberg et al., 1968). The lactate produced can be oxidized by red muscle (or other fully aerobic tissues like heart and brain) to maintain energy balance and swimming performance when insufficient oxygen is available to keep all tissues aerobic (Omlin and Weber, 2010; Weber, 1991; Weber et al., 2016). Future studies on striped bass should incorporate the measurement of post-exercise oxygen consumption (Marras et al., 2010; Zhang et al., 2018), muscle and blood biochemistry (Zhang et al., 2018), and regional blood flow during swimming under hypoxic conditions to better understand how striped bass partition metabolism to be able to perform so well under hypoxia.

Relationships between HT and swimming respiration

HT was not related to oxygen consumption rate under any condition tested. Excluding the possibility that there is no relationship between HT and $\dot{M}_{O_2}$, there are several alternatives: (1) the flow levels we chose did not sufficiently challenge the aerobic capacities of these striped bass to an extent where trade-offs could be discerned (e.g. in a centrarchid, fish with a higher U_{crit} have been shown to have lower HT; Crans et al., 2015); (2) selecting speed based on size alone instead of each individual's empirically determined U_{crit} may have obscured the results; and (3) metabolism during recovery from exercise, which was not measured in this study, may be the key metabolism under selection (Marras et al., 2010), and more relatable to an individual's HT. However, if this result holds up to further experimental scrutiny, it suggests that being hypoxia tolerant does not necessarily diminish an individual's capacity to consume oxygen while swimming.

Multiple studies have drawn attention to potential trade-offs or complementarity between HT and aerobic swimming ability in fish (Zhou et al., 2019; Crans et al., 2015; Fu et al., 2011; Fu et al., 2014); however, there is no consensus on how these trade-off within individuals or across species (Zhou et al., 2019; Crans et al., 2015; Fu et al., 2011; Fu et al., 2014; Nilsson, 2007). Among the traits suspected to be critical to both HT and swimming capacity are the ability to exchange respiratory gases at the gill, $\dot{M}_{O_2}$ transport to tissues and efficiency of ATP generation and allocation (Crans et al., 2015; Hochachka et al., 1996; Randall, 1982). In contrast, some traits may favour only one of these physiological characteristics. For example, HT is often associated with a high Hb–O_2 affinity (low P_{50}; Mandic et al., 2008), but that could limit O_2 unloading at the working muscle thereby compromising aerobic swimming. Similarly, increasing blood [Hb] and haematocrit can promote HT (Nelson et al., 2019) but the consequential increase in blood viscosity may limit cardiac performance at high exercise levels (Wells and Weber, 1991).

Metabolic rate can be an important determinant of an individual's response to hypoxia (Killen et al., 2012), with high metabolic rate animals generally faring worse (Zambonino-Infante et al., 2017). However, having a high aerobic metabolic rate can also facilitate recovery from the type of exhaustive exercise that taxes anaerobic capacity (Marras et al., 2010), suggesting that these animals might have a selective advantage if they are able to escape encroaching hypoxic waters. Here, although $\dot{M}_{O_2}$ was somewhat predictive of an animal's capacity to change HT, it was not directly correlated to HT. The rate of oxygen consumption was also poorly predictive of HT in the co-familiar European sea bass yet was significantly associated with survival in a simulated natural estuary that experiences hypoxia (J.A.N., unpublished observation).

In contrast to hypoxia tolerance per se, the increase of each individual's hypoxia tolerance (ΔHT) with three hypoxia exposures

was significantly and negatively correlated with $\dot{M}_{O_2}$ at low flow. One explanation for this result may be that animals with low routine $\dot{M}_{O_2}$ have more resources (e.g. aerobic scope) to allocate to the plastic response to hypoxia (i.e. new gill tissue, erythrocyte synthesis, etc.). Alternatively, those fish with the lowest routine $\dot{M}_{O_2}$ may have been the most habituated to the laboratory and been de-sensitized to stress (Reid et al., 1994), and so the energy not invested in stress responses could go into physiological plasticity. In either case, the plastic response to hypoxia is bound to have significant physiological trade-offs, otherwise it would be the default trait value measurable under any conditions; for instance, increased gill surface area would substantially increase the cost of osmoregulation and acid–base balance (Sardella and Brauner, 2007). Future research should focus on the HT of swimming striped bass and whether the plastic responses induced by hypoxia exposure have fitness consequences for this iconic fish of the North American east coast.

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Competing interests
The authors declare no competing or financial interests.

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

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