Hypoxia and Sprint Swimming Performance of Juvenile Striped Bass, *Morone saxatilis*

Krista Kraskura*
Jay A. Nelson
Department of Biological Sciences, Towson University, Towson, Maryland 21252

Accepted 9/21/2017; Electronically Published XX/XX/2017

Online enhancements: appendix figure.

ABSTRACT

q1 Annual hypoxia in the Chesapeake Bay has expanded to the point where Darwinian fitness of juvenile striped bass (*Morone saxatilis*) may depend on their ability to perform in low-oxygen environments. The locomotion they use in predator/prey dynamics relies primarily on white (type II) muscle that is powered by anaerobic metabolic pathways and has generally been thought to be immune to aquatic hypoxia. We tested the sprint performance of 15 juvenile striped bass twice under acute hypoxia (20% air saturation [AS]) 5 wk apart and once under normoxia (>85% AS) in between. Average sprint performance was lower under the first hypoxia exposure than in normoxia and increased in the second hypoxia test relative to the first. The rank order of individual sprint performance was significantly repeatable when comparing the two hypoxia tests but not when compared with sprint performance measured under normoxic conditions. The maximum sprint performance of each individual was also significantly repeatable within a given day. Thus, sprint performance of striped bass is reduced under hypoxia, is phenotypically plastic, and improves with repetitive hypoxia exposures but is unrelated to relative sprint performance under normoxia. Since energy to fuel a sprint comes from existing ATP and creatine phosphate stores, the decline in sprint performance probably reflects reduced function of a part of the reflex chain leading from detection of aversive stimuli to activation of the muscle used to power the escape response.

Keywords: Morone saxatilis, hypoxia, sprint, swimming.

*Corresponding author; e-mail: k.kraskura@gmail.com.

Physiological and Biochemical Zoology 91(1):000–000. 2018. 1522-2152/2018/9101-17034$15.00. DOI: 10.1086/694933

Introduction

Low-oxygen concentrations in aquatic systems are increasing worldwide (Breitburg et al. 2009). Nutrient loading, elevated temperatures, low water mixing, and decomposing bacteria combine to produce large hypoxic hypolimnetic regions in many lakes and estuaries. In Chesapeake Bay, these regions may persist for up to 6 mo (Hagy et al. 2004; Kemp et al. 2005; Scully 2016). Unfortunately, these hypoxic hypolimnetic regions are not static because strong winds and tidal currents can quickly drive hypoxic waters into littoral zones that are usually well oxygenated (Breitburg 1992; Kemp et al. 2005; Scully 2016). Thus, aquatic organisms can be rapidly exposed to acute hypoxia that may limit or overwhelm their ability to escape or acclimate (Domenici et al. 2007; Rice et al. 2013). When this happens, the survivorship and therefore Darwinian fitness of a fish may be directly associated with their ability to perform routine biological functions under hypoxia. Swimming performances powered primarily by aerobic metabolism—generally measured as U_{crit} or U_{max}—has been studied in multiple species under hypoxic conditions (reviewed in Domenici et al. 2013). The general finding from these studies is that hypoxia constrains aerobic scope (AS) and thus metabolic power of individuals (Fry 1971; Claireaux et al. 2000; Claireaux and Chabot 2016), resulting in a reduced maximum sustained swimming speed (Dahlberg et al. 1968; Bushnell et al. 1984; Dutil et al. 2007; Petersen and Gamperl 2010), which is not unexpected for a swimming performance powered mostly by type I (i.e., red) muscle fibers that rely on oxygen for energy transduction (McKenzie 2011). Only a few studies have explored whether hypoxia influences fish swimming performances powered mostly by type II (i.e., white) muscle fibers that function without an immediate need for oxygen (Weber and Haman 1996). These studies all examined fast-start escape responses that generally encompass only the first 70–100 ms of an aversive response (Lefrançois et al. 2005; Lefrançois and Domenici 2006; Gotanda et al. 2012). To our knowledge, no studies have investigated sprint performance—as defined by Reidy et al. (2000) and Nelson et al. (2002)—of fish under hypoxia. This type of locomotor performance is thought to be critical for survival in many fish species (Nelson et al. 2002; Handelsman et al. 2010; Oufiero et al. 2011; Vandamm et al. 2012), although the direct benefit of high sprint performance in fish has rarely been tested. Oufiero et al. (2011) and Handelsman et al. (2010) report positive relationships between fish sprinting capacity and their success in environments with high predation, and Katzir and Camhi (1993) and Walker et al. (2005) report similar results for laboratory fish. Thus, sprint performance might be an important fitness component for many fish species because it is often critical in escaping from

predators or capturing food (Domenici and Blake 1997; Nelson et al. 2002).

The Chesapeake Bay is the nursery for the Atlantic Ocean's population of the culturally and commercially valuable striped bass, *Morone saxatilis* Walbaum; up to 90% of the Atlantic's striped bass population originates in the Chesapeake Bay (Berggren and Lieberman 1978). Juvenile striped bass in Chesapeake Bay often occupy the hypolimnion (Setzler-Hamilton et al. 1981), an area that frequently becomes hypoxic. Because these hypoxic zones are mobile, it is unlikely that fish will always be able to escape them and thus will have to perform in these waters to survive. When water-breathing fish in hypoxic waters reach their critical oxygen tension ($P_{O_{2crit}}$; a point at which they can no longer maintain their ordinary metabolic demand aerobically), they must rely on supplemental anaerobic metabolism, metabolic arrest, aquatic surface respiration, or some combination of these to survive until they return to oxygenated waters (Chapman and McKenzie 2009). The water oxygen tension at which this occurs and also the duration during which fish can operate aerobically at various levels of hypoxia varies between and within a species, including striped bass (Nelson and Lipkey 2015). Fish that experience hypoxic conditions often have reduced growth (reviewed by Diaz and Breitburg 2009), suppressed immune systems (Burt et al. 2012; Lapointe et al. 2014), and changes in escape-associated behaviors, presumably reflecting altered brain function (Killen et al. 2012; Lucon-Xiccato et al. 2014). Because the ability to carry out many routine biological functions can be impaired in hypoxic waters, we hypothesize that the sprint performance that is critical in success in predator/prey interactions could also be affected by hypoxia. Here we test the null hypothesis that sprint performance of juvenile striped bass is unaffected by acute hypoxia. Furthermore, because we examined the same individuals twice under hypoxia and once in normoxia trials (which were conducted between the two hypoxia exposures), we can report on the repeatability of sprint performance within and across different environmental oxygen levels (Killen et al. 2016).

Methods

The fish handling protocol was approved by Towson University's Institutional Animal Care and Use Committee (12042013JN-02). Young of the year striped bass ($n = 15$) were collected in the summer of 2015 from the upper Chesapeake Bay and transported to Towson University, where they were held in Chesapeake Bay water for 2 d and gradually acclimated to 20°C and 10‰ salinity with a maximum temperature change of 2.5°C per day. Fish were held in three 285-L tanks ($n = 5 \pm 1$, temperature = 20° ± 1.5°C, salinity = 10‰ ± 1‰) with biweekly 20%–30% water exchanges on a 12L:12D cycle. All animals were fed to satiation at least 5 d a week with commercial pellet food (Hikari tropical food sticks), which they readily accepted. At 8 wk after capture, individuals were anesthetized with tricaine methanesulfonate, MS-222 (100 mg L^{-1}, buffered 1:1 with Na^+; HCO_3), weighed (g), measured for total length (TL) and fork length (FL), and marked with passive integrated transponder tags (Biomark) for individual identification. Fish were allowed a minimum of 4 wk to recover from handling and surgery before any experimentation.

Sprint performance was measured in a sprint performance chamber (SPC; Nelson et al. 2008). Briefly, the dimensions of the SPC were 1.5 m × 0.15 m × 0.15 m. Light-emitting laser diodes (OnPoint Lasers) were placed on one side of the chamber at positions of 0, 1, 3, 7, 15, 23, 31, and 39 cm from the sprint starting point, thus creating seven intervals, the last four of which were 8 cm in length (7–15, 15–23, 23–31, and 31–39 cm from the start of the SPC) and were the only ones analyzed here. A 5-mm glass rod was transversely attached to the laser lens to refract the laser beam and project a vertical light plane across the raceway. The light plane penetrated through a clear plexiglass window on one side of the SPC and was detected by eight arrays of Photodarlington detectors (Honeywell International; 18 sensors per array, 144 sensors total). One sensor was placed vertically every 0.5 cm, starting at 0.5 cm from the bottom and up to a depth of 8.5 cm. The light beam activated a photoreceptor array that put out a signal to one of eight digital inputs on a PowerLab /4S (ADInstruments 2009) interfaced with a computer (MacBook Pro; Apple) running LabChart7 (ADInstruments) software. Any disruption between the laser light source and the array (e.g., a fish swimming through) was detected and recorded by the software. Breakage of the first laser beam acted as the trigger, with subsequent beam breakages being recorded to an accuracy of 0.1 ms. Sprint swimming velocity was calculated using recorded times and known distances between arrays. Minor modifications were made to the SPC to allow control of the oxygen tension. Water was supplied to the SPC via an external circuit connected to an oxy-stat system (Loligo Systems). To maintain oxygenation level, a slight flow (<1 cm s^{-1}) was directed against the swimming path of the fish so that reported sprint speeds may be a slight underestimation of what would have been recorded in purely static water.

Before sprinting, randomly selected individuals were fasted for a minimum of 24 h and a maximum of 48 h before being transferred without air exposure to the SPC and allowed a 1-h acclimation ($T = 20.03° \pm 0.09$°C, mean ± SEM; Nelson and Claireaux 2005; Handelsman et al. 2010; Killen et al. 2014). For hypoxia tests, the oxygen concentration was progressively lowered during the second hour (60 min) period to 22.9% ± 0.3% (mean ± SEM) air saturation (AS; equivalent to 64.4 ± 0.8 μmol O_2 L^{-1}), a rate of hypoxia development close to what can be found in Chesapeake Bay (Breitburg 1992), by bubbling nitrogen gas into the external circuit of the sprint chamber. For normoxic tests, air was substituted for nitrogen to keep any bubbling disturbance constant between trials. Oxygen tension was monitored with a galvanic oxygen-sensing probe (OxyGuard Mini Probe, Loligo Systems) placed near the holding area of the SPC but in a manner so as not to disturb the fish. Sprinting was initiated after 60 min of oxygen depletion (hypoxia test) or no change (normoxia test) by lifting a retaining gate and chasing the fish by hand. Each individual was tested a minimum of five times with ≥5 min of recovery between each trial until three quality sprints with a straight path and investigator-perceived motivation of the fish were obtained. To avoid overexposure to hypoxia, fish under

hypoxia were sprinted a maximum of eight times. The average number of trials (mean ± SD) was 4 ± 2 under hypoxia and 7 ± 4 in normoxia. No signs of exhaustion or habituation was observed in any of the sprints (see "Results"), and no fish lost equilibrium during experimentation under hypoxia. The 5-min interval between tests was justified by previous studies showing no trial effect with a similar between-trial interval, including those with the congeneric European sea bass (Nelson and Claireaux 2005; Claireaux et al. 2007; Handelsman et al. 2010; Killen et al. 2014). Each individual had its sprint performance tested on three separate occasions, but the order that an individual fish was tested within a given occasion was selected randomly. In chronological order, the sprint tests that were performed were an initial hypoxia (H1) followed by normoxia (N) and a second hypoxia test (H2). The minimum and average times between tests were as follows: 14 and 19 d between H1 and N, 19 and 28 d between N and H2, and 37 and 48 d between H1 and H2. All fish were weighed and measured a total of three times; measurements were obtained for each individual on the day when the last sprint trial within a sprint test (H1, N, H2) was performed (mean ± SEM [range]: TL_{H1} = 139 ± 6 mm [97–183 mm], $mass_{H1}$ = 28 ± 4 g [7.6–65.6 g]; TL_N = 144 ± 6 mm [100–186 mm], $mass_N$ = 31 ± 5 g [8.3–69 g]; TL_{H2} = 153 ± 6 mm [107–194 mm], $mass_{H2}$ = 37 ± 5 g [11–74.4 g]).

Data were analyzed and tested for significance using R v3.3.1 (R Development Core Team 2016) software. The level of significance in all tests was $\alpha = 0.05$. Repeatability between the best and second-best sprints by each individual within each sprint test (N, H1, and H2; i.e., daily repeatability) was tested using Pearson correlation, and individual repeatability between means of the three best sprints from each sprint test (H1–N, N–H2, H1–H2; i.e., long-term repeatability) was analyzed using Spearman's rank order correlation, and Kendall's coefficient of concordance (Kendall's *W* for H1-N-H2). We used linear mixed-effect models (R package lme4:lmer) to test (1) whether size (TL; mm) and growth rate (GR; mm [TL] d^{-1} and g d^{-1}) were significant interaction terms affecting sprint speed, (2) for the effect of which tank the fish were being held in and observed sprint performance of individuals, and (3) for significance differences in sprint performance among the three sprint tests (H1, N, H2). In models testing for covariation, the fixed effects were sprint test (H1, N, H2), with the interactions being TL, GR, and holding tank (tested separately) and a random effect of individuals. The final model was simply designed with a fixed effect of sprint test and a random effect of individuals. We also used the same final model to test for global differences in sprint performance under hypoxia (with H1 and H2 combined) and normoxia (N). The random effect of individuals was used to account for the repeated measures design. In all models, the slope and intercept were allowed to vary for each individual (random effect) in each swim test. We used a χ^2 test to test whether within each sprint test (H1, N, H2, H combined) an animal's maximum sprint speed was equally likely to occur in each of the four 8-cm sprint intervals (25% probability) and also to test whether maximum sprint speed was independent of trial on a given day (12.5% probability). Furthermore, ANOVA was used to detect the significance level of fixed effects on sprint speed for all mixed-effect models; the tests used Satterthwaite approximations to degrees of freedom. Finally, post hoc Tukey's honest significant difference test on the final model was used to identify differences between least squares mean sprint speeds in all tests. Sprint swimming performance is reported as the mean ± SEM of each individual's top three sprints throughout the remainder of the article.

Results

Repeatability

Sprint performance of juvenile striped bass was significantly repeatable on a daily basis for all three of the sprint tests (H1 r [13] = 0.78; N r [13] = 0.94; H2 r [13] = 0.96; $P < 0.001$; data not shown). The rank order of mean sprint performance by each individual was also repeatable over an average of 5 wk, when both sprints were conducted in water of approximately 20% AS (fig. 1; Spearman's ρ [13] = 0.56, $P = 0.03$). Interestingly, the rank order of performance was shuffled for the sprint trial conducted in normoxic water in between the two hypoxia trials (i.e., the best sprinter under normoxia was not the best sprinter under hypoxia); thus, sprint performance between trials conducted in hypoxic water and the trial conducted in normoxic water was not significantly repeatable (Spearman's ρ; N–H1 ρ [13] = 0.13, P = 0.65; N–H2 ρ [13] = −0.46, $P = 0.08$; Kendall's W [H1-N-H2] = 0.38, $P = 0.31$).

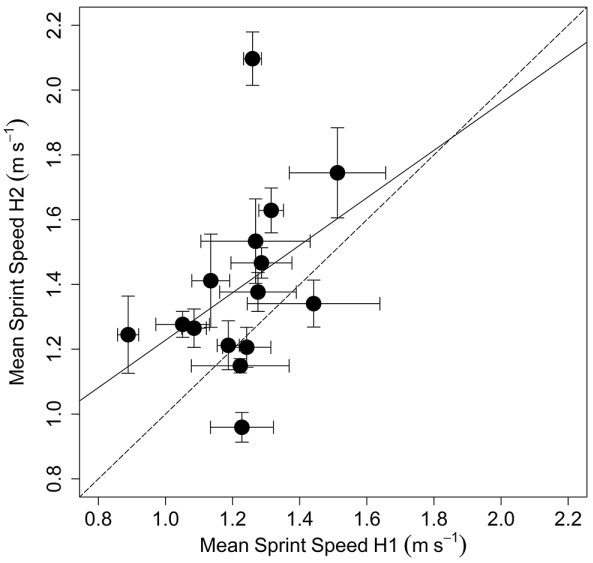

Figure 1. Repeatability of sprint performance of 15 juvenile striped bass in water with oxygen content regulated at 20% of air saturation. The mean sprint performance of each individual in its first hypoxia trial (H1) is plotted against its mean performance in a second identical trial (H2) approximately 5 wk later. Mean sprint speeds were calculated from the three highest velocity intervals taken from each of three separate sprints. Means ± 1 SEM are plotted, with the solid line representing the correlation between the two trials (Spearman rank order coefficient $\rho = 0.56, P = 0.03$) and the dashed line representing the line of identity.

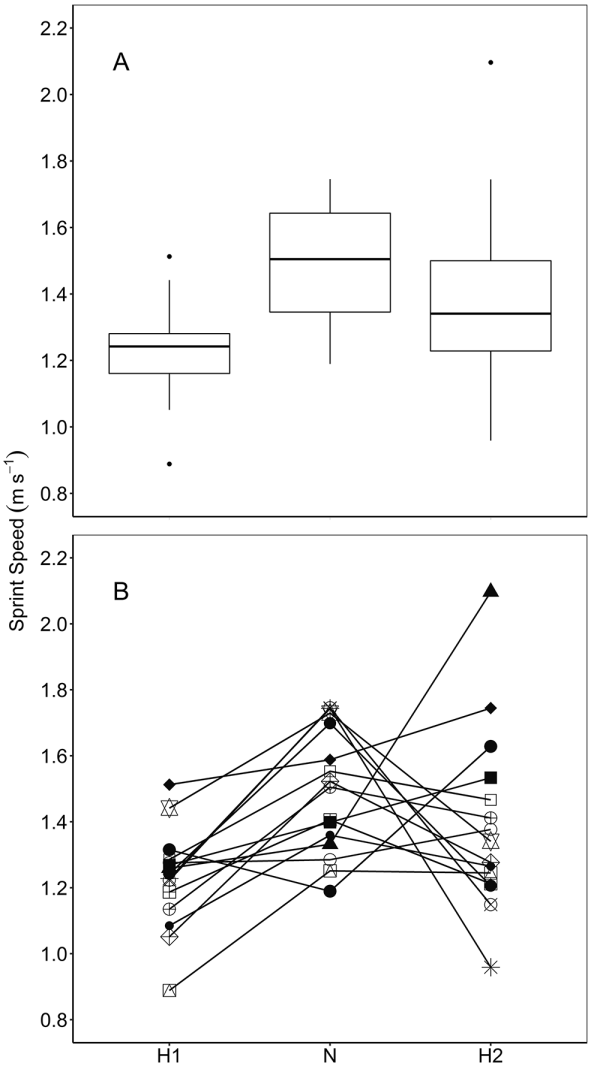

Figure 2. Mean sprint performance of 15 juvenile striped bass in three separate trials ordered chronologically. Each fish was first tested in water with an oxygen content of 20% of air saturation (AS; H1), followed by a sprint in normoxia ($[O_2] > 85\%$ AS; N) at least 14 d later, followed by a second hypoxic sprint (H2) at least 19 d after that. *A* shows a box plot where solid lines are median values of all individual mean sprint speeds ($n = 3$), boxes represent interquartile range (IQR), and whiskers show the full range of data excluding outliers (black circles), or values more than ± 1.5 IQR outside of the box. In *B*, each symbol represents an individual, and lines connect that individual's points (mean of three best sprints) across sprint tests. Sprint speed was significantly affected by oxygen conditions (ANOVA: $F_{2,23.12} = 31.74$, $P < 0.001$); the H1 and N tests were significantly different from each other (Tukey post hoc test: $P < 0.001$).

Effects of Hypoxia

The mean sprint speeds of juvenile striped bass were 1.23 $\pm$ 0.04 m s^{-1} in H1, 1.49 $\pm$ 0.05 m s^{-1} in N, and 139 $\pm$ 0.07 m s^{-1} in H2 (fig. 2*A*), and they were significantly different from each other, as indicted by ANOVA ($F_{2,23.12} = 31.74$, $P < 0.001$). Post hoc Tukey's analyses indicated that mean sprint speed in H1 was significantly lower than in N ($P < 0.001$) and that fish sprint performance was significantly better in the second hypoxia trial than the first ($P = 0.02$). Nine individuals sprinted consistently slower under hypoxia than in normoxia, four individuals were not affected by environmental oxygen level, and two individuals sprinted better under hypoxia (fig. 2*B*). Eleven out of 15 individuals sprinted better under hypoxia the second time they were exposed to it (figs. 1, 2*B*). We also found a global significant difference between sprints recorded under hypoxia (H1 and H2 combined) and normoxia ($F_{2,120} = 16.54$, $P < 0.001$; post hoc Tukey's test: $P < 0.001$).

Fish behaved differently when tested under hypoxia than when tested in normoxic water. For all sprint tests, an animal was significantly less likely to have its maximum velocity recorded from the final of the four 8-cm intervals at the end of their sprint. For the two hypoxia tests, an animal was also significantly less likely to have its maximum velocity recorded from the third 8-cm interval in the SPC, which was not true in normoxic water (χ^2 test: H1 χ^2 [3] $= 10.56$, $P = 0.014$; H2 χ^2 [3] $= 16.96$, $P < 0.001$; N χ^2 [3] $= 10.56$, $P = 0.014$; H combined χ^2 [3] $= 26$, $P < 0.001$; fig. 3). There was no effect of trial number within a given day as to when a fish recorded its maximum velocity for any trial under any environmental condition

Mistake?
N=H1 and is significant

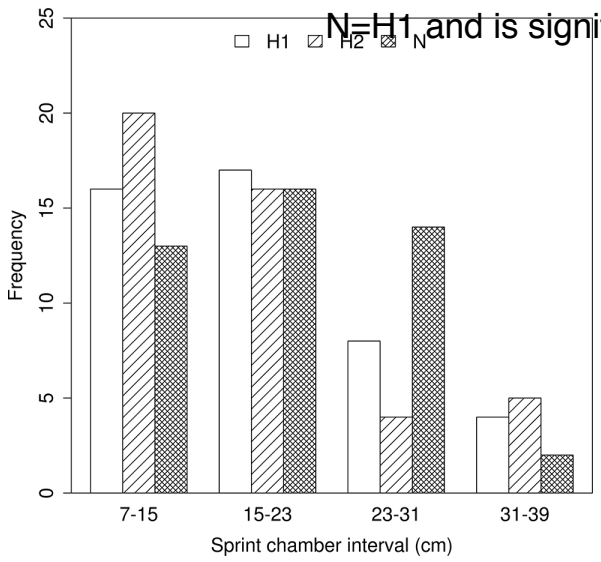

Figure 3. Frequency distribution of which 8-cm interval of the sprint performance chamber (SPC) recorded a maximum sprint speed. The numbers 7–15, 15–23, 23–3, and 31–39 correspond to four 8-cm intervals from the sprint start point (0 cm) in the SPC. A total of 45 sprints (the three best for each fish, $n = 15$) are plotted for each sprint test (first hypoxia exposure [H1], second hypoxia exposure [H2], and then normoxic [N]). Fish under hypoxia (oxygen content approximately 20% of that at air saturation) were significantly less likely to have their maximum velocity recorded from the third 8-cm interval (23–31 cm in the SPC). The probability of obtaining a maximum sprint speed in any of the 8-cm intervals was significantly affected by hypoxia (χ^2 test of independence: H1 χ^2 [3] $= 10.56$, $P = 0.014$; H2 χ^2 $=$ [3] $= 16.96$, $P < 0.001$; N χ^2 [3] $= 10.56$, $P = 0.014$; H combined χ^2 [3] $= 26$, $P < 0.001$).

decimal pt. missing

Mistake?
N is significant

(χ^2 test: H1 χ^2 [7] = 6.91, P = 0.44; H2 χ^2 [7] = 10.9, P = 0.14; N χ^2 [7] = 1.77, P = 0.97; data not shown); that is, a fish was equally as likely to have its top sprint performance recorded in its first trial as in its last.

Effects of Size, Growth, and Rearing Conditions

Neither fish size nor growth rate significantly affected or interacted with sprint performance for any of the sprint trials under any environmental condition. A mixed-effect model analyses showed size (TL) to be an insignificant interaction term (ANOVA: $F_{2, 22.90}$ = 1.31, P = 0.29). In addition, growth rate (whether measured as g d^{-1} or mm [TL] d^{-1}) was also an insignificant interacting predictor (ANOVA: g d^{-1}: $F_{2, 30.57}$ = 1.91, P = 0.17; mm [TL] d^{-1}: $F_{2, 63.44}$ = 1.38, P = 0.26) in determining the sprint speed of juvenile striped bass over the 9-wk duration of the experiment. Thus, size and growth rate were removed from the final mixed-effect models (see "Methods"). There was a slightly significant interaction between sprint performance and rearing tank (ANOVA: $F_{4, 23.80}$ = 3.05, P = 0.04) attributable to one individual having an extraordinary improvement in sprint performance during the second hypoxia test (fig. A1, available online). A single fish can have this effect because of the small sample size and their division into three holding tanks. The main effect of hypoxia remained clear, such that the tank effect was not included in the primary analyses (fig. A1).

Discussion

Repeatability

The significant individual repeatability of striped bass sprint performance in hypoxic water over 5 wk suggests within-context stability of this trait (Killen et al. 2016). Long-term repeatable sprint performances have also been reported for the cofamiliar European sea bass (*Dicentrarchus labrax*; Claireaux et al. 2007), blacknose dace (*Rhinichthys atratulus*; Nelson et al. 2015), guppy (*Poecilia reticulata*; Oufiero and Garland 2009), and Atlantic cod (*Gadus morhua*; Reidy et al. 2000; Martínez et al. 2002) in normoxic waters, but to our knowledge, this is the first report of long-term significant rank order repeatability for sprint locomotion of striped bass or any fish species under hypoxic conditions. This is also the first record of daily repeatability for sprint performance of fish under hypoxia. The approximate 5-wk repeatability of performance under adverse environmental conditions not only testifies to the utility of the method but also points to the potential of this trait as a fitness parameter for fish inhabiting or acutely encountering hypoxic waters (Boake 1989; Oufiero and Garland 2009). Considering the prevalence and mobility of hypoxic zones in Chesapeake Bay (Breitburg 1992), it is likely that successful ontogeny for resident striped bass will include sprint swimming in hypoxic waters.

Effects of Hypoxia

Sprint performance of juvenile striped bass was phenotypically plastic with respect to hypoxia; individuals generally sprinted better during their second hypoxia exposure, but when their two hypoxia performances were considered in tandem, they were still significantly slower than their intervening sprints conducted in normoxic water. Because most but not all fish had reduced sprint performance under hypoxia, whatever caused this average diminution of performance under hypoxia was not uniform across this group of fish and could therefore contribute to fitness differences on intrusion of hypoxic water into their habitat (Breitburg 1992). This average loss of sprint performance elicited by reducing the oxygen saturation of water to approximately 20% AS is not predictable from the energetics of swimming. Sprint performance is powered by the type II (white) epaxial and hypaxial musculature that will use on-board ATP and then ATP rapidly regenerated from the creatine phosphokinase (EC 2.7.3.2) and myokinase (EC 2.7.4.3) reactions to fuel contraction. Muscle contraction fueled this way should theoretically be independent of the environmental [O_2] (Weber and Haman 1996; Kieffer 2000). Although oxygen itself is not required to directly power a sprint, acute hypoxia exposure could be indirectly affecting sprinting through its action on other physiological systems. Hypoxia reduces an individual's metabolic scope (Claireaux et al. 2000; Claireaux and Chabot 2016) and changes its energy use patterns, metabolic byproduct removal (Weber et al. 2016), oxygen extraction from the environment, and delivery to tissues (Randall 1982; Sandblom and Axelsson 2006; Petersen and Gamperl 2010). Any combination of these effects may lead to deficits along the pathway from sensory detection to sprint execution (see Lefrançois et al. 2005; Lefrançois and Domenici 2006; Lucon-Xiccato et al. 2014). In the chain of events from stimulus detection, through signal transmission and redirection via the peripheral and central nervous systems and finally to muscle contraction, there are multiple sites where hypoxia could impede function. Several hypoxia-induced nervous system defects have already been reported in fish. Visual acuity is impaired in snapper (*Pagrus auratus*) at 40% and 25% AS (Robinson et al. 2013), severe hypoxia disrupts population-level lateralization of staghorn sculpin (*Leptocottus armatus*; Lucon-Xiccato et al. 2014), and hypoxia-induced distress can act on sensory channels and impede maneuverability resulting in disturbed schooling behavior in several fish species (reviewed in Domenici et al. 2007). In mammalian systems, one response to acute hypoxia is a neurotransmitter-mediated decrease in synaptic transmission (Corcoran and O'Connor 2013) that could be relevant here. Finally, fish may reprioritize energy-demanding tasks behaviorally and/or physiologically to optimize energy expenditure (e.g., Axelsson et al. 2002; Jourdan-Pineau et al. 2010), so that initiation and/or continuation of the sprint may be compromised (Lefrançois et al. 2005; Lefrançois and Domenici 2006). Support for this idea can be gleaned from the significant difference between where in the SPC a striped bass's maximum velocity was recorded under hypoxia versus normoxia. Fish seemed unwilling to sprint further than 23 cm when in hypoxic water, despite being chased by a human. That same fish sprinting in normoxia was equally as likely to have its maximum velocity recorded at any point up to 31 cm. The cofamiliar *D. labrax* also has demonstrable behavioral changes under hypoxia, showing increases in boldness and risk-taking behavior (Killen et al. 2012).

"upon" sounds better to me here

Phenotypic plasticity in response to hypoxia was fairly uniform among this group of wild striped bass collected from the same location. Despite differences between sprint performance during an initial hypoxia exposure and their subsequent performance under normoxia, performance during a second exposure to 20% AS water increased by a similar degree in most individuals ($\overline{X} = 0.17 \pm 0.07$ m s^{-1}; fig. 2; also readily observable as the parallel nature of the lines in fig. A1). The significant repeatability of rank order between the two hypoxia trials affirms this uniformity of change. A similar relatively uniform improvement in hypoxia tolerance (HT) was also observed among a different group of juvenile striped bass repetitively subjected to hypoxia challenge tests where loss of equilibrium was the end point (J. A. Nelson and G. K. Lipkey, personal observations). Although plastic responses to hypoxia at the whole-animal level often appear to be a species-level characteristic (e.g., Chapman et al. 2000), the interaction between HT and swimming ability are certainly individual characteristics in striped bass. This was manifest here by the significant reordering of rank order between sprint performances under hypoxia versus normoxia but not in the replicate hypoxia tests. Individual-level interactions between HT and swimming were also seen in earlier studies, where the rank HT measured as loss of equilibrium was significantly repeatable across multiple hypoxia challenge tests months apart (J. A. Nelson and G. K. Lipkey, personal observations) yet was unrelated to the rank order of HT measured while the animal was swimming at 50% of its estimated U_{crit} (Nelson and Lipkey 2015). Several physiological and morphological traits have been associated with hypoxia-induced plasticity in hypoxia tolerance in fish; these factors could contribute to the plasticity in sprint swimming under hypoxia reported here (see Chapman et al. 2000; Nelson and Lipkey 2015; Borowiec et al. 2016).

The lack of repeatability between an individual's rank order sprint performance across hypoxic and normoxic conditions suggests that different individuals may be selected for in environments with different oxygen availability (for review, see Killen et al. 2016). As important as sprint performance undoubtedly is to a pelagic predator like striped bass, it is unlikely that selection will act on a single trait, especially in a dynamic ecosystem like Chesapeake Bay. Several interlinked traits—including thermal tolerance (e.g., oxygen and capacity-limited thermal tolerance; Pörtner 2010), AS (for a review, see Farrell 2016), capacity to recover from environmental stressors (e.g., excess post-exercise oxygen consumption; Marras et al. 2010), morphology (Conradsen et al. 2016), and ability to fight diseases (Lapointe et al. 2014)—under variable oxygen conditions will potentially play roles in directing selection. Future studies should investigate the role that these traits play in determining the Darwinian fitness of striped bass in variable oxygen environments and their coupling or interaction with sprint and other types of swimming performances as well as their role in phenotypically plastic responses that enhance survival.

Despite the relative preservation of rank order across duplicate sprint trials in hypoxic waters, the variance in performances provides some evidence for individual differences in the hypoxia tolerance of sprint performance and in the phenotypic response to hypoxia exposure. The intraspecific variation in sprint performance of striped bass in normoxic water (coefficient of variation [CV] N = 14.0%) was similar to that reported for *D. labrax* (14.3%; Claireaux et al. 2007), but the intraspecific variation in sprint performance was greater when the sprint was conducted in hypoxic waters, especially the second hypoxia test (CV H1 = 17.0%; CV H2 = 21.3%). This variance undoubtedly has some basis in the genotype but may also be associated with individual-specific life histories, variance in the plastic response to hypoxia exposure, or, most likely, combinations of the above (Killen et al. 2013; Conradsen et al. 2016). All fish were reared under the same conditions and experienced equal experimental hypoxia induction twice (e.g., Regan and Richards 2017), but since these fish were wild caught at ~4 mo of age, it is entirely possible that they were differentially exposed to hypoxia during early ontogeny. So while the initial decrement of sprint performance under hypoxia definitely varies by individual, there may be additional variance that accrues with multiple exposures that is individual specific and needs further investigation. Thus, just as proposed trade-offs between aerobic and anaerobic swimming are poorly identified (Oufiero and Garland 2009; Marras et al. 2010, 2013), the relationships between plasticity of HT, aerobic, and anaerobic swimming performances require more investigation if we are to predict the fitness of fish that frequently encounter hypoxic waters.

Effects of Size, Growth, and Rearing Conditions

There were no significant effects of size or growth rate on sprint performance in either normoxia or hypoxia. A lack of allometry in sprint performance was also found for a large sample of European sea bass covering a similar size range (Handelsman et al. 2010). Hypoxia tolerance has been generally shown to be a size-independent trait in fish (Nilsson and Östlund-Nilsson 2008), although there are exceptions (Pan et al. 2016). There is, however, some evidence of species-specific allometric relationships of several traits (e.g., acceleration, metabolic capacity, and metabolic enzyme activity) that may significantly influence sprint performance (Goolish 1991; Norton et al. 2000; Everett and Crawford 2010; Vandamm et al. 2012; Urbina and Glover 2013; Gerry et al. 2016). Overall, because of the small size range of our fish, relatively short time intervals between tests, and a lack of statistical indication of any effects, we concluded that influences of individual size and growth do not merit further discussion.

Although laboratory residence can alter sprint performance (Nelson et al. 2015), the results here do not appear to be artifacts of laboratory residence. The juvenile striped bass used were kept at low densities and were generally quite active in the 285-L tanks. The improved performance in the second (normoxic) test and the general improvement from H1 to H2 suggest that performance deficits were not induced by laboratory residence.

Summary and Perspectives

The mean sprint performance of juvenile striped bass was on average lower in hypoxia sprint tests than in a normoxia sprint test. The rank order of sprint performance was repeatable be-

- here

delete

tween the two hypoxia tests, and individual sprint performance under hypoxia generally improved with a second hypoxia exposure. The rank order of maximal sprint performance under hypoxia was different than that in normoxia, demonstrating interindividual variance in response to whatever diminished sprint performance under hypoxia. Although the rank order of sprint performance was repeatable between the two trials conducted under hypoxic conditions, there was some evidence that the fish were differentially responding to multiple exposures. The size of individual juvenile striped bass or their growth rate in the laboratory had no significant effect on sprint performance either within or across the three sprint tests. The ability of striped bass to tolerate hypoxia with minimally affected swimming performances is likely to be an integral component of Darwinian fitness in waters such as Chesapeake Bay that experience oxygen deprivation in large volumes of water for extended periods. Future research should focus on understanding individual-level aerobic and anaerobic limitations, energetic trade-offs, and energy allocation pathways of fish swimming in water of varying oxygen content.

Acknowledgments

We thank Eric Durell and his team from the Maryland Department of Natural Resources for collecting fish and C. E. Oufiero for helpful advice on statistical analyses. We also thank K. Whitlow for useful discussions, K. Ricci for help in the lab, and anonymous reviewers for their helpful comments on an early version of the manuscript.

Literature Cited

ADInstruments. 2009. LabChart data acquisition and data analysis software. ADInstruments, Bella Vista, Australia. https://www.adinstruments.com/products/labchart.

Axelsson M., J. Altimiras, and G. Claireaux. 2002. Post-prandial blood flow to the gastrointestinal tract is not compromised during hypoxia in the sea bass *Dicentrarchus labrax*. J Exp Biol 205:2891–2896.

Berggren T.J. and J.T. Lieberman. 1978. Relative contribution of Hudson, Chesapeake, and Roanoke striped bass, *Morone saxatilis*, stocks to the Atlantic coast fishery. Fish Bull 76: 335–345.

Boake C.R.B. 1989. Repeatability: its role in evolutionary studies of mating behavior. Evol Ecol 3:173–182.

Borowiec B.G., K.D. Crans, F. Khajali, N.A. Pranckevicius, A. Young, and G.R. Scott. 2016. Interspecific and environment-induced variation in hypoxia tolerance in sunfish. Comp Biochem Physiol A 198:59–71.

Breitburg D.L. 1992. Episodic hypoxia in Chesapeake Bay: interacting effects of recruitment, behavior, and physical disturbance. Ecol Monogr 62:525–546.

Breitburg D.L., D.W. Hondorp, L.A. Davias, and R.J. Diaz. 2009. Hypoxia, nitrogen, and fisheries: integrating effects across local and global landscapes. Annu Rev Mar Sci 1:329–349.

Burt K., D. Hamountene, J. Perez-Casanova, A. Gamperl, and H. Volkoff. 2012. The effect of intermittent hypoxia on growth, appetite and some aspects of the immune response of Atlantic salmon (*Salmo salar*). Aquac Res 45:124–137.

Bushnell P.G., J.F. Steffensen, and K. Johansen. 1984. Oxygen consumption and swimming performance in hypoxia-acclimated rainbow trout *Salmo gairdneri*. J Exp Biol 113: 225–235.

Chapman L.J., F. Galis, and J. Shinn. 2000. Phenotypic plasticity and the possible role of genetic assimilation: hypoxia-induced trade-offs in the morphological traits of an African cichlid. Ecol Lett 3:387–393.

Chapman L.J. and D.J. McKenzie. 2009. Behavioral responses and ecological consequences. Pp. 25–77 in J.G. Richards, A.P. Farrell, and C.J. Brauner, eds. Fish physiology. Vol. 27. Academic Press, New York.

Claireaux G. and D. Chabot. 2016. Responses by fishes to environmental hypoxia: integration through Fry's concept of aerobic metabolic scope. J Fish Biol 88:232–251.

Claireaux G., C. Handelsman, E. Standen, and J.A. Nelson. 2007. Thermal and temporal stability of swimming performance in the European sea bass. Physiol Biochem Zool 80: 186–196.

Claireaux G., D.M. Webber, J.-P. Lagardère, and S.R. Kerr. 2000. Influence of water temperature and oxygenation on the aerobic metabolic scope of Atlantic cod (*Gadus morhua*). J Sea Res 44:257–265.

Conradsen C., J.A. Walker, C. Perna, and K. Mcguigan. 2016. Repeatability of locomotor performance and morphology: locomotor performance relationships. J Exp Biol 219:2888–2897.

Corcoran A. and J.J. O'Connor. 2013. Hypoxia-inducible factor signaling mechanisms in the central nervous system. Acta Physiol 208:298–310.

Dahlberg M.L., D.L. Shumway, and P. Doudoroff. 1968. Influence of dissolved oxygen and carbon dioxide on swimming performance of largemouth bass and coho salmon. J Fish Res Board Canada 25:49–70.

Diaz R.J. and D.L. Breitburg. 2009. The hypoxic environment. Pp. 1–23 in J.G. Richards, A.P. Farrell, and C.J. Brauner, eds. Fish physiology. Vol. 27. Academic Press, New York.

Domenici P. and R.W. Blake. 1997. The kinematics and performance of fish fast-start swimming. J Exp Biol 200:1165–1178.

Domenici P., N.A. Herbert, C. Lefrançois, J.F. Steffensen, and D.J. Mckenzie. 2013. The effects of hypoxia on fish swimming performance and behavior. Pp. 129–159 in A.P. Palstra and J.V. Planas, eds. Swimming physiology of fish. Springer, Berlin.

Domenici P., C. Lefrançois, and A. Shingles. 2007. Hypoxia and the antipredator behaviours of fishes. Philos Trans R Soc B 362:2105–2121.

Dutil J.-D., E.L. Sylvestre, L. Gamache, R. Larocque, and H. Guderley. 2007. Burst and coast use, swimming performance and metabolism of Atlantic cod *Gadus morhua* in sub-lethal hypoxic conditions. J Fish Biol 71:363–375.

Everett M.V. and D.L. Crawford. 2010. Adaptation versus allometry: population and body mass effects on hypoxic me-

tabolism in *Fundulus grandis*. Physiol Biochem Zool 83:182–190.

Farrell A.P. 2016. Pragmatic perspective on aerobic scope: peaking, plummeting, pejus and apportioning. J Fish Biol 88:322–343.

Fry F.E.J. 1971. The effects of environmental factors on physiology of fish. Pp. 1–98 in W.S. Hoar and D.J. Randall, eds. Fish physiology. Academic Press, New York.

Gerry S.P., J. Belden, M. Bisaccia, K. George, T. Mahoney, and D.J. Ellerby. 2016. Scaling of the fast-start escape response of juvenile bluegills. Zoology 119:518–525.

Goolish E.M. 1991. Aerobic and anaerobic scaling in fish. Biol Rev 66:33–56.

Gotanda K.M., E.E. Reardon, S.M.C. Murphy, and L.J. Chapman. 2012. Critical swim speed and fast-start response in the African cichlid *Pseudocrenilabrus multicolor victoriae*: convergent performance in divergent oxygen regimes. Can J Zool 554:545–554.

Hagy J.D., W.R. Boynton, C.W. Keefe, and K.V. Wood. 2004. Hypoxia in Chesapeake Bay, 1950–2001: long-term change in relation to nutrient loading and river flow. Estuaries 27: 634–658.

Handelsman C., G. Claireaux, and J.A. Nelson. 2010. Swimming ability and ecological performance of cultured and wild European sea bass (*Dicentrarchus labrax*) in coastal tidal ponds. Physiol Biochem Zool 83:435–445.

Jourdan-Pineau H., A. Dupont-Prinet, G. Claireaux, and D.J. McKenzie. 2010. An investigation of metabolic prioritization in the European sea bass, *Dicentrarchus labrax*. Physiol Biochem Zool 83:68–77.

Katzir G. and J.M. Camhi. 1993. Escape response of black mollies (*Poecilia sphenops*) to predatory dives of a pied kingfisher (*Ceryle rudis*). Copeia 1993:549–553.

Kemp W.M., W.R. Boynton, J.E. Adolf, D.F. Boesch, W.C. Boicourt, G. Brush, J.C. Cornwell, et al. 2005. Eutrophication of Chesapeake Bay: historical trends and ecological interactions. Mar Ecol Prog Ser 303:1–29.

Kieffer J.D. 2000. Limits to exhaustive exercise in fish. Comp Biochem Physiol A 126:161–179.

Killen S.S., B. Adriaenssens, S. Marras, G. Claireaux, and S.J. Cooke. 2016. Context dependency of trait repeatability and its relevance for management and conservation of fish populations. Conserv Physiol 4:cow007, doi:10.1093/conphys/cow007.

Killen S.S., S. Marras, and D.J. McKenzie. 2014. Fast growers sprint slower: effects of food deprivation and re-feeding on sprint swimming performance in individual juvenile European sea bass. J Exp Biol 217:859–865.

Killen S.S., S. Marras, N.B. Metcalfe, D.J. McKenzie, and P. Domenici. 2013. Environmental stressors alter relationships between physiology and behaviour. Trends Ecol Evol 28:651–658.

Killen S.S., S. Marras, M.R. Ryan, P. Domenici, and D.J. Mckenzie. 2012. A relationship between metabolic rate and risk-taking behaviour is revealed during hypoxia in juvenile European sea bass. Funct Ecol 26:134–143.

Lapointe D., W. Vogelbein, M. Fabrizio, D. Gauthier, and R. Brill. 2014. Temperature, hypoxia, and mycobacteriosis: effects on adult striped bass *Morone saxatilis* metabolic performance. Dis Aquat Organ 108:113–127.

Lefrançois C. and P. Domenici. 2006. Locomotor kinematics and behaviour in the escape response of European sea bass, *Dicentrarchus labrax* L., exposed to hypoxia. Mar Biol 149: 969–977.

Lefrançois C., A. Shingles, and P. Domenici. 2005. The effect of hypoxia on locomotor performance and behaviour during escape in *Liza aurata*. J Fish Biol 67:1711–1729.

Lucon-Xiccato T., J.J.H. Nati, F.R. Blasco, J.L. Johansen, J.F. Steffensen, and P. Domenici. 2014. Severe hypoxia impairs lateralization in a marine teleost fish. J Exp Biol 217:4115–4118.

Marras S., G. Claireaux, D.J. McKenzie, and J.A. Nelson. 2010. Individual variation and repeatability in aerobic and anaerobic swimming performance of European sea bass, *Dicentrarchus labrax*. J Exp Biol 213:26–32.

Marras S., S.S. Killen, P. Domenici, G. Claireaux, and D.J. McKenzie. 2013. Relationships among traits of aerobic and anaerobic swimming performance in individual European sea bass *Dicentrarchus labrax*. PLoS ONE 8:e72815.

Martínez M., H. Guderley, J.A. Nelson, D. Webber, and J.-D. Dutil. 2002. Once a fast cod, always a fast cod: maintenance of performance hierarchies despite changing food availability in cod (*Gadus morhua*). Physiol Biochem Zool 75:90–100.

McKenzie D.J. 2011. Swimming and other activities: energetics of fish swimming. Pp. 1636–1644 in A.P. Farrell, ed. Encyclopedia of Fish Physiology. Elsevier, San Diego, CA.

Nelson J.A., F. Atzori, and K.R. Gastrich. 2015. Repeatability and phenotypic plasticity of fish swimming performance across a gradient of urbanization. Environ Biol Fishes 98:1431–1447.

Nelson J.A. and G. Claireaux. 2005. Sprint swimming performance of juvenile European sea bass. Trans Am Fish Soc 134:1274–1284.

Nelson J.A., P.S. Gotwalt, S.P. Reidy, and D.M. Webber. 2002. Beyond Ucrit: matching swimming performance tests to the physiological ecology of the animal, including a new fish "drag strip." Comp Biochem Physiol A 133:289–302.

Nelson J.A., P.S. Gotwalt, C.A. Simonetti, and J.W. Snodgrass. 2008. Environmental correlates, plasticity, and repeatability of differences in performance among blacknose dace (*Rhinichthys atratulus*) populations across a gradient of urbanization. Physiol Biochem Zool 81:25–42.

Nelson J.A. and G.K. Lipkey. 2015. Hypoxia tolerance variance between swimming and resting striped bass *Morone saxatilis*. J Fish Biol 87:510–518.

Nilsson G.E. and S. Östlund-Nilsson. 2008. Does size matter for hypoxia tolerance in fish? Biol Rev 83:173–189.

Norton S.F., Z.A. Eppley, and B.D. Sidell. 2000. Allometric scaling of maximal enzyme activities in the axial musculature of striped bass, *Morone saxatilis* (Walbaum). Physiol Biochem Zool 73:819–828.

Oufiero C.E. and T. Garland. 2009. Repeatability and correlation of swimming performances and size over varying time-scales in the guppy (*Poecilia reticulata*). Funct Ecol 23:969–978.

Oufiero C.E., M.R. Walsh, D.N. Reznick, and T. Garland. 2011. Swimming performance trade-offs across a gradient

in community composition in Trinidadian killifish (*Rivulus hartii*). Ecology 92:170–179.

Pan Y.K., R. Ern, and A.J. Esbaugh. 2016. Hypoxia tolerance decreases with body size in red drum *Sciaenops ocellatus*. J Fish Biol 89:1488–1493.

Petersen L.H. and A.K. Gamperl. 2010. Effect of acute and chronic hypoxia on the swimming performance, metabolic capacity and cardiac function of Atlantic cod (*Gadus morhua*). J Exp Biol 213:808–819.

Pörtner H. 2010. Oxygen- and capacity-limitation of thermal tolerance: a matrix for integrating climate-related stressor effects in marine ecosystems. J Exp Biol 213:881–893.

Randall D. 1982. The control of respiration and circulation in fish during exercise and hypoxia. J Exp Biol 100:275–288.

R Development Core Team. 2016. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. https://www.R-project.org/.

Regan M.D. and J.G. Richards. 2017. Rates of hypoxia induction alter mechanisms of O_2 uptake and the critical O_2 tension of goldfish. J Exp Biol 220:2536–2544.

Reidy S.P., S.R. Kerr, and J.A. Nelson. 2000. Aerobic and anaerobic swimming performance of individual Atlantic cod. J Exp Biol 203:347–357.

Rice J.A., J.S. Thompson, J.A. Sykes, and C.T. Water. 2013. The role of metalimnetic hypoxia in striped bass summer kills: consequences and management implications. Am Fish Soc Symp 80:121–145.

Robinson E., A. Jerrett, S. Black, and W. Davison. 2013. Hypoxia impairs visual acuity in snapper (*Pagrus auratus*). J Comp Physiol A 199:611–617.

Sandblom E. and M. Axelsson. 2006. Adrenergic control of venous capacitance during moderate hypoxia in the rainbow trout (*Oncorhynchus mykiss*): role of neural and circulating catecholamines. Am J Physiol 291:R711–R718.

Scully M.E. 2016. Mixing of dissolved oxygen in Chesapeake Bay driven by the interaction between wind-driven circulation and estuarine bathymetry. J Geophys Res Ocean 121:5639–5654.

Setzler-Hamilton E.M., W.R. Boynton, J.A. Mihursky, T.T. Polgar, and K.V. Wood. 1981. Spatial and temporal distribution of striped bass eggs, larvae, and juveniles in the Potomac estuary. Trans Am Fish Soc 110:121–136.

Urbina M.A. and C.N. Glover. 2013. Relationship between fish size and metabolic rate in the oxyconforming inanga *Galaxias maculatus* reveals size-dependent strategies to withstand hypoxia. Physiol Biochem Zool 86:740–749.

Vandamm J.P., S. Marras, G. Claireaux, C.A. Handelsman, and J.A. Nelson. 2012. Acceleration performance of individual European sea bass *Dicentrarchus labrax* measured with a sprint performance chamber: comparison with high-speed cinematography and correlates with ecological performance. Physiol Biochem Zool 85:704–717.

Walker J.A., C.K. Ghalambor, O.L. Griset, D. McKenney, and D.N. Reznick. 2005. Do faster starts increase the probability of evading predators? Funct Ecol 19:808–815.

Weber J.-M., K. Choi, A. Gonzalez, and T. Omlin. 2016. Metabolic fuel kinetics in fish: swimming, hypoxia and muscle membranes. J Exp Biol 219:250–258.

Weber J.-M. and F. Haman. 1996. Pathways for metabolic fuels and oxygen in high performance fish. Comp Biochem Physiol A 113:33–38.

 DOI: 10.1086/694933

Appendix from K. Kraskura and J. A. Nelson, "Hypoxia and Sprint Swimming Performance of Juvenile Striped Bass, *Morone saxatilis*" (Physiol. Biochem. Zool., vol. 91, no. 1, p. 000)

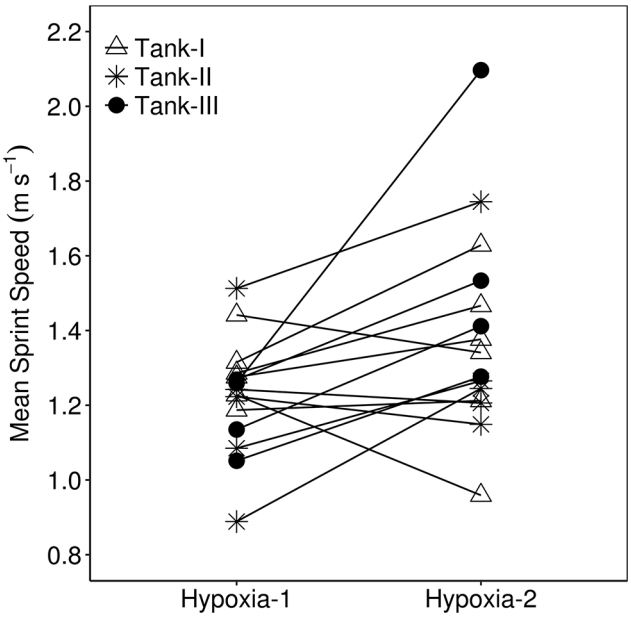

Figure A1. Mean sprint performance of 15 individual juvenile striped bass under hypoxic conditions from three different holding tanks. Each individual was sprinted twice under hypoxic conditions (20% air saturation) approximately 5 wk apart; the order of individuals sprinted in each test was selected randomly. Mixed-effect modeling revealed a slightly significant interaction between sprint performance and holding tank (ANOVA: $F_{4,23.80} = 3.05$, $P = 0.04$), attributable to the small sample size and all animals in tank III improving, including one dramatically.

QUERIES TO THE AUTHOR

Q1. Your article has been edited by University of Chicago Press manuscript editors for grammar, clarity, consistency, and conformity to PBZ style. Please read the proof of your article carefully to make sure that your meaning has been retained. Note that we may be unable to make revisions that conflict with journal style or create grammatical problems. This proof is your final check of the text; once it has been placed on the web as an ahead-of-print article, it is considered published. Thank you.
Q2. Is added citation to ADInstruments 2009 correct?
Q3. Figure A1 in the appendix is currently typeset online only. Is this correct, or should it be included in print?