TOWSON UNIVERSITY
OFFICE OF GRADUATE STUDIES

LOCOMOTOR AND PHYSIOLOGICAL PERFORMANCES OF STRIPED BASS RELEVANT TO DARWINIAN FITNESS UNDER NATURAL LEVELS OF HYPOXIA

by
Krista Kraskura

A thesis
Presented to the faculty of
Towson University
in partial fulfillment
of the requirements for the degree of
Masters of Science
Department of Biological Sciences

Towson University
Towson, Maryland 21252

August, 2017

TOWSON UNIVERSITY
OFFICE OF GRADUATE STUDIES

THESIS APPROVAL PAGE

This is to certify that the thesis prepared by Krista Kraskura entitled Locomotor and physiological performances of striped bass relevant to Darwinian fitness under natural levels of hypoxia has been approved by the thesis committee as satisfactory completing the thesis requirements for the degree of Masters of Science

Dr. Jay A. Nelson
Chairperson, Thesis Committee
Date: 7/29/17

Dr. Christopher E. Outiero
Committee Member
Date: 7/24/17

Dr. Andrij Z. Horodysky
Committee Member
Date: 07/27/2017

Dr. Janet V. Delany
Dean of Graduate
Date: 8/21/17

Acknowledgements

First and foremost, I would like to express my gratitude to my advisor Dr. Jay Nelson. Thanks to his irreplaceable guidance and support through my master's journey, thanks for his valuable academic advice and motivation, help designing and analyzing research studies, and for his endless patience to help me improve in scientific writing. Big thanks to Bradley G. Anderson for his emotional support, inspiration for independent problem solving, and extensive help with computer learning that all have accelerated my progression, which wouldn't have been possible otherwise. Many thanks to my great graduate committee members, Dr. Christopher E. Oufiero and Dr. Andrij Z. Horodysky for their advice and support, as well as Dr. Colleen Winters for her guidance through my teaching assistantship responsibilities. Lastly, but not least importantly, thanks to my family in Latvia that was always with me in spirit. This work would not have been possible without any of these people.

Abstract

Locomotor and physiological performances of striped bass relevant to Darwinian fitness under natural levels of hypoxia

Krista Kraskura

Due to climate change and anthropogenic influences, aquatic hypoxia is occurring more frequently, is more severe and it persist for longer periods of time. Juvenile striped bass being obligate aerobes and occupying an increasingly more hypoxic Chesapeake Bay, may be under additional pressures to meet their metabolic needs to support activities crucial for Darwinian fitness. The goals here were to investigate several such performances under hypoxia: sprint swimming, prey capture, escape response, hypoxia tolerance, and respiration rate under low and high flow conditions. Juvenile striped bass sprinted slower under hypoxia *vs.* normoxia in a repeatable manner. Kinematics of prey capture and escape response seemed to be unaffected when exposed to hypoxia, but motivation to escape and feed was reduced. Their hypoxia tolerance increased over time, but was not obviously related to their metabolic rates while swimming. Aerobic swimming metabolism was suppressed in hypoxic water. And lastly, all performances varied substantially between individuals.

Table of Contents

Chapter 3

List of Tables

Chapter 3: Hypoxia Tolerance, Swimming, and Respiration Rate

List of Figures

Chapter 2: Escape Response and Prey Capture

.

CHAPTER 1

Sprint Swimming

"Hypoxia and sprint swimming performance of juvenile striped bass, *Morone saxatilis.*"

Krista Kraskura and Jay A. Nelson

Abstract

Annual hypoxia in the Chesapeake Bay has expanded to the point where Darwinian fitness of juvenile striped bass (*Morone saxatilis*) may depend upon 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, thus it has generally been thought to be immune to aquatic hypoxia. We tested the sprint swimming performance of fifteen juvenile striped bass twice under acute hypoxia (20 % air saturation (AS)) five weeks apart and once under normoxia (> 85 % AS) in between. Most individuals had a significantly lower sprint swimming performance under the first hypoxia exposure than in normoxia, and most individuals sprinted significantly better in their second hypoxia test. The rank order of individual sprinting performances was significantly repeatable when comparing the two hypoxia tests, but not when comparing normoxic sprint performance with that under hypoxia. The maximum sprinting ability of each individual was also significantly repeatable within a given day. Thus, sprinting ability of striped bass under hypoxia is less than in normoxic waters, is phenotypically plastic and improves with repetitive hypoxia exposures but is unrelated to relative sprinting ability 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.

Introduction

Low oxygen concentrations in aquatic systems are increasingly common in today's world (Breitburg et al. 2009). A combination of high nutrient loading, warmth, low water mixing, and decomposing bacteria produce large hypolimnetic regions devoid of oxygen (dead zones) in many lakes and estuaries. In the Chesapeake Bay, for example, these dead zones may persist for as much as ½ of the year (Hagy et al. 2004; Kemp et al. 2005; Scully 2016a). Unfortunately, these dead zones are not static, as strong winds and tidal currents can quickly drive hypoxic bottom-water upwards into usually normoxic littoral zones (Breitburg 1992; Kemp et al. 2005; Scully 2016a; Scully 2016b). Consequently, aquatic organisms can experience acute hypoxia exposure that may significantly disturb or completely overwhelm the ability of even motile organisms such as fish to escape or acclimate (Domenici et al. 2007; Rice et al. 2013). On such occasions, the survivorship and therefore Darwinian fitness of a fish may be directly associated with its ability to perform routine biological functions under hypoxic conditions. Aerobic swimming performance, measured as U_{crit} or U_{max} (critical swimming speed, and maximum swimming speed, respectively), has been studied often and in multiple species under hypoxic conditions (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 capacity (Dahlberg et al. 1968; Bushnell et al. 1984; Dutil et al. 2007; Petersen and Gamperl 2010), not unexpected for swimming powered mostly by Type I muscle fibers (red) that rely on oxygen for energy transduction (McKenzie 2011). Only a few studies (Lefrançois et al. 2005; Lefrançois and Domenici 2006;

Gotanda et al. 2012 that all measured fast-start escape responses, generally only the first 70-100 ms of an aversive response) have explored whether hypoxia influences fish swimming performances powered mostly by Type II muscle fibers (white) that are fueled primarily with anaerobically produced energy (Weber and Haman 1996). To our knowledge, no studies have investigated sprint swimming 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 mortality selection in many fish types (Nelson et al. 2002; Handelsman et al. 2010; Oufiero et al. 2011; Vandamm et al. 2012), but the direct benefit of high sprinting ability 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. Therefore, sprinting ability might be an important fitness component for many fish species as it is often used in escaping from predators and capturing food (Domenici and Blake 1997; Nelson et al. 2002), and is likely of equal importance to fishes unwittingly being exposed to hypoxic waters.

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 with high risk of exposure to hypoxia. Because these hypoxic zones are mobile, it is unlikely that fish will always be able to escape them, and thus will have to be able to perform in these waters if they are to survive. When

water-breathing fish in hypoxic waters reach their critical oxygen tension (PO_{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). However, the water oxygen tension at which this occurs, and also the duration during which fish can operate aerobically at various levels of hypoxia varies remarkably among 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). Since, the ability to carry out many routine biological functions can be impaired in acute and chronic exposure to hypoxic waters, it is possible that sprint swimming, which is critical to success in predator/prey interactions, could also be affected by hypoxia. Here we test the null hypothesis that sprint swimming performance of juvenile striped bass is unaffected by acute hypoxia. Furthermore, because we examined the same individuals twice in hypoxia and once in normoxia between the two hypoxia exposures, we can report on the repeatability of sprinting performance within and across the contexts of different environmental oxygen levels (Killen et al. 2016).

Methods

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 days 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 ± 1, temperature = 20 ± 1.5°C, salinity = 10 ± 1 ‰) with biweekly 20 – 30 % water exchanges on a 12D:12L light cycle. All animals were fed to satiation at least five days a week with commercial pellet food (Hikari® tropical food sticks) which they readily accepted. At eight weeks post-capture, individuals were anesthetized with tricaine methanesulfonate, MS-222 (100 mg L^{-1}, buffered 1:1 with Na^{+}; HCO_3), weighed (g), and measured for total length (TL) and fork length (FL), and marked with passive integrated transponder tags (PIT-tags; Biomark® Inc.) for individual identification. Fish were allowed a minimum of four weeks to recover from handling and surgery before any experimentation.

Sprint swimming performance was measured in a sprint performance chamber (SPC) described by Nelson et al. (2008). Only minor modifications were required to allow hypoxia exposure. Briefly, the dimensions of the sprint chamber were 1.5 m (length) X 0.15 m (width) X 0.15 m (height). Light-emitting laser diodes (OnPoint Lasers, Inc.) were placed on one side of the chamber at 0, 1, 3, 7, 15, 23, 31, and 39 cm positions from the sprint starting point. A five 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, Inc.; 18 sensors/array, 144 sensors total). One sensor was placed vertically at every 0.5 cm starting at 0.5 cm from the bottom up to 8.5 cm depth. The light beam activated a photoreceptor array that put out a signal to one of eight digital inputs on a Powerlab®/4S (ADInstruments©, Inc.) interfaced with a computer (Apple®, Inc.;

MacBook Pro) with LabChart7® (ADInstruments©, Inc.) 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 0.1 msec accuracy. Sprint swimming velocity was calculated using recorded times and known distances between arrays. Only velocity data from 8 cm intervals (the last four) were analyzed. To control the oxygen tension of the sprint chamber, water was supplied to the SPC via an external circuit connected to an OXY-REG instrument (Loligo® Systems). To maintain oxygenation level, a slight flow (< 1 cm sec^{-1}) was directed against the swimming path of the fish in the SPC that may have contributed to a slight underestimation of sprint speeds that would have been recorded in purely static water.

Before sprinting, randomly selected individuals were fasted for a minimum of 24 and a maximum of 48 hours before being transferred without air exposure to the SPC and allowed a 1-hour acclimation to the SPC (T = 20.03 ± 0.09 ºC, mean ± s.e.m.) (Nelson and Claireaux 2005; Handelsman et al. 2010; Killen et al. 2014). Then, for hypoxia tests, the oxygen concentration was progressively lowered during the second hour (60 min) period to 22.95 ± 0.28 % (mean ± s.e.m.) air saturation (AS; equivalent to 64.4 ± 0.79 µmol O_2 L^{-1}), a rate of hypoxia development reported by Breitburg (1992) to occur in the Chesapeake Bay, by bubbling nitrogen gas into the external circuit of the sprint chamber. For the control tests in normoxia, air was bubbled in place of nitrogen to keep any bubbling disturbance constant between normoxic and hypoxic tests. Oxygen tension was monitored with a galvanic oxygen-sensing probe 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 sprinted a minimum of 5 times with ≥ 5 min recovery between each trial until 3 quality sprints with a straight path and investigator perceived motivation of the fish were obtained. To avoid over-exposure to hypoxia, fish in hypoxia were sprinted a maximum of 8 times. The average number of trials in hypoxia was: 4.5 ± 2.4 (mean ± SD); and in normoxia was: 6.6 ± 3.8 (mean ± SD). No signs of exhaustion or habituation was observed in any of the sprints (see "Results" below), and no fish lost equilibrium during experimentation under hypoxia. The five-minute interval between tests was justified by previous studies showing no trial effect with a similar between trial interval, including those with the congener 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 individual fish were tested within a given occasion was selected randomly. In chronological order, the sprint tests that were performed were: hypoxia (H1); followed by normoxia (N); and a second hypoxia test (H2). The minimum and average time between tests were: 14 and 19 days between H1-N, respectively; 19 and 28 days between N-H2, respectively; 37 and 48 days between H1-H2, respectively. Each fish was weighed and measured on the final day of each sprint test (TL_{H1} = 138.9 ± 6.5 (97 - 183) mm, $mass_{H1}$ = 27.7 ± 4.3 (7.6 - 65.6) g; TL_N = 143.7 ± 6.4 (100 - 186) mm, $mass_N$ = 31.3 ± 4.6 (8.3 - 69) g; TL_{H2} = 153.3 ± 6.3 (107 - 194) mm, $mass_{H2}$ = 37.1 ± 4.6 (11 - 74.4) g; mean ± s.e.m. (range) for all). The fish handling protocol was approved by Towson University's Institutional Animal Care and Use Committee (12042013JN-02).

Data were analyzed and tested for significance using R v3.3.1 (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's correlation, and individual repeatability between means of three best sprints from each sprint test (H1-N, N-H2, H1-H2; *i.e.* long-term repeatability) were 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 (1) test whether size (TL, mm) and growth rate (GR, mm (TL) day^{-1} and g day^{-1}) were covariates affecting sprint speed, to (2) test for the effect of which tank the fish were being held in and observed sprinting ability of individuals, and to test (3) for significance differences in sprint swimming performance among the three sprint tests (H1, N, H2). In models testing for covariates 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 sprinting ability 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 Chi-squared test of independence to test whether within each sprint test (H1, N, H2) an animal's maximum sprint speed was equally likely to occur in any of the 8 cm laser intervals (7-15 cm, 15-23 cm, 23-31 cm, 31-39 cm from the start of the SPC) and also to test whether maximum sprint speed was independent of swim trial on a given day.

Furthermore, we used Analysis of Variance (ANOVA) to find 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 HDS test on the final model was used to identify differences between least square mean sprint speeds in all tests. Sprint swimming performance is reported as the mean of each individual's top three sprints ± s.e.m. throughout the remainder of the manuscript.

Results

Repeatability

Sprint swimming performance of juvenile striped bass was significantly repeatable on a daily basis for all three of the sprint tests (r_{H1} (13) = 0.78, r_N (13) = 0.94, r_{H2} (13) = 0.96, $p < 0.001$ for all, data not shown). The rank order of mean sprint performance by each individual was also repeatable over an average of five weeks, 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 *etc.*); thus sprint performance between trials conducted in hypoxic water and the trial conducted in normoxic water was not considered significantly repeatable (Spearman's $\rho_{N\text{-}H1}$ (13) = 0.13, $p = 0.65$; $\rho_{N\text{-}H2}$ (13) = -0.46, $p = 0.08$; Kendall's W (H1-N-H2) = 0.38, $p = 0.31$).

Effects of hypoxia

The mean sprint speed of juvenile striped bass was 1.23 ± 0.04 m s^{-1} in H1, 1.49 ± 0.05 m s^{-1} in N, and 1.39 ± 0.07 m s^{-1} in H2 (Fig. 2A), which 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 sprinting performance was significantly better in the second hypoxia trial than the first ($p = 0.02$). Nine individuals sprinted consistently slower in hypoxia than in normoxia, four individuals were not affected by environmental oxygen level, and two individuals sprinted better in hypoxia (Fig. 2B). Eleven out of 15 individuals sprinted better in hypoxia the second time they were exposed to it (Figs. 1 & 2B). 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 sprinted under hypoxia than when sprinted in normoxic water. We only used animal velocities recorded from the final four 8 cm intervals of the SPC for all analyses and Chi-square analyses showed that for all sprint tests, an animal was significantly less likely to have its maximum velocity recorded from the final 8 cm interval (Fig. 3). For the two hypoxia tests, the animal was also significantly less likely to have its maximum velocity recorded from the third 8 cm interval, which was not true in normoxic water (Chi-square test: χ^2H1 (3) = 10.56, $p = 0.014$; χ^2H2 (3) = 16.96, $p < 0.001$; χ^2N (3) = 10.56, $p = 0.014$) (Fig. 3). There was no effect of trial number within a given day on when a fish recorded its maximum velocity for any trial under any environmental condition (Chi-square test: χ^2H1 (7) = 6.91, $p = 0.44$; χ^2H2 (7) = 10.9, $p = 0.14$; χ^2N (7) = 1.77, $p = 0.97$; data not shown), *i.e.* a fish was equally as likely to have its best sprint recorded in its first trial as in its last.

Effects of size, growth, and rearing conditions

Neither fish size or growth rate significantly affected or interacted with sprinting ability 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 day^{-1} or mm (TL) day^{-1} was also an insignificant covariate (ANOVA: g day^{-1}: F (2, 30.57) = 1.91, p = 0.17; mm (TL) day^{-1}: F (2, 63.438) = 1.38, p = 0.26) in determining the sprint speed of juvenile striped bass over the nine week duration of the experiment. Therefore, neither individual size or growth rate was included in the primary statistical analyses. However, there was a slightly significant interaction between sprinting ability and rearing tank (ANOVA: F (4, 23.80) = 3.05, p = 0.04) attributable to one individual having an extraordinary improvement in sprinting performance during the second hypoxia test. 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 (data not shown).

Discussion

Repeatability

The significant individual repeatability of striped bass sprint performance in hypoxic water over five weeks suggests within-context stability of this trait (Killen et al. 2016). Long-term repeatable sprinting performances have also been reported for the co-familiar 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 swimming performance of fish under hypoxia. The approximate five-week 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 the 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 swimming 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 sprinting ability in hypoxia, whatever caused this average dimunition of performance in hypoxia was not uniform across this group of fish and could, therefore, contribute to fitness differences upon intrusion of hypoxic water into their habitat (Breitburg 1992). This average diminished sprinting performance elicited by reducing the oxygen saturation of water to approximately 20 % AS, is not predictable from the energetics of swimming. Sprint swimming 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), changes its energy use patterns, metabolic by-product 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 energy 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 re-direction 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 laterization 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 by 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' maximum velocity was recorded in hypoxia versus normoxia. Fish seemed unwilling to sprint further than 23 cm when in hypoxic water, despite being chased by a human. However, that same fish sprinting in normoxia was equally as likely to have its maximum velocity recorded at any point up to 31 cm. The co-familiar *D. labrax* also has demonstrable behavioral changes under hypoxia, showing increases in boldness and risk-taking behavior (Killen et al. 2012).

Phenotypic plasticity in response to hypoxia exposure was fairly uniform amongst this group of wild striped bass collected from the same location. Despite a quite variable difference between sprint swimming performance during an initial hypoxia exposure and their subsequent normoxic performance, performance during a second exposure to 20 % AS water increased by a similar degree in most individuals ($\bar{X}$ = 0.17 ± 0.07 m s^{-1}; Fig. 2AB). The significant repeatability of rank order between the two hypoxia trials statistically 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 endpoint (J.A. Nelson and G.K. Lipkey unpublished 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 in the present study by the significant reordering of rank order between sprinting 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 unpublished 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 likely 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 sprinting ability 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). However, as important as sprinting 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 inter-linked traits, including thermal tolerance (*e.g.* oxygen and capacity-limited thermal tolerance, OCLTT; Pörtner 2010), aerobic scope (AS; for a review see Farrell 2016), capacity to recover from environmental stressors (*e.g.* excess post-exercise oxygen consumption, EPOC; 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 sprinting performance and in the phenotypic response to a 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 before arriving to the lab (Killen et al. 2013; Conradsen et al. 2016), variance in the plastic response to a single hypoxia exposure or, most likely, combinations of the above. All fish were reared under the same conditions and experienced equal amounts of hypoxia exposure in the laboratory, but since these fish were wild-caught at ~ 4 months of age, it is entirely possible that they were differentially exposed to hypoxia during early ontogeny. So, while the initial decrement of sprinting ability under hypoxia definitely varies by individual, there may be additional variance that accrues with multiple exposures that is individual-specific but is beyond the scope of this experiment to ascertain. Thus, just as proposed trade-offs between aerobic and anaerobic swimming are poorly identified (Oufiero and Garland 2009; Marras et al. 2010; Marras et al. 2013), the relationships between plasticity of HT, aerobic and anaerobic

swimming performance of fish 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 was no significant effect of size or growth on sprint performance either in normoxia or hypoxia. A lack of allometry in sprint performance was also found for a large sample of the European sea bass covering a similar size range as the striped bass measured here (Handelsman et al. 2010). Even though there is some species-specific evidence of existing allometric relationships of several traits that may significantly influence sprint swimming performance (*e.g.* acceleration: Vandamm et al. 2012; Gerry et al. 2016, metabolic capacity: Everett and Crawford 2010; Urbina and Glover 2013, metabolic enzyme activity: Goolish 1991; Norton et al. 2000), 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). Overall, due to 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 on the observed results did not merit further discussion. These juvenile striped bass were generally quite active in the 285 L tanks, and low densities they were held at and the general improvement in sprint performance from H1 to H2 suggests that they weren't suffering any performance deficits from the lethargy of laboratory residence. A significant tank effect arose from a dramatic improvement in performance by one fish during its second hypoxia exposure.

Summary and perspectives

In summary, the mean sprinting 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 significantly repeatable between the two hypoxia tests, and individual sprinting ability under hypoxia generally improved with a second hypoxia exposure. The rank order of swimming ability in hypoxia was different than that in normoxia, demonstrating inter-individual variance in response to whatever diminished sprint swimming performance in hypoxia. Although the rank order of sprint performance was significantly repeatable between the two sprints 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 swimming 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 like Chesapeake Bay that experience oxygen deprivation in large volumes of water for extended periods of time. Future research should focus on understanding individual-level aerobic and anaerobic limitations, energetic trade-offs, and energy allocation pathways of striped bass while swimming in water of varying oxygen content.

Figure Legends

Figure 1. Repeatability of sprint swimming performance of fifteen juvenile striped bass in water with oxygen regulated at 20 % of air saturation. The mean sprint swimming performance of each individual in its first hypoxia trial (H1) is plotted against its mean performance in a second identical trial (H2) approximately five weeks later. Mean sprint speeds were calculated from the three top velocity intervals taken from each of three separate sprints. Means ± 1 s.e.m. are plotted with a 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 swimming performance of fifteen juvenile striped bass in three separate trials ordered chronologically. Each fish was sprinted first in water with an oxygen content 20 % of air saturation, AS, (H1) followed by a sprint in normoxia ($[O_2]$ > 85 % AS; N) at least 14 days later, followed by a second hypoxic sprint (H2) at least 19 days later. Panel (A) shows a boxplot where horizontal 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 dots), or values more than ± 1.5 IQR outside of the box. In panel (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 sprint test (ANOVA, F (2, 23.12) = 31.74, $p < 0.001$); Tukey post-hoc test indicated significant difference between H1 and N tests ($p < 0.001$), but non-significant difference between N and H2 ($p = 0.56$).

Figure 3. Frequency distribution of which 8 cm laser interval of the 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 (approximately 20 % of 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 8 cm intervals was significantly affected by hypoxia (Chi square test of Independence: χ^2H1 (3) = 10.56, p = 0.014; χ^2H2 (3) = 16.96, p < 0.001; χ^2N (3) = 10.56, p = 0.014).

Figure 1

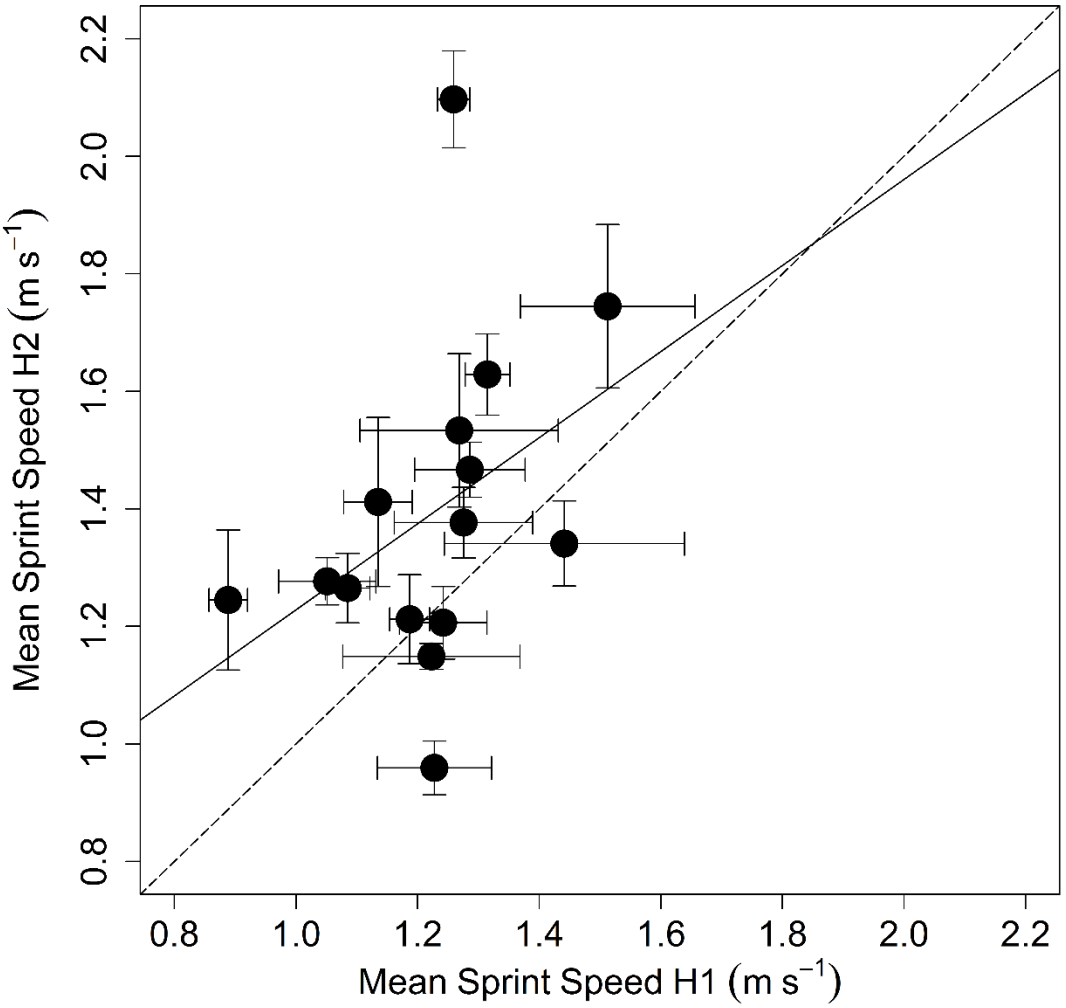

Mean Sprint Speed H2 (m s^{-1})
Mean Sprint Speed H1 (m s^{-1})
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2

Figure 2.

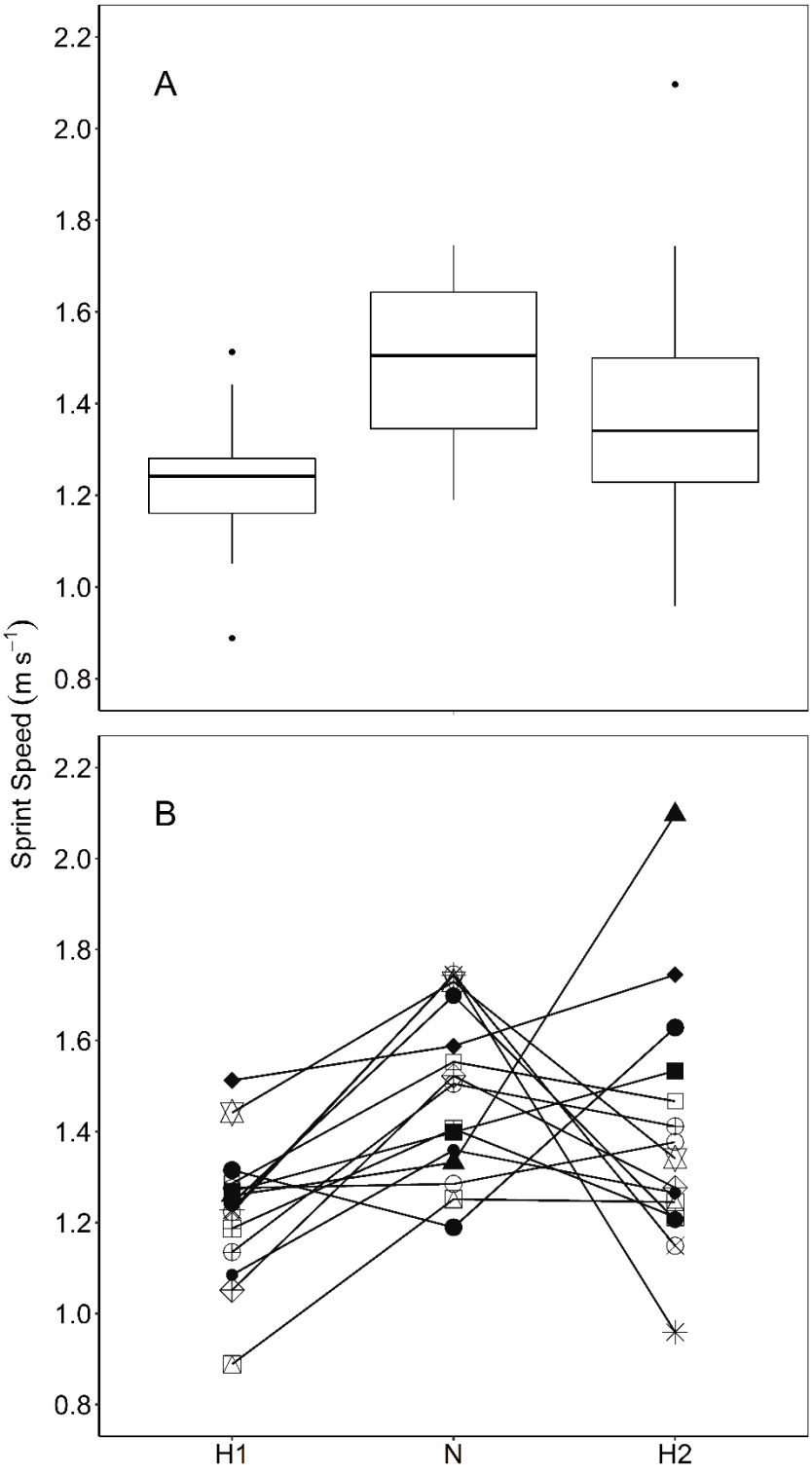
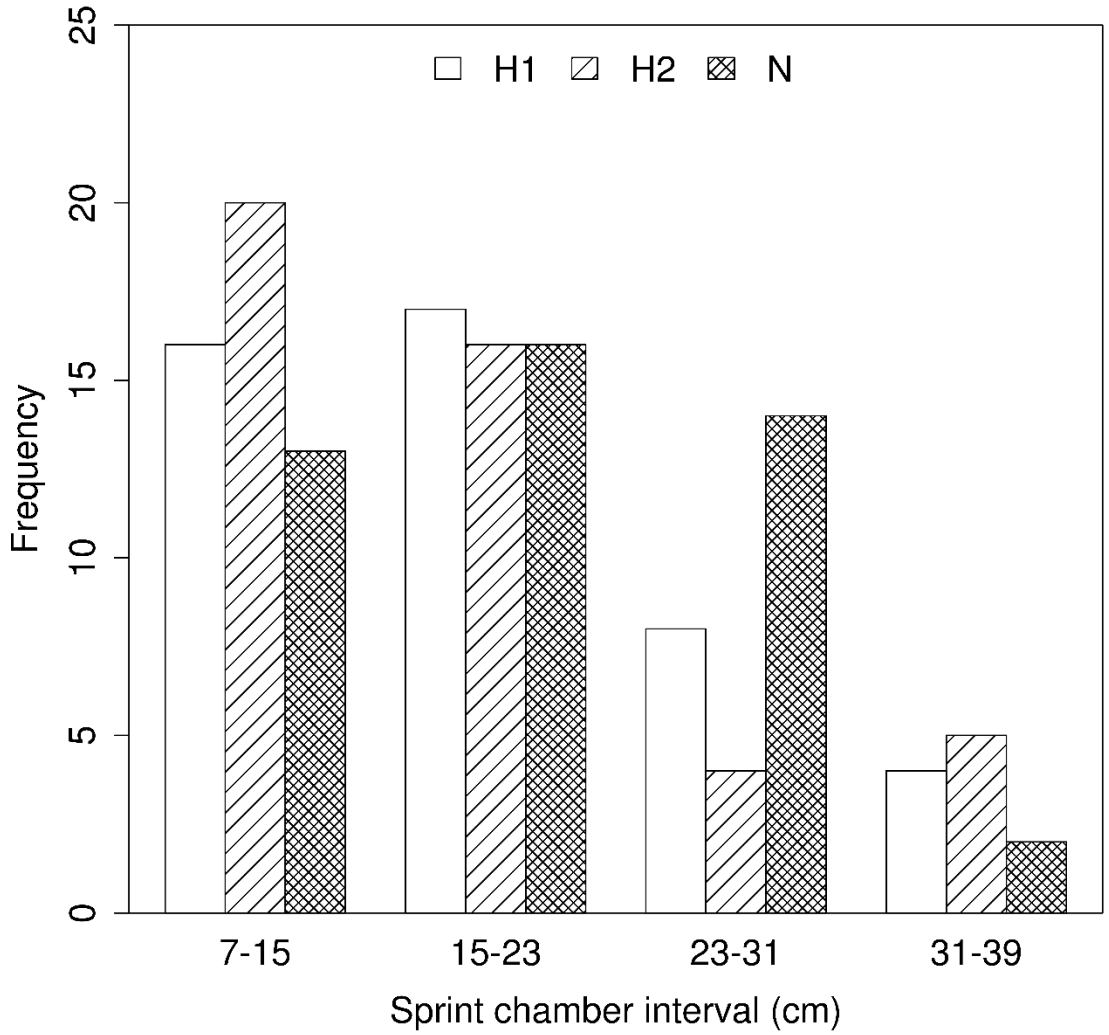


Figure 3


H1
H2
N
Frequency
0
5
10
15
20
25
7-15
15-23
23-31
31-39
Sprint chamber interval (cm)

CHAPTER 2

Escape Response and Prey Capture

"Escape response and prey capture ability of juvenile striped bass: hypoxia alters behavior not kinematics."

Krista Kraskura

Abstract

Successful engagement in predator-prey relationships is a crucial part of an individual's fitness, which comprises both the ability to escape from predation and capture prey. These performances may become extremely important and determinant when environmental conditions are not optimal. Juvenile striped bass inhabiting the Chesapeake Bay may become unavoidably exposed to hypoxia, and if they are to survive they must remain motivated and successful in catching prey and escaping from predators. Thus, the aim of this study was to investigate the kinematics and behavior of fast-start escape responses and prey capture performances of juvenile striped bass exposed to realistic hypoxic (20 % air saturation, AS) and optimal, normoxic conditions (>90 % AS). The responsiveness to escape inducing stimuli was significantly reduced under hypoxia, but when responsive, the locomotor ability seemed to remain unaffected. Similarly, with the prey capture performance, only three out of five individuals engaged in feeding when oxygen levels dropped near 20 % AS. While kinematics and locomotion were largely unaffected by hypoxia, a principal component analysis on prey capture revealed that 'timing variables' explained 44.5 % of the total variance, and maximum kinematic excursion variables explained 18.5 % of the variance observed in this dataset. From the available data, we may suggest that if juvenile striped bass are going to remain active in predator-prey interaction under hypoxia, their performance may be only minimally affected.

Introduction

Seasonal hypolimnetic hypoxic zones as well as anoxic (dead) zones in many aquatic systems, including the Chesapeake Bay, continue to expand in volume, becoming more severe, and persistent (Hagy et al. 2004; Diaz and Rosenberg 2008; Breitburg et al. 2009). Hypoxia may affect fish directly by altering their physiological processes (for reviews see Claireaux and Chabot 2016; Farrell 2016), thus setting limits to engage in energetically expensive activities such as swimming, feeding and reproduction (Chapman and McKenzie 2009), or indirectly via population level and ecosystem changes, *e.g.* relocation and redistribution of conspecifics as well as individuals from other species inhabiting the ecosystem (Breitburg 1992; Bell and Eggleston 2005; Chapman and McKenzie 2009). As a result, species interactions, including predator-prey interactions, may be affected by hypoxic water (Breitburg et al. 1994; Chapman and McKenzie 2009). But because not all species and individuals within a species are affected similarly by hypoxia, there will be "winners" and "losers" when the environment becomes hypoxic; the identity of these winners or losers can vary according to species tolerances but also substantially according to the individual tolerances within a species (Abrahams et al. 2007; Nelson and Lipkey 2015). Furthermore, several limiting variables, such as the rate of oxygen displacement, persistence of low oxygen levels, availability of refuge, and ability to acclimate will likely dictate or contribute to the animals' responses or their potential success in hypoxia (Breitburg 1992; Breitburg et al. 1994).

Two thirds of the Chesapeake Bay waters are hypoxic from early spring until fall (Hagy et al. 2004; Breitburg 2002). Although highly motile species such as fish can often avoid the hypolimnetic dead zone, strong winds and tidal currents can quickly draw these

severely hypoxic waters upwards into surface layers (Breitburg 1992; Kemp et al. 2005; Scully 2016a) consequently exposing fish in the epilimnion to acute hypoxia. Striped bass are an ecologically, recreationally, and economically valuable species in the Chesapeake Bay; because the Chesapeake Bay is the nursery for up to 90% of the Atlantic population (Berggren and Lieberman 1978), their seasonal abundance means they are important components of Bay food webs at all ages (Fay et al. 1983). At the juvenile stage, they are preyed upon by large predatory piscivores, and if close to the surface they may become prey for piscivorous birds (Fay et al. 1983). On the other hand, striped bass are voracious predators mostly feeding on highly motile smaller pelagic fish and zooplankton (Cooper et al. 1998; Walter et al. 2003). Hypoxic conditions may not only force striped bass to relocate to possibly more vulnerable locations, but also their prey densities, composition, and distribution may change, likely increasing their challenge to acquire food (Breitburg et al. 1994). In addition, hypoxia-exposed individuals will be coping physiologically with the lack of oxygen that can potentially affect any predator-prey associated locomotive and behavioral performances, including unexpected ones like sprinting performance (see chapter: *Sprint swimming*; Domenici et al. 2007; Chapman and McKenzie 2009; Domenici et al. 2010; Domenici et al. 2013). To survive and maintain their fitness under hypoxic conditions, individuals will have to find a way to effectively engage in predator-prey interactions, *i.e.* both feeding and escaping predation.

Predator evasion is crucial to survival, and the escape response in fish morphologically similar to striped bass typically starts with a C-bend (stage 1), followed by a contralateral muscle contraction (stage 2) and sprint or steady coasting away from

the threat (stage 3) (Domenici and Blake 1997; Blake 2004; Nowroozi and Brainerd 2013). Fast-start responses are primarily powered by anaerobically produced energy and mediated by a large pair of neurons called Mauthner cells that allow fish to respond within a couple of ms (~5-10) (Domenici 2011). It is a well conserved trait, but also evolves quickly in the presence of selective pressures (Domenici and Blake 1997; Blake 2004). The fast-start consists of behavioral components (*e.g.* responsiveness, latency, directionality) and locomotive-kinematic components (*e.g.* escape velocity and acceleration, turning angle and rate) (Domenici and Blake 1993; Domenici 2011), and can be influenced by factors such as body form and size (Webb 1978; Blake 2004; Gerry et al. 2012), ontogeny (Wakeling et al. 1999; Gerry et al. 2016) and the type and direction of the stimulus initiating the response (Eaton and Emberly 1991; Domenici 2010). Furthermore, fast-start escape responses can vary among- and within- species (Jornod and Roche 2015; for review see Domenici 2010). The fast-start escape response of fishes has been heavily studied, however, the effects of environmental conditions on escape response have only been minimally addressed. For example, temperature is known to affect fast-start response in some species (reviewed by Domenici and Blake 1997; Lyon et al. 2007; Nasuchon et al. 2016), and hypoxia affects fast-starts (Lefrançois et al. 2005; Lefrançois and Domenici 2006; Gotanda et al. 2012), but these results remain controversial and more studies on different species are needed to make ecologically relevant conclusions. The present study investigates and characterizes the escape response of juvenile striped bass under hypoxia and normoxia, with the complementary goals of evaluating whether fast-start performance could explain the previously observed decrease in sprint swimming under hypoxic water in the same individuals examined

earlier (see chapter 1: *Sprint swimming*) and whether it relates to their ability to capture prey. This study can also address the question of whether relative performance of individuals is the same across different levels of environmental [O_2].

Prey capture performance is another measure of potential importance to Darwinian fitness for fish feeding under any environmental conditions, including hypoxia. Generally, three well conserved motor pattern strategies (often in some combination) can be employed to capture prey, those include suction feeding, ram feeding, and biting (Liem 1980; Wainwright 2002). Based on their morphology and ecological niche, striped bass are best suited for suction feeding by approaching elusive highly motile prey from a distance with high attack (ram) speed (Cook 1996; Grubich 2001; Carroll 2004; Day et al. 2015). Both cranial and axial fast glycolytic muscles power expansion of the buccal cavity to accelerate prey and water into the mouth (Camp et al. 2015; Day et al. 2015, Westneat and Olsen 2015). Although, kinematics and behavior of prey capture are considered by many to be stereotypical traits acquired through evolution (Grubich 2001, Wainwright 2002), a number of species can exhibit modulatory multiplicity of feeding styles under direct effects of varying biological conditions (Wainwright 2002). Common examples include modulated prey capture in the presence of different prey types and sizes (Nemeth 1997; Wainwright and Friel 2000; Ferry-Graham et al. 2001), and level of satiation (Sass and Motta 2002; reviewed by Wainwright 2002). There are very few documented studies on prey capture modulation under altered physical-environmental conditions, such as those associated with global climate change and/or anthropogenic influences like O_2, pH, CO_2 levels, temperature, and (or) turbidity (*e.g.* Wintzer and Motta 2004; Meager et al. 2005; deVries and Wainwright

2006; Sloan and Turingan 2012, Allan et al. 2017). To our knowledge, no study reports on prey capture under varying oxygenation levels for any fish species. In this study, the goals were to characterize the kinematics of prey capture of juvenile striped bass under normoxic and environmentally realistic hypoxic conditions, to compare recorded behavior and kinematics across two environmental oxygen levels, and to integrate these results with those from fast-start analysis.

Both prey capture and fast-start are explosive performances predominantly powered by white glycolytic muscles, thus are not expected to be affected by environmental hypoxia. But some studies have shown behavioral and locomotive changes in fish under hypoxic conditions; the underlying mechanisms are still unknown but hypothesized to be neural. This is the first report of prey capture and suction feeding performance for any fish under hypoxia, and the first study where both performances, escape response and prey capture, are examined in the same individuals allowing to explore context dependent performances intra-individually as well as inter-individually. Overall, the goal of this study is to help expand our understanding of predator-prey performances in hypoxic water for juvenile striped bass, thus helping evaluate their future success in an increasingly hypoxic Chesapeake Bay.

Methods

Animals

The juvenile striped bass that were used for all tests here (sample sizes for each test indicated below) were the same individuals that were tested for maximal sprint performance (chapter 1: *Sprint swimming*). Fish were collected in the summer of 2015 from the upper Chesapeake Bay and transported to Towson University, where they were

gradually acclimated over 2 days to the rearing conditions of 20°C and 10 ‰ salinity with a maximum temperature change of 2.5°C per day. Fish were held in three 285 L tanks (temperature = 20 ± 1.5°C, salinity = 10 ± 1 ‰), water was exchanged biweekly (20-30 % volume) to maintain optimal water quality conditions. Photoperiod was set to 12D:12L. Animals were fed to satiation at least five days a week with commercial pellet food (Hikari® tropical food sticks). Four weeks before the first prey capture test, fish diet was switched to live guppies, *Poecilia reticulata,* and minnows, *Pimephales promelas,* and they were kept on this diet until the end of all experiments. For identification purposes, fish were implanted with passive integrated transponder (PIT-tagged) at eight weeks post-capture. Individuals were anesthetized in MS-222 (100 mg L^{-1}, buffered 1:1 with Na^{+}; HCO_3), weighed (g), and measured for total length (TL) and fork length (FL), then marked with the PIT-tag (Biomark® Inc.). Fish were allowed a minimum of four weeks to recover from handling and surgery before they were subjected to tests of any kind, and they were not handled for 8 weeks before any of the tests in this study. Experimentation took place in May-June 2016, and August-September 2016 examining escape response and prey capture, respectively. The fish handling protocol and research procedures were approved by Towson University's Institutional Animal Care and Use Committee (12042013JN-02 and 03312014CO-02).

Escape response (ER)

Experimental set up

A fast start tank (h = 40 cm, d = 130 cm) with supplemental hypoxia circuit was used for all escape response tests. The tank had a restricted circular experimental area (84 cm in diameter). A submersible LED light strip was placed on the side walls to make it a

non-preferable location for the fish to reside and to help provide adequate illumination for filming. A one cm square grid was placed on the bottom of the experimental area and used for distance calibration in digital analysis. We used a scaffold built on the tank to center the camera and attach a PVC pipe above the experimental area. The PVC pipe terminated 10 cm above the water level (depth = 15 cm) and was used to direct a rubber ball to a fixed spot to initiate an escape response while avoiding escape behaviors due to visual stimulation by the falling ball. Two submersible pumps and airstones were used to facilitate gas (nitrogen and air) distribution, thus ensuring uniform oxygen conditions within the tank. The experimental setup is represented graphically in the Figure 1. Oxygen concentration (% AS) in the tank was monitored and recorded during all experiments using galvanic oxygen probes (OxyGuard Mini Probe, Loligo® Systems). The flow in the experimental area was negligible. This experimental set up allowed oxygen level control without noticeable disturbance to the fish.

Escape response (ER) test

Eleven individuals had their ER performance tested under two conditions: hypoxia (H) and normoxia (N). The experimental order of individuals and tests, *e.g.* which individual was subjected to which test first was selected randomly. Fish that were tested under hypoxia first were given a minimum of seven days of recovery, and those tested under normoxia first were given a minimum of two days of recovery before being tested again. Feeding was discontinued at least 24 hours before any experimentation. The selected individual was transferred without air exposure to the experimental area in the fast start tank and allowed to acclimate for 60 min (air saturation, AS = 98.9 ± 1.32 %, salinity = 10.6 ± 0.84 ‰, T = 19.9 ± 0.69 ^{o}C; mean ± SD for all). For ER tests in hypoxic

water, AS % was gradually lowered at an ecologically relevant rate to 20 % AS (equivalent to 1.8 - 2.0 mg O_2L^{-1}; Breitburg 1992) over 60 min, by bubbling N_2 gas in the circulating water. The oxygen level was monitored and maintained at 20 ± 1 AS % while testing using an OXY-REG instrument (Loligo® Systems). In normoxic tests, air was bubbled in place of N_2 gas and the AS % in water was maintained at > 95 % AS for the ensuing 60 min. The LED lights were turned on at the start of the experiment. An escape response stimulus was given only when fish were in a static position or gliding slowly and positioned a minimum of one estimated fish length away from the wall but not directly under the PVC pipe. A test subject was given 60 min to voluntarily move around the experimental area before initiating a fast-start. If the fish never took a position as described above, a non-aggressive, but motivating stimulus (*e.g.* calm tapping on the other side/against the restrictive wall) was applied to encourage the individual to reposition. Escape responses were recorded with a GoPro Hero 4 camera (GoPro, Inc.) at 240 frames s^{-1} and all videos analyzed afterwards.

Digital analysis

Recorded videos were converted from mp4 format to frame-image sequence using ImageMagic® software. The following selected parts from each video were used for analysis: (1) the frame when the ball first comes in contact with the water and (2) frame sequence starting with the first detectable movement of fish and covering a minimum of stages 1 and 2 of the fast-start (stages defined and described in-text and reviewed in detail by Domenici and Blake 1997). All images were imported in ImageJ software and digitized on an (x, y) coordinate plane using MtrackJ plugin. Three following points were tracked: the estimated center of mass of fish (CoM; point at the beginning of first dorsal

fin), the tip of the snout, and the stimulus (approximate middle point of the ball at the time it came in contact with water).

All further analyses were done in R v3.3.1 software (2016). First, each derivative step of CoM and head was smoothed using a local polynomial regression fitting function statistic to minimize human tracking errors. A list of calculated kinematic and behavioral variables of interest with their descriptions are provided in Table 1.

Statistical analysis

Pearson's Chi-square test with Yates' continuity correction was used to test the null hypothesis that the probability that fish will or will not respond to provided stimulus is independent of environmental oxygen levels (N and H), and a Chi-square test for given probabilities (50%) was used to test whether the probability to respond within each test (N and H) was the same. All locomotive-kinematic variables were tested for significance using Welch's two sample t-test.

Prey capture

Experimental set up

Prey capture experiments, both in hypoxic and normoxic water, were conducted in the housing fish tanks. During tests under hypoxia, submersible pumps were used to create uniform oxygen conditions. The flow created during a hypoxia test was equal to that fish experienced on a daily basis and while tested under normoxia. Currents were measured using a flowmeter (Flo-mate 2000, Marsh-McBirney Inc.) in at least 5 randomly selected locations throughout the tank to ensure that conditions were similar. Prior to each test, a galvanic oxygen probe (OxyGuard Mini Probe, Loligo® Systems) was

calibrated and placed in the tank to control and monitor [O_2] in the water using the OXY-REG instrument (Loligo® Systems). Disturbances to the fish were minimized at all times.

Prey capture test

Juvenile striped bass were tested in 2 groups (n = 3; n = 2) to maintain competition for food between individuals and help motivate strikes. Each group was tested a total of 4 times, in chronological order: under normoxic conditions (N1), hypoxic (H1), repeated normoxic (N2), and repeated hypoxic (H2) conditions. Time periods between experimentation was as follows: 10-13 days between N1-H1, 5-8 days between H1-N2, and 7-9 days between H2-N2. Temperature and salinity in all tests were maintained at 20 ± 0.5 ^{o}C (mean ± SD) and 10 ‰, respectively. During normoxic tests (N1, N2), oxygen level in the water was maintained at > 90 % AS. For H1 and H2, the oxygen level was gradually lowered to 20% AS over a period of 1 h by bubbling nitrogen gas (followed the same procedure as described above for the escape response tests); hypoxia level was kept stable at 20 ± 1 % AS during feeding. After completion of the test in hypoxic water, the tank was re-oxygenated (optimal AS for this species approximately >80 %AS was reached in approximately 40 min). No fish lost equilibrium during experimentation.

All prey capture events were filmed using a high speed camera (Fastec IL3-100S, Fastec Imaging, Corp.) at 1000 frames s^{-1}. A Nila Zaila LED light (Nila, Inc.) was used to provide proper lighting condition for filming at given speeds. Before each series of tests, an image of a ruler placed in the filming frame were captured and used in later data analysis to calibrate distance variables. Individual prey fish, one at the time, were introduced to the tank using a plastic tube. Two prey species were used: minnow,

Pimephales promelas, for the larger striped bass (n = 3) and guppy, *Poecilia reticulata*, for smaller individuals (n = 2); prey adjustments were made to minimize the potential confounding effects of different predator to prey size ratio (Osse et al. 1997; Lundvall et al. 1999). We did not observe any changes in motivation or behavioral differences in feeding between individuals fed different prey. All prey fish had their caudal fin cut before being introduced in the tank to limit movement and spatial dispersion of feeding strikes. Feeding was discontinued 48 hrs before any testing. All strikes and capture success (captured/ not captured with the first attempt) of each individual were reported, but only explosive motivated strikes in which fish were oriented laterally to the camera were used for digital analysis.

Digital analysis

All videos were digitized on an (x, y) coordinate plane using ImageJ software with the MtrackJ plugin. The following nine landmarks were tracked (Fig. 2; Oufiero et al. 2012): 1- the upper tip of the jaw, 2- the lower tip of the jaw, 3- fixed point on the upper dorsum, 4- fixed point on the lower dorsum, 5- the estimated CoM of the prey, 6- the posterior spot on mandible, 7- fixed point on the upper cranium, 8- fixed point on the lower cranium, 9- fixed point on the lower jaw, between points 2 and 6.

Further analysis was done in R software (2016). Using the landmark points (Fig. 2) several kinematic variables were calculated. For all kinematic-displacement variables, the corresponding speed was calculated using the generic formula, speed = derivative distance traveled at each times step / 0.001 s. Table 2 summarizes all calculated variables, which were smoothed using a local polynomial regression method (R package stats:loess) to minimize human tracking error. Lastly, all maximum (peak) excursion values and their

timing within the prey capture events were also calculated. Prey capture is defined here as the event beginning at the time point when gape is 30 % of the maximum opening and ending at the time point when the prey crosses the line between upper and lower jaws (points 1 and 2).

Statistical analysis

We used R software and principal component analysis (PCA; R package stats::pca) to analyze 10 variables simultaneously and find the relationships among them. All maximum kinematic displacement variables, their timing, and attack speed were used to create the correlation matrix. We also ran a PCA excluding attack speed from the original 10 variables selected to evaluate the role of attack speed during prey capture. The broken stick criterion was used to justify our selection of PC axes, and their use in further analysis and discussion.

Results

Escape response

Responsiveness was dependent on environmental oxygen levels (Chi-square test: χ^2 (1) = 12.07, $p < 0.001$). A fish was equally likely to respond or not respond to stimuli under normoxia, whereas under hypoxia responsiveness was significantly reduced (Chi-square test: χ^2 H(1) = 28.17, $p < 0.0001$; χ^2 N(1) = 12.07, $p = 0.86$; Fig. 3). Among 33 given stimuli in normoxic water (total for all fish) 16 induced ER, while only 5 out of 41 stimuli induced an ER while under hypoxia (Fig. 3). There were 9 ‘responders’ under normoxia, and only 4 under hypoxia. Two individuals that where 100% responsive in normoxia never engaged in an ER under hypoxia. The statistical sensitivity investigating

locomotive and behavioral components of the ER was too low due to the low sample size and (or) low responsiveness in hypoxic water; thus no significant differences were found (data summarized in Table 3).

Prey capture

All individuals were highly motivated to feed under normoxic conditions, but only three out of five tested individuals engaged in feeding under hypoxia (Fig. 4AB). When feeding, the prey capture success, that is the prey was captured with the first attempt, was >99 % regardless of ambient oxygen levels. Two principal component (PC) axes were identified that explained a total of 63 % of the variance in our data with performances from all tests included, N1, N2, H1, H2. The first axis (PC1) loaded heavy on all timing variables and explained 44.5 % of the total variation; more negative values indicate faster displacement of the neurocranium and more rapid prey capture overall (Fig. 5, Table 5). The second axis was interpreted as an "explosiveness-displacement" axis as indicated by positive loading of maximum kinematic excursion values and explained 18.5 % of the variance (Fig. 5, Table 5). The attack speed had very little influence on prey capture, as inferred from the negligible change in PCA results when attack speed was included or not included (data not shown). No clear indication of separation between prey capture/suction feeding performances in hypoxic and normoxic water was found (Fig. 5). And none of the mixed effect model results indicated significant difference between any performance measures.

Discussion

Escape response

Responsiveness to startle stimuli under hypoxia was significantly reduced in juvenile striped bass. Under aerobically, constrained conditions, engaging in escape response may be energetically very costly (Webb 1978). Furthermore, there is a proposed trade-off between energy spent during escape and the level of perceived risk of getting eaten by a predator, which may determine the response decisions in fish (reviewed by Domenici 2010). Therefore, the reduced responsiveness observed here is likely a result of fish minimizing their energy expenditure and/or the stimulus was not threatening enough. The reduced % responsiveness under hypoxia is reported in two other fish species: an approximately 50% reduction in the co-familiar European sea bass, *Dicentrarchus labrax* (Lefrançois and Domenici 2006) and in golden grey mullet, *Liza aurata* (Lefrançois et al. 2005). In these studies, the responsiveness in normoxic conditions was close to 100% which is higher than we observed (Lefrançois et al. 2005; Lefrançois and Domenici 2006; Marras et al. 2011). This difference may arise from the experimental set up, the personality or motivation of the fish (Webb 1984), or may be a species-specific factor (Webb 1986). Studies examining escape response in fish rarely report % responsiveness, thus more extended comparisons to our reported levels of responsiveness even under normoxia is limited. Other behavioral measures, latency and directionality, were not affected by the 20 % AS hypoxic conditions of this study. Similarly, no change in latency was found in *L. aurata* (tested at 10 and 20 % AS; Lefrançois et al. 2005) and *D. labrax* (tested at 10 and 20 % AS; Lefrançois and Domenici 2006) when exposed to acute hypoxia, but decreased latency (individuals responded sooner) was found in African

cichlid, *Pseudocrenilabrus multicolor victoriae,* that was reared in hypoxic water, 1.1 mg O_2 L^{-1} (approximately 13 % AS) (Gotanda et al. 2012). Despite the lack of more comparable studies in fish, the inconsistency between these few studies may be a result of species-specific physiologies, their ecology and avoidance strategies, or the variation in methods (reviewed by Domenici et al. 2010). Lastly, hypoxia has shown to decrease or alter escape behaviors in a few other systems, including juvenile and adult scallops (Brokordt et al. 2013) and freshwater clam (Saloom and Duncan 2005), suggesting that the hypoxia-effects on short explosive movements like fast-start in fish are not universal and require more research. Which hypothesized intrinsic factors, *i.e.* energetics or neural impairment, may causes any alterations in escape behaviors and decisions made is still unknown and may not be the same across taxa.

No significant difference between any locomotive performances of ER under hypoxia *vs.* normoxia was found. Since only 4 fish responded to stimuli, the small sample size may have limited our evaluation, but our results suggest that, if responsive, juvenile striped bass will be able to evade predation under hypoxia with similar success to that under normoxia. Walker et al. (2005) demonstrated that successful evasion of predation is associated with ability of prey to turn rapidly and at high speeds during the first part of the fast-start. Therefore, the main performance measures critical to successful escape are hypothesized to be velocity, acceleration, turning angle and rate (Webb 1986; Walker et al. 2005). The maximum velocities (1.36 m s^{-1}, mean in normoxia) recorded here are comparable to those attained by similar size *D. labrax* (Lefrançois and Domenici 2005; Marras et al. 2011), and also other fish species (short-horned sculpin: Beddow et al. 1995, golden grey mullet: Lefrançois et al. 2005, bluegill: Gerry et al. 2012, tropical spiny

chromis: Ramasamy et al. 2015, and tropical damselfish: Jornod and Roche 2015). Also, the mean turning rates, 2256 ^{o} s^{-1} (Turesson et al. 2009; Marras et al. 2011; Jornod and Roche 2015), maximum acceleration, 53 m s^{-2} (Beddow et al. 1995; Walker et al. 2005; Lefrançois et al. 2005; Lefrançois and Domenici 2006; Marras et al. 2011, Gerry et al. 2012; Ramasamy et al. 2015), and turning angle, 86.5^{o} (Marras et al. 2011) recorded here under normoxia are consistently within the same ranges as reported in other studies. But noteworthy, the between and within species variance for these performances are very high (Marras et al. 2011; Jornod and Roche 2015).

To our knowledge, this is the first report describing escape response of striped bass of any age at any condition. In natural environments, both juvenile striped bass and approaching predators would be under the influences of hypoxia (Breitburg 1992), therefore the potential for survival of juvenile striped bass will likely depend on hypoxia effects on both themselves and the predator (Robb and Abrahams 2003). Furthermore, in the wild, hypoxia rarely, if ever, will affect physiology and behavior of individual fish in an independent manner. Particularly, in the Chesapeake Bay system where environmental conditions, including dead zones, are changing on a seasonal basis (Najjar et al. 2010), other factors like temperature, turbidity, light conditions, species distribution and their population characteristics will all interact with low oxygen availably to further determine how striped bass may respond to predation threats (for reviews see Domenici 2011; McBryan et al. 2013). Nevertheless, much more research is needed to objectively evaluate the escape ability of juvenile striped bass under any ecologically relevant conditions.

Prey capture

Not all individuals tested were interested in feeding when ambient oxygen levels were dropped to 20 % AS. Decreased food consumption under hypoxia is not an uncommon finding in striped bass and other fish (Breitburg et al. 1994; Pichavant et al. 2001; Brandt et al. 2009; Townhill et al. 2017). Breitburg et al. (1994) reports on decrease in amount of food (fish larvae) consumed by juvenile striped bass with decreasing oxygen availability, and when exposed to the same level of hypoxia (~ 2 mg O_2 L^{-1}) as used here the total food consumption was half of that under normoxia (Breitburg et al. 1994). However, in Breitburg et al. (1994) the food consumption is reported for groups of fish, lacking individual specific evaluation. Despite the small sample size in our study, the inter-individual variance in feeding behavior is obvious (Fig. 4AB). This finding may be associated with their relative hypoxia tolerances (not tested here) or the available scope for aerobic activity, for example digestion (Fry 1971; Claireaux and Chabot 2016). Striped bass are a social species forming schools at this stage (Fay et al. 1983), it is likely that established dominances in the tank played a role in their feeding behavior. In our study, the two individuals who did not capture prey were both from the same housing tank, but in the meantime, feeding also was drastically decreased in one individual from a separate group (Fig. 4AB). The amount of food consumed by each individual in a group is a common measure of dominance in social fish like striped bass (*e.g.* Metcalfe et al. 1995), therefore it would be interesting to explore whether dominance hierarchies in juvenile striped bass or other fish are changed when encountering hypoxia. Especially, because previous studies in our lab reported no

correlation between juvenile HT and individuals social rank in juvenile striped bass (Lipkey 2012).

Neither, kinematics or locomotion of juvenile striped bass differed significantly between prey captures performed in hypoxic and normoxic water. It may be that our sample size was too small to indicate any significant differences, or the kinematic and locomotive components of prey capture are mostly unaffected by low (20% AS, Table. 5) environmental oxygen levels in this species. Performance scores on timing axis (PC1) from all tests (N1, N2, H1, H2) overlap in PCA multidirectional space, but prey captures performed in both hypoxia tests (H1 and H2) tend to be more restricted in the space they occupy along the displacement axis (CV (N) = -506.02 %, CV (H) = 116.10 %; PC2; performance scores under hypoxia all lay on the upper half indicating larger displacement) (Fig. 5). Reduced variability of locomotive performances in fish under challenging conditions *vs.* optimal conditions has been observed before (*e.g.* Killen et al. 2013; Killen et al. 2016; Norin et al. 2016).

With a focus on the performance in normoxia, the prey capture kinematic measures analyzed here are well within the range of those found in literature (Table 4) (Richard and Wainwright 1995; Cook 1996; Ferry-Graham et al. 2001; Gibb and Ferry-Graham 2005; deVries and Wainwright 2006; Higham et al. 2006; Tran et al. 2010; Oufiero et al. 2012; Sloan and Turingan 2012). To our knowledge, this is the first report describing prey capture performance of any fish under hypoxic conditions, which limits any cross-species comparison. Poulin et al. (1987) reported a partly relevant study where an individual cichlid, *Astronotus ocellatus*, was given 12 prey fish, guppies, *P. reticulata*, under hypoxic conditions; they found increased capture latency, decreased probability to

capture prey and loss of interest in prey. The authors hypothesized that changes in feeding behavior were a result of increased aquatic surface respiration (ASR) by a predator and possible task prioritization within their scope of activity (Poulin et al. 1987). However, individuals in our study never engaged in ASR, and for individuals who did feed under hypoxia the motivation and capture latency remained high for all times. Interestingly, modulation and/or negative effects on suction feeding in fish has been shown under different environmental temperature levels (Wintzer and Motta 2004; Allan et al. 2017, but see deVries and Wainwright 2006; Sloan and Turingan 2012), and CO_2 (Allan et al. 2017). What are, if any, the effects of hypoxia on suction feeding in fish may provide a new yet unexplored research avenue. The interest on rapidly changing environment effects on ecologically crucial performances like prey capture is growing, and despite the lack of significant alterations in feeding kinematics and locomotion in juvenile striped bass, the potential for it should not be ignored and more research across different species be performed.

Summary

The kinematics and locomotive ability of either escape response and prey capture of juvenile striped bass were not significantly altered under hypoxic conditions. However, the responsiveness or engagement in escape behavior was significantly lower when exposed to 20 % AS. Similarly, when oxygen level dropped to 20 % AS interest in feeding halted in some individuals, was clearly reduced in others, but was maintained in some tested individuals. Differential behavioral responses were observed between individuals in both tests, suggesting that different individuals may be selected for in 'fitness' advantage under differential oxygen levels, *i.e.* normoxic *vs.* hypoxic. Because

of low sample sizes and low responsiveness in these studies, no further ecologically relevant conclusion may be inferred. Meanwhile, the results of this study also don't exclude the potential of hypoxia (20 % AS) to have an effect on predator-prey relationships of juvenile striped bass in the Chesapeake Bay. Future studies should investigate feeding behavior and escape response of a higher number of individuals across a gradient of hypoxia, and throughout ontogeny.

Table 1. Kinematic and speed variables measured to describe escape response.

Parameter	Definition
% responsiveness	the fraction of the total provided stimulus that induced an ER in fish
latency (s)	the time interval between the stimulus onset (t_0; time when ball hits the water) and the first detectable movement of fish (t_1)
Distance, Dist (m)	the distance between CoM and stimulus at t_0
ER_{type}	the type of response: double bend (DB) or single bend (SB) (Domenici 1997). Associated time points: t_1 – time at the beginning of fast-start, t_2 – time at the end of "C" formation, also end of stage 1 and SB escape response, and t_3 – time at the end of stage 2 and DB response
A_{max}, A_{mean} (m s^{-2})	the maximum and mean acceleration measured during ER
V_{max}, V_{mean} (m s^{-1})	the maximum and mean speed measured during ER
TR_{max}, TR_{mean} (^{o} s^{-1})	the maximum turning rate during formation of "C", and return
TA (^{o})	the turning angle; the angle between CoM and head at t_0 and t_2 if ER_{type} = SB, and between t_0 and t_3 if ER_{type} = DB
S1A, S2A (^{o})	the bending angles of stages 1 and 2; the angle between CoM and head at t_1 and t_2, and t_1 and t_2, respectively
$S1_{time}$, $S2_{time}$ (s)	the duration of stage 1 and 2, the time interval between t_1 and t_2, and t_2 and t_3, respectively
$S1_{dist}$, $S2_{dist}$ (m)	the net distance traveled during stages 1 and 2, respectively
ET (^{o})	the escape trajectory; the angle created between CoM and stimulus at t_0 and CoM and head at the end of ER
ER_{dist} (m)	the net distance traveled during the escape response
ER_{time} (s)	the time interval between t_1 and t_3 if ERtype = DB, and between t_1 and t_2 if ERtype = SB

Table 2. Kinematic and speed variables measured to describe prey capture.

Kinematic variables:	
gape (cm)	the distance between points 1 and 2
jaw protrusion (cm)	the distance between a midpoint between points 1 and 2 (mid-gape) and point 8
lower jaw rotation (^{o})	the angle formed between points 9, 6, and 8
cranial rotation (^{o})	the angular rotation of head, points 7 and 8, in relation to the body, points 3 and 4
strike distance (cm)	the distance between mid-gape and estimated CoM of the prey, point 5, at the time when gape is open at 30% of max
Speed variables:	
attack speed (cm s^{-1})	the speed at which midpoint between points 3 and 4 (mid-body) moved
gape speed (cm s^{-1})	the speed of displacement of mid-gape point
jaw protrusion speed (cm s^{-1})	the speed of displacement of mid-gape in relation to the mid-body
lower jaw rotation speed (^{o} s^{-1})	angular speed of angle formed between points 9,6, and 8
cranial rotation speed (^{o} s^{-1})	the angular speed of cranial rotation

Table 3. Escape response performance under normoxia (> 90 % air saturation) and hypoxia (approximately 20 % air saturation).

	Normoxia (n = 14)	Hypoxia (n = 5)
Dist (m)	0.31 ± 0.024	0.29 ± 0.063
ERtype	SB = 7; DB = 7	DB = 3; SB = 2
Escape Trajectory (º) ‡	130.35 ± 21.56	158.82 ± 39.0
TA (º) ‡	86.15 ± 14.37	69.53 ± 30.12
TRmean ($^{o}\,s^{-1}$)	1654.9 ± 184.6	1210.2 ± 531.7
TRmax ($^{o}\,s^{-1}$)	2256.9 ± 393.8	2958.2 ± 307.4
Vmean ($m\,s^{-1}$)	0.84 ± 0.06	0.82 ± 0.03
Vmax ($m\,s^{-1}$)	1.36 ± 0.12	1.52 ± 0.09
Amean ($m\,s^{-2}$)	26.87 ± 2.77	29.06 ± 4.48
Amax ($m\,s^{-2}$)	53.02 ± 6.3	76.89 ± 18.9
ERdist (m)	0.031 ± 0.004	0.035 ± 0.005
S1A (º) ‡	96.41 ± 13.55	85.67 ± 24.07
S2A (º) ‡	26.12 ± 4.87	31.80 ± 2.86
S1time (s)	0.04 ± 0.005	0.04 ± 0.009
S2time (s)	0.02 ± 0.002	0.03 ± 0.007
S1dist (m)	0.019 ± 0.004	0.016 ± 0.006
S2dist (m)	0.021 ± 0.001	0.023 ± 0.003
Latency (s)	0.06 ± 0.007	0.05 ± 0.01
ERtime (s)	0.05 ± 0.005	0.06 ± 0.008
t1 (s)	0.06 ± 0.007	0.06 ± 0.014
t2 (s)	0.102 ± 0.009	0.095 ± 0.019
t3	0.109 ± 0.007	0.122 ± 0.019

The sample size indicated represents the total escapes analyzed, not individuals. Reported are the mean values and standard error of all analyzed escape response parameters. For the description of variables see Table 1. No significant differences between given performance measures were found.
‡ The angles reported above are adjusted to a 180-degree scale. TA: it eliminated the right left turn; Escape trajectory: this adjustment allows evaluation of whether most escapes were away or towards the stimulus (Domenici and Blake 1997)

Table 4. Mean prey capture performance measures of all individuals (n = 5) under normoxic (> 90 % air saturation) conditions.

	Normoxia (n = 64)
Max gape (cm)	2.14 ± 0.043
Max jaw protrusion (cm)	0.39 ± 0.016
Max cranial rotation (^{o})	13.58 ± 0.93
Max lower jaw rotation (^{o})	20.44 ± 1.38
Max attack speed (cm s^{-1})	62.70 ± 2.22
Max jaw protrusion speed (cm s^{-1})	31.54 ± 1.36
Max gape speed (cm s^{-1})	109.88 ± 4.43
Max cranial rotation speed (^{o} s^{-1})	1220.13 ± 82.25
Strike distance (cm)	3.13 ± 0.11
TTPC (s)	0.043 ± 0.002
TTPG (s)	0.041 ± 0.001
Time to max jaw protrusion (s)	0.040 ± 0.002
Time to max lower jaw rotation (s)	0.039 ± 0.002
Time to max cranial rotation (s)	0.035 ± 0.002

n indicate the total prey capture events analyzed. See table 2 for full description of performance parameters.

Table 5. The comparison between prey capture performances of three individuals that engaged in feeding under both, normoxic and hypoxic conditions.

	Normoxia (n = 21)	Hypoxia (n = 18)	PC1‡	PC2‡
Max gape (cm)	2.45 ± 0.054	2.37 ± 0.065	-	0.51
Max jaw protrusion (cm)	0.39 ± 0.033	0.37 ± 0.035	-	0.23
Max cranial rotation (^{o})	11.99 ± 1.08	11.57 ± 1.75	-	-0.44
Max lower jaw rotation (^{o})	23.45 ± 2.87	25.59 ± 2.50	-	0.57
Max attack speed (cm s^{-1})	76.54 ± 3.94	69.53 ± 3.91	-	0.30
Max jaw protrusion speed (cm s^{-1})	31.83 ± 2.30	29.36 ± 3.00	-	-
Max gape speed (cm s^{-1})	131.96 ± 8.53	107.72 ± 8.48*****	-	-
Max cranial rotation speed (^{o} s^{-1})	1505.81 ± 144.02	1051.9 ± 151.10******	-	-
Strike distance (cm)	3.69 ± 0.14	3.29 ± 0.24	-	-
TTPC (s)	0.043 ± 0.003	0.046 ± 0.004	-0.45	-
TTPG (s)	0.040 ± 0.003	0.044 ± 0.004	-0.45	-
Time to max jaw protrusion (s)	0.038 ± 0.003	0.042 ± 0.005	-0.45	-
Time to max lower jaw rotation (s)	0.037 ± 0.003	0.042 ± 0.004	-0.45	-
Time to max cranial rotation (s)	0.031 ± 0.002	0.035 ± 0.005	-0.39	-

The sample sizes indicated if for total analyzed videos

The air saturation levels were >90 % air saturation, AS, and approximately 20 % AS for normoxia and hypoxia tests, respectively

* t-test results: t = -2.02, df = 36.79, p = 0.05 (Welch approximation for d.f.)

** t-test results: t = -2.17, df = 36.39, p-value = 0.04 (Welch approx. for d.f.)

‡ only PC scores above 0.2 or below -0.2 are reported

Figure Legends

Figure 1. Graphical representation of the escape response experimental set up. The numbers indicate the following: 1) the experimental area with 1 cm grid bottom; fish can freely move around this area, 2) the external area used to create uniform hypoxia with minimal disturbance to fish, 3) air stones (4 total), used to bubble air during a normoxia test or nitrogen gas to create hypoxia, 4) an external hypoxia circuit tank filled with glass beads to optimize gas diffusion in water, 5) a computer interfaced with OXY-REG instrument (Loligo® System) used to control and monitor [O_2], 6) nitrogen gas tank, 7) galvanic oxygen probe (OxyGuard Mini Probe, Loligo® Systems) monitoring oxygen level in the experimental area. The X represents the location where ball was dropped to induce an escape response in fish. The arrows indicate direction of the water flow, and the fine-dashed line represents the air or N_2 gas flow.

Figure 2. An image of a juvenile striped bass, *Morone saxatilis*, capturing prey showing all landmark points used in digital analysis. The 9 landmark points were tracked over the time of the full prey capture event and used to extract the kinematic-speed variables (see Table 1). The points correspond to the following: 1- the upper tip of the jaw, 2- the lower tip of the jaw, 3- fixed point on the upper dorsum, 4- fixed point on the lower dorsum, 5- the estimated CoM of the prey, 6- the posterior spot on mandible, 7- fixed point on the upper cranium, 8- fixed point on the lower cranium, 9- fixed point on the lower jaw, between points 2 and 6.

Figure 3. Responsiveness (%) of startled juvenile striped bass. The responsiveness is expressed as the fraction of individuals that responded to the simulated predator stimulus under normoxic conditions (N; air saturation. AS > 90 %) and hypoxic conditions (H; AS

= 20 %). Responsiveness was dependent on environmental oxygen availability (Chi-Square tests: χ^2 (1) = 12.07, $p < 0.001$). It was less likely that fish responded to stimuli under hypoxia (Chi square test: χ^2 H (1) = 28.17, $p < 0.0001$; χ^2 N (1) = 12.07, $p = 0.86$).

Figure 4. Prey consumption by each individual striped bass exposed to normoxia and hypoxia. Panel A: The percent prey from total introduced consumed by each individual under hypoxic (H, approximately 20 % air saturation, AS) and normoxic (N: > 90 % AS) conditions. Panel B: A Plot representing individual specific variance in engagement or interest in feeding across different environmental [O_2]. Each fish is marked with a different symbol and line; fish marked with squares (Indiv. 3 and 5) were housed/tested together, and all others were kept/tested together in a different tank.

Figure 5. Axes of a principal component analysis on prey capture performance under hypoxic, 20 % air saturation (AS), and normoxic, > 90 % AS, conditions. Two axes, PC1, and PC2, as retained by broken stick criterion are plotted and explained 63% of variance in our data. PC1 was a "timing axis" (higher values indicate longer kinematic displacement time, *e.g.* it takes longer to open the jaw, or longer to catch a prey), and PC2 was identified as a "displacement-explosiveness axis", in which higher scores represent larger maximum displacement of neurocranium parts involved in feeding (*e.g.* maximum gape, jaw rotation, jaw protrusion). In this multidimensional space, no significant separation between in prey capture performance measures under normoxia (N, black circles) and hypoxia (H, grey triangles) was identified.

Figure 1.

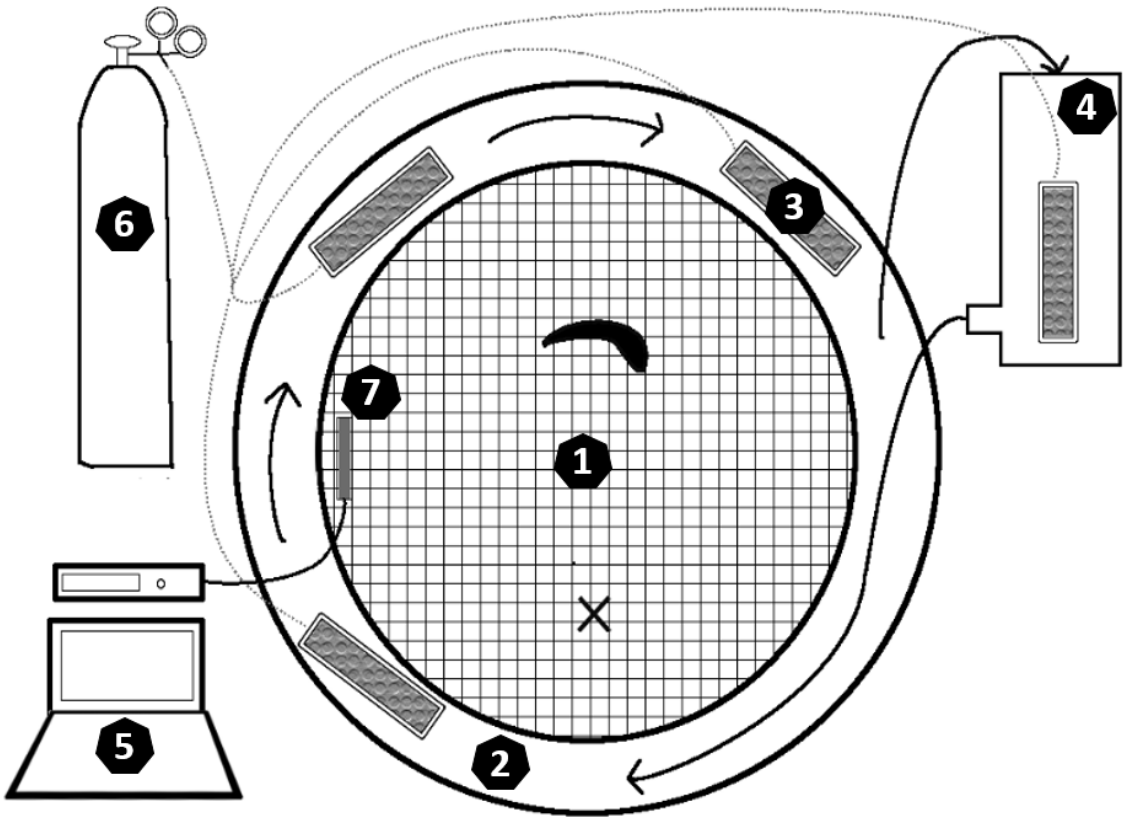


Figure 2.

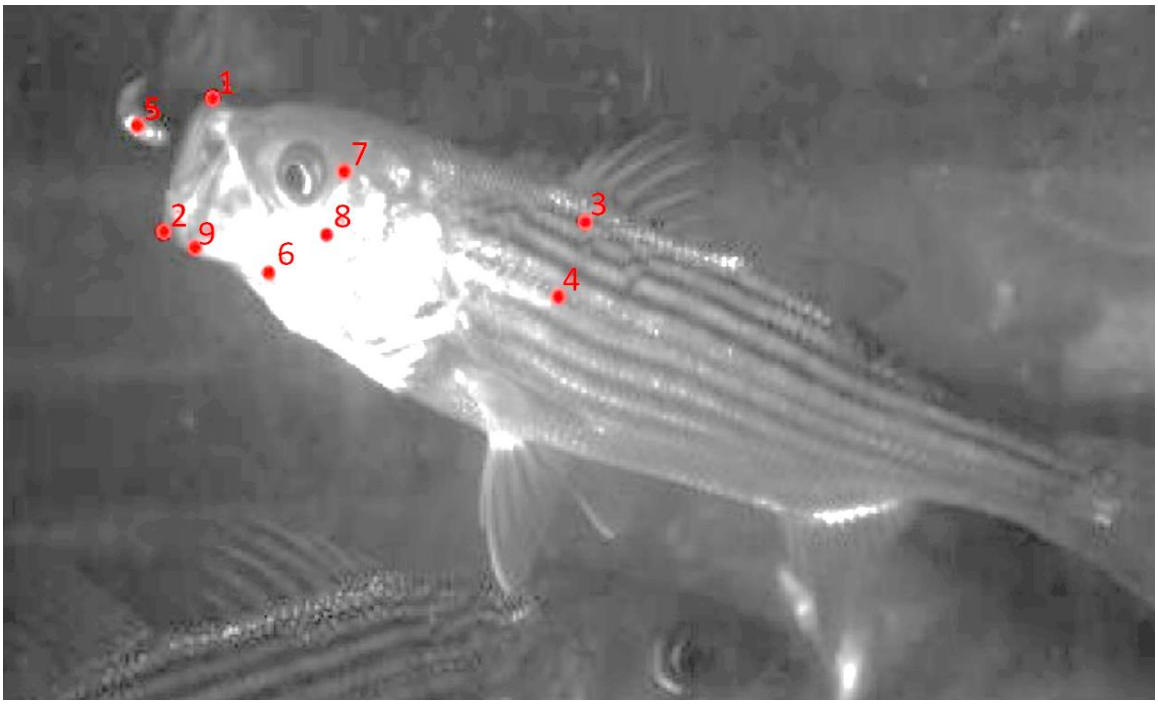


Figure 3.

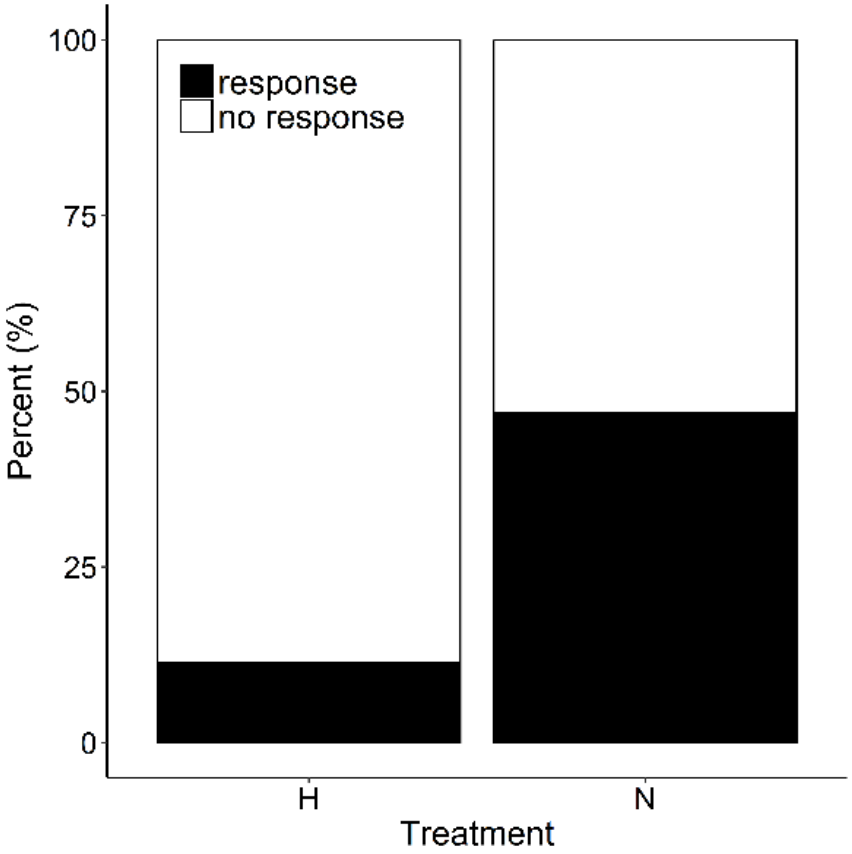


Figure 4.

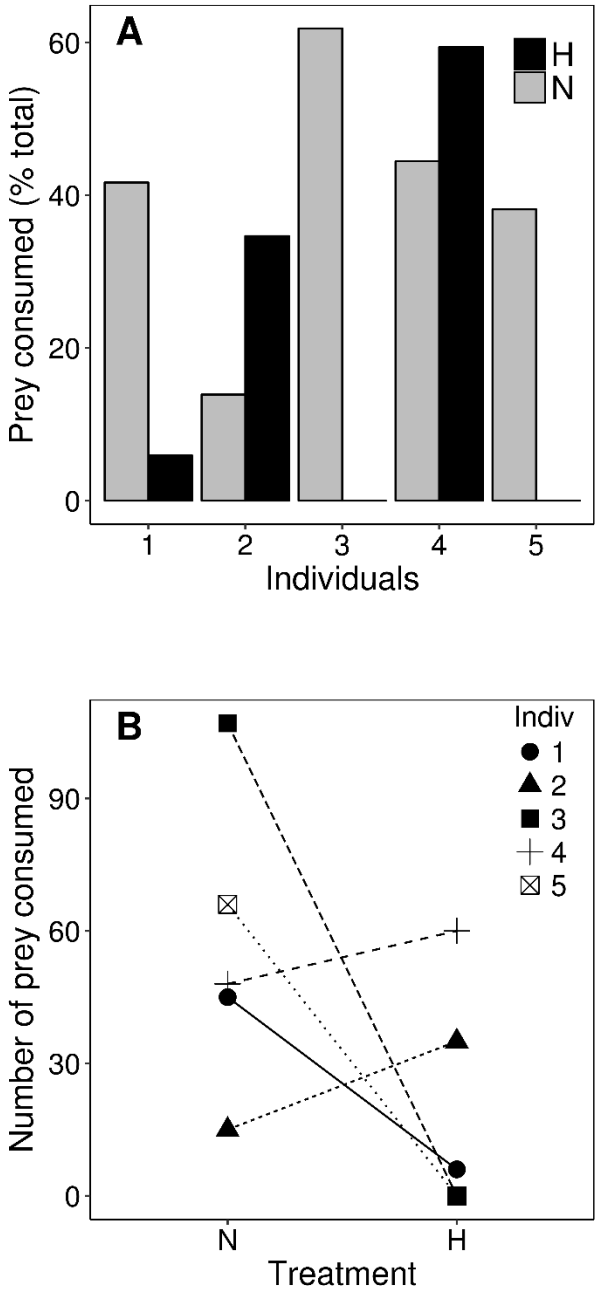
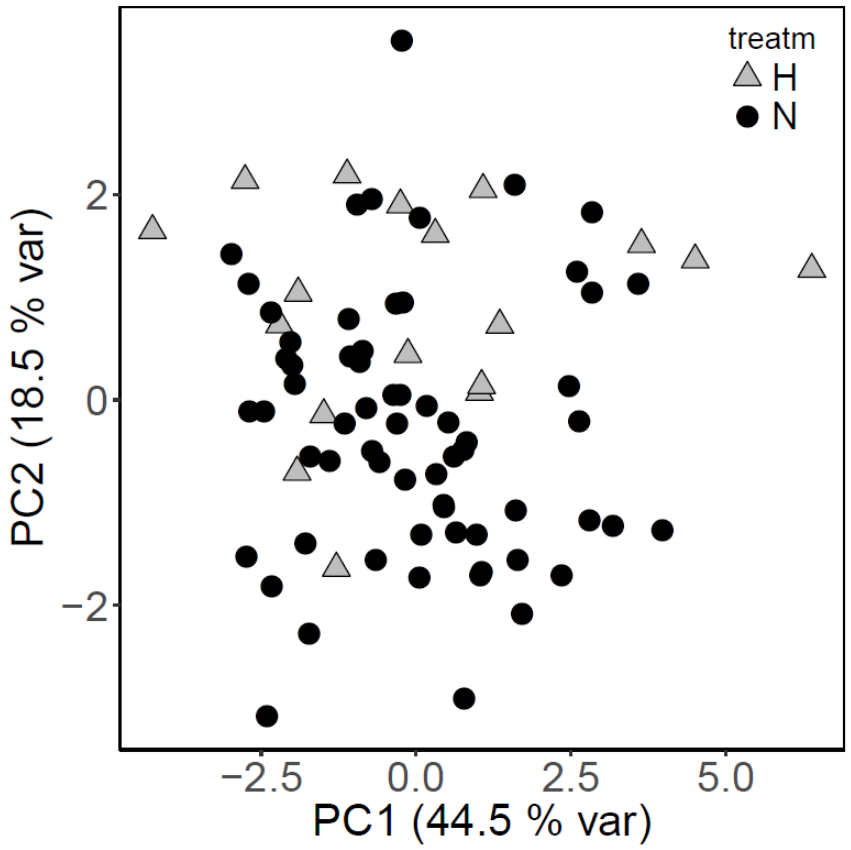

A
H
N
Prey consumed (% total)
0
20
40
60
1
2
3
4
5
Individuals
B
Indiv
1
2
3
4
5
Number of prey consumed
0
30
60
90
N
H
Treatment

Figure 5.



CHAPTER 3

Hypoxia Tolerance, Swimming, and Respiration rate

"Hypoxia tolerance and swimming respiration rate in juvenile striped bass under hypoxic conditions, and the relationships between them."

Krista Kraskura

Abstract

Hypoxia in the Chesapeake Bay has become an emerging concern over the last decades as regions with low oxygen availability are increasing and expanding in volume. For juvenile striped bass that rely on oxygen for survival, this will add an additional metabolic pressure to perform energetically expensive activities, including swimming. When under hypoxia their Darwinian fitness may depend on their hypoxia tolerance (HT) and/or ability to escape the hypoxic region by swimming away from it. In this study we measured i) HT expressed as cumulative oxygen deficit D_{CO} of 18 juveniles striped bass twice, 11 weeks apart, and ii) measured their oxygen consumption rate while swimming in low flow (10.2 cm s^{-1}) and high flow (estimated 67 % U_{max}, maximum swimming speed) under normoxia (> 90 % air saturation) and hypoxia (20 % AS). The rank order of individual HT was significantly repeatable over the 11-week period, and HT increased significantly in all but two individuals. Expectedly i) metabolism was lower while swimming against low flow regardless of available oxygen levels, and ii) oxygen consumption rate was significantly lower in high flow hypoxia tests, than it was under normoxia. There were no significant relationships between individual HT and their metabolism while swimming under any conditions. No trade-offs between ability to increase HT (ΔHT) and metabolic performances were identified, except for a significant negative relationship between ΔHT and respiration rate in low flow. Lastly, both HT and respiration rate varied substantially among individuals suggesting that they may respond to, and be affected by, hypoxia differently. Future research is required to elucidate our findings and make any ecologically relevant conclusions.

Introduction

Metabolism is a series of chemical processes that enables animals to extract energy and essential nutrients from foodstuffs to be used in biological structures and functions. Metabolic rate (MR), typically measured as oxygen consumption (Nelson and Chabot 2011) is one of the most commonly studied physiological parameters that is often directly related to an animal's ecology and its fitness (Fry 1971; Claireaux and Lefrançois 2007; Killen et al. 2010). Metabolic capacity determines an organism's ability to perform and efficiently budget all oxygen-requiring activities critical to fitness, such as food acquisition, digestion and assimilation, growth, reproduction, locomotion, and immunity (Breitburg et al. 2009; Jourdan-Pineau et al. 2010; Domenici et al. 2013; Killen et al. 2013; Lapointe et al. 2014; Claireaux and Chabot 2016). However, these costly activities will have to trade-off within aerobic scope (AS) or the window between an animal's standard metabolic rate (SMR – the basal metabolism required to sustain life) and maximum metabolic rate (MMR – maximum aerobic metabolic activity attainable) (Holt and Jørgensen 2015).

Aquatic hypoxia or low dissolved oxygen availability has become a serious ecological and economical concern in coastal areas (Diaz and Rosenberg 2008; Breitburg et al. 2009; Diaz and Rosenberg 2011). A combination of factors influence the severity, magnitude, and persistence of hypoxic or dead zones (Breitburg 1992; Scully 2016a; Scully 2016b). In most cases, warmth and nutrients, primarily nitrogen from anthropogenic activities, boosts planktonic production in the epilimnion of stratified water bodies; the unconsumed fraction dies, sinks and adds to the organic matter for bacterial decomposition that gradually removes oxygen from the hypolimnion (Diaz and

Rosenberg 2008). Yet, strong winds and tidal currents can acutely drive the severely hypoxic bottom waters to the otherwise normoxic surface layer (Breitburg 1992, Kemp et al. 2005, Scully 2016a, Scully 2016b). Different organisms have varying hypoxia tolerances (HT) (Mandic et al. 2009a; Bickler and Buck 2007), and they may differentially respond to hypoxia. For water breathing fish, the ability to tolerate and escape hypoxia in a manner that is energetically efficient will become crucial if they are to survive (Rice et al. 2013). Therefore, an individual's success under hypoxia depends on its ability to tolerate hypoxia, that is access environmental and internal oxygen stores, and respond behaviorally (*e.g.* become quiescent, engage in aquatic surface respiration, ASR) and/or physiologically (increase ventilation, reprioritize and/or limit metabolic processes or undergo some degree of metabolic arrest) before hypoxia overwhelms their system (Rice et al. 2013). When energetically feasible, fish will likely try to avoid encroaching hypoxia by swimming away towards more oxygenated locations, thus physiological responses to hypoxia while swimming are equally important to those measured in static water and probably more ecologically relevant (McKenzie et al. 2007; Nelson and Lipkey 2015).

Swimming in fish is one of the major determinants of an individual's Darwinian Fitness (Blake 2004; Langerhans and Reznick 2010). Two modes, aerobic and anaerobic swimming, are used by fish to perform their daily tasks. Migration, foraging, and maintenance of position against the current rely primarily on aerobic swimming using slow-oxidative muscles (red) and theoretically can be carried out indefinitely if enough oxygen and fuel substrates (lipids, carbohydrates, and protein) are obtained and distributed throughout the working organism (McKenzie 2011). During anaerobic

swimming, fast-twitch muscles (white) are used to power fast, explosive, burst-type performances after which recovery is required to replenish exhausted endogenous energy stores (McKenzie 2011). The two swimming modes may trade-off (Blake 2004, but see Claireaux et al. 2007; Marras et al. 2013) or be supplementary to each other when conditions require, *e.g.* burst-and-coast-swimming or swimming under hypoxic conditions (Dutil et al. 2007). Both maintenance of swimming ability and the efficiency of swimming are crucial in hypoxic waters when aerobic potential suffers. Metabolic rate measured as oxygen consumption rate ($\dot{M}O_2$) is used to determine energetic costs of physiological and locomotive activities in fish (Nelson 2016). Furthermore, swimming ability is dependent on metabolic power that in turn relies on cardiovascular and respiratory capacity (Randall 1982). Therefore, hypothetically, individuals with low metabolic rates per unit mass performing the same task may be at an advantage when oxygen availability is limited. Metabolic performance and requirements during swimming under hypoxia may be under selective pressures in fish that frequently encounter oxygen deprived environments.

Hypoxia tolerance will play an important role in determining when individuals leave hypoxic conditions (more tolerant individuals are shown to leave hypoxia last), how internal energy stores are partitioned or prioritized (Claireaux and Chabot 2016), and the extent of physiological homeostasis perturbation, thus also recovery or even overall survival. Quantification of loss of equilibrium (LOE) is one of the valuable methods used to measure or express ecologically relevant HT in fish (Crans et al. 2015; Nelson and Lipkey 2015; Borowiec et al. 2016). The baseline levels of HT vary between (Chapman et al. 1995; Nilsson and Östlund-Nilsson 2003; Mandic et al. 2009a) and within fish

species (Pihl et al. 1991; Nelson and Lipkey 2015), and can be limited by O_2 uptake at the gills (Nilsson 2007), tissue O_2 demand (Mandic et al. 2009a), and blood O_2 carrying capacity (Nikinmaa 2001). Hypoxia tolerance can be gained through phenotypic plasticity that will occur over individuals' lifetime or through evolution over generations (Chapman et al. 2000). Phenotypically, HT will be gained through some combination of molecular, morphological, and physiological changes that enhance any of the steps in the cardiovascular and respiratory cascade (Randall 1982; Hochachka et al. 1996).

All physiological performances, swimming ability, HT, and metabolic capacity, are closely linked. While each has been studied individually from an evolutionary and ecological perspective for several decades, the likely existing trade-offs and interdependencies between any of these traits are not commonly addressed and are still unclear (Fu et al. 2011; Crans et al. 2015; Nelson and Lipkey 2015; Pang et al. 2015). Moreover, swimming performance, hypoxia tolerance, and metabolism are plastic traits in most fish and will change to best suit the given environment (Fu et al. 2011; Killen et al. 2013; Fu et al. 2014; Norin et al. 2016; Killen et al. 2016). Yet, it is unlikely that plasticity and selection will work in isolation on one trait. Given how rapidly aquatic environments are changing, it has become especially important to investigate variation, phenotypic plasticity, and their associated underlying mechanisms in fish to better understand and predict an individual's Darwinian fitness.

The aim of this study was to investigate hypoxia tolerance, expressed as time to loss of equilibrium (LOE) when exposed to hypoxia, metabolic rate (oxygen consumption) during low and medium flow conditions under normoxic and hypoxic conditions, and any possible interaction between these physiological measures. In this

study, I used wild juvenile striped bass, *Morone saxatilis* (Walbaum, 1972), native to the Chesapeake Bay where they naturally encounter hypoxia. Previous research has shown that HT of juvenile striped increases when exposed to hypoxia multiple times (Nelson and Lipkey, unpublished data) and also found a lack of relationship between an individual's HT in minimal flow conditions versus that while swimming (Nelson and Lipkey 2015). Similar results were presented by McKenzie et al. (2007) who reported modulated responses to hypoxia of sturgeon while resting *vs*. swimming. It is evident that different physiological mechanism may be responsible for HT under the different physiological states of resting and swimming. This study further investigates the potential relationships between swimming, metabolic activity, and HT. The major questions posed here are: 1) *is metabolic rate while resting or swimming predictive of an individual's hypoxia tolerance?* 2) *how does an individual's metabolic rate differ when swimming under hypoxia or normoxia* 3) *how are individual differences in metabolic rate, if any, correlated to their HT and plasticity of HT? and*, 4) *how variable and repeatable are the physiological traits measured here?* All of these questions are ecologically and evolutionary relevant, and are addressed in the following experiments.

Methods

Fish collection and maintenance

Fish (n = 18) were collected in the summer of 2016 from the Potomac River near its confluence with the Chesapeake Bay and transported in river water (T = 26^{o}C, salinity = 6 ‰) to Towson University. Fish were acclimated to lab conditions by lowering the temperature 2.5 ^{o}C per day, and kept in three 285 L tanks (n = 6 per tank) under optimal conditions (T = 18.7 ± 0.7 ^{o}C (mean ± SD); salinity = 10 - 11 ‰) with a photoperiod

cycle of 12L:12D. Water quality was maintained by weekly water exchanges (30 – 40 % total volume) with resultant [NH_3/NH_4^+] = 0 ppt; [NO_2] = 0.28 ± 1.7 ppt and [NO_3^-] = 62.9 ± 33.62 ppt (mean ± SD). Fish were fed once daily with commercial food (Hikari® tropical food sticks), at least 6 times a week. After > 10 weeks of acclimation all individuals were anesthetized with MS-222 (100 mg L^{-1}, buffered 1:1 with Na^+; HCO_3), weighed (g), measured (total length, TL; fork length, FL) and PIT tagged (PIT-tags; Biomark® Inc.) for identification purposes. Fish were given 2 months of recovery from PIT-tagging before any experimentation. The fish handling protocol was approved by Towson University's Institutional Animal Care and Use Committee (12042013JN-02).

Hypoxia challenge test (HCT)

A HCT was performed twice; HCT1 was performed at the beginning of the experiment, 4 weeks before the first respirometry trial (see next section *Respirometry*), and HCT2 was performed 10 days after the final respirometry trial (HCT1 and HCT2 were 11 weeks apart; Fig. 1). The methods described below were followed in both HCTs. All individuals (n = 18) were transferred to the experimental tank (T_{HCT1} = 18.8 ºC, T_{HCT2} = 18.0 ºC, $salinity_{HCT1,\ HCT2}$ = 10 ‰, $[O_2]_{HCT1,HCT2}$ > 90 % air saturation, AS) without air exposure and were allowed to acclimate for 24 hours. Feeding was discontinued 24 hrs before transfer and while in the experimental tank. Oxygen levels at the start of the HCTs were $[O_2]_{HCT1,\ HCT2}$ = 92 % AS, which was reduced to 10 ± 2% AS over an hour, a rate reflective of the most rapid hypoxia incursions into Chesapeake Bay littoral zones (Breitburg 1992), by bubbling nitrogen gas directly into the tank. Two calibrated oxygen sensing optodes PreSens® were used to monitor oxygen concentration during experimentation; the % AS was recorded using a Powerlab®/4S (ADInstruments©, Inc.)

interfaced to a computer running LabChart7® (ADInstruments©, Inc.) software. Oxygen concentration was maintained at 10.82 ± 0.74 % AS and 10.23 ± 0.30 % AS (means ± SD) for 4 hours during HCT1 and HCT2, respectively. No individuals lost equilibrium up to this point, so oxygen concentration was further gradually lowered at an average rate of 1.03 % AS hr^{-1} during HCT1, and 0.86 % AS hr^{-1} during HCT2 until they did. At the point of loss of equilibrium (LOE; when individual fish could not maintain an upright position for > 10 s) individual fish were immediately removed from the experimental tank, identified, placed in fully oxygenated water, and the time of the LOE was recorded to the closest minute. 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_{OC} is the difference between the area under the hypothetical curve in normoxic water (initial % AS at the beginning of experiment) and an actual % AS until the LOE (Nelson and Lipkey 2015). D_{CO} for each individual was calculated by summing integrated areas between every two time points recorded, every second, and reported in %*h (%*min in Nelson and Lipkey, 2015). The 30th second of the min recorded for LOE was used as the stop point for integration. All individuals fully recovered from both HCTs. Fish were measured and weighed after each HCTs (TL_{HCT1} = 133 ± 9 (119 - 145) mm, $mass_{HCT1}$ = 23.2 ± 5 (15.6 - 31.3) g; TL_{HCT2} = 157 ± 13.5 (134 - 179) mm, $mass_{HCT2}$ = 37.3 ± 10. 8 (21.2 - 57.9) g; mean ± SD (range) for all).

Respirometry

Oxygen consumption was measured in Brett-type swim tunnel-respirometer Loligo® with working area (length = 28 cm, width = 7.8 cm, d = 8.2 cm) and volume

5.25 L. The swim tunnel-respirometer bath was supplied with UV sterilized (UV sterilizer, Vecton ®) and biologically filtered water. Airstones were placed in an external circuit and respirometer bath to control oxygen concentration. Water temperature was maintained at 19.8 ± 1 ^{o}C with a circulating water bath (Polytemp, Polyscience®).

A randomly selected individual was transferred to the swim tunnel-respirometer without air exposure and acclimated for 24 hrs in a 10.2 cm s^{-1} current. Acclimation time was selected based on a preliminary test of juvenile striped bass investigating oxygen consumption rate over a period of 48 hrs; fish calmed and O_2 consumption normalized within 24 hrs after handling. The working area containing the fish was darkened at all times to reduce the stress of the fish. After acclimation, oxygen consumption rate ($\dot{M}O_2$, μmol min^{-1}, see eq. 1) was measured in normoxic water (N) at low flow (LF, 10.2 cm s^{-1}), then the water velocity was increased at the rate of 6 cm s^{-1} min^{-1} to the high flow (HF) regimen 28.9 ± 1.7 cm s^{-1} min^{-1} (velocity equivalent to 67 ± 3.5 % of individuals estimated U_{max} (maximum swimming speed) as determined from a dataset on largemouth bass (*Micropterus salmoides*) of similar size swum at the same temperature; Beamish 1970). The fish were allowed to adjust to the HF for 10 min before measuring oxygen consumption (N-HF test). Next, the current was decreased to LF at the same rate (6 cm s^{-1} min^{-1}), and $\dot{M}O_2$ was measured immediately to monitor recovery from the HF exercise (N-LF2 test). Following this test, the oxygen concentration in the water was lowered to approximately 20 % AS over a period of 1 hour, and the same experimental procedure described above was repeated under hypoxic conditions (H-LF and H-HF tests; recovery was not measured). Figure 1 graphically represents the experimental progression. The fish were immediately removed from the working area after completion of last test under

hypoxia, or if fish was unable to maintain its position in the respirometer and was resting against the back grid (only one individual). Oxygen levels were maintained between 93.5 - 100 % AS in normoxia tests (N-LF, N-HF, N-LF2; Fig. 1), and 17 - 26 % AS in hypoxia tests (H-LF, H-HF; Fig. 1). All closed respirometry tests consisted of three cycles of 10 min of measuring and a 5 min flush. Before being returned to their housing tank, each individual was anesthetized in MS-222, measured and weighed. Background or bacterial oxygen consumption was measured after every experiment and each individual's $\dot{M}O_2$ was corrected to account for it (see eq. 1). The oxygen consumption rate was calculated using the following equation:

$$\dot{M}O_2 = \left(slope_{fish} - slope_{bacteria}\right) * \left(V_{resp} - m_{fish}\right) \qquad \text{eq. (1)}$$

where $\dot{M}O_2$ is oxygen consumption rate (μmol min^{-1}); slopes were obtained by fitting linear regression on [oxygen] decrease in water over time (μmol L^{-1} s^{-1}); V_{resp} = volume of respirometer (measured manually, 5.25 L); m = mass of fish measured in kg. All methods complied with the protocols approved by the Towson University's Institutional Animal Care and Use Committee (New IACUC approved protocol # 1611000152).

Statistical analysis

All data were analyzed in R v3.3.1 software (2016). The rank order repeatability of hypoxia tolerance (D_{CO}) across 11 weeks and 4 days was statistically determined using Spearman's rank order and Kendall rank correlation tests. The significance between sample mean hypoxia tolerance between two HCTs was tested with a paired t-test. Coefficient of variation (CV % = (SD/mean)*100 %) was calculated to determine variation in the HT for each test.

The significance level of the correlations between oxygen consumption rate and individual size (g) and growth rate (GR, g day^{-1}) was determined using Pearson's correlation. Because the metabolic scaling coefficient may change depending on the swimming activity and oxygen availability (Everett and Crawford 2010; Urbina and Glover 2013), only the data from the normoxic low flow tests were plotted and used to analyze size and growth effects. To correct for this size effect, the oxygen consumption rate for all individuals in all tests was size-adjusted to mean sized fish in our sample (29.3 g; 18.2 - 46.6 g range) using allometric relationship $\dot{M}O_2$ = 3.39*mass(g) + 27.5 (Fig. 2). Whether the mean oxygen consumption rate between tests was repeatable was tested using Spearman's rank order correlation. The non-parametric test was chosen because it allows to test for repeatability or stability in each individual's relative rank position in each test while avoiding artificially inflating variance associated with different flow and oxygen regimens. Coefficient of variation, CV %, was also calculated for all respirometry tests.

Linear mixed effect model, LMM, (R package lme4::lmer) analysis was used to examine differences between respiration rate in different respirometry tests: N-LF, N-HF, N-LF2, H-LF, H-HF (Fig. 1). First models were designed to test for possible interacting factors (temperature and water velocity, estimated 67 % U_{max}) with the fixed effect (respirometry test), but none were significant and excluded from further analyses. Linear mixed effect models were further used to test for the main effect of respirometry test on oxygen consumption rate. Individual was included as a random effect in all mixed effect models to account for the repeated measures design. ANOVA with Satterthwaite approximation for degrees of freedom and Post hoc Tukey's tests were used to determine

significance levels in any differences between $\dot{M}O_2$ under different oxygen and flow conditions. For LMM statistical analyses we used a dataset containing each individual's mean respiration rate (n = 3) for each treatment.

Results

Hypoxia tolerance (HT)

HT expressed as cumulative oxygen deficit (D_{CO}) was significantly repeatable over 11 weeks (Spearman's $\rho = 0.59$, $p = 0.012$; Kendall's $\tau = 0.45$; $p = 0.009$; Fig. 3A). All except two individuals were more hypoxia tolerant in the second HCT; the average increase of HT (D_{CO}) of each individual was 127.44 ± 24.58 (%h, mean ± s.e.m.) (Fig. 3AB). This increase was statistically significant in our sample (paired t-test: $t(17) = -5.18$, $p < 0.001$). HT did not correlate with an individual's body size in any of the tests (HCT1: Pearson's $r = 0.14$, $p = 0.6$; HCT2: Pearson's $r = 0.21$, $p = 0.4$; data not shown), but the increase in hypoxia tolerance (ΔHT) was significantly positively correlated with growth rate, in other words individuals with higher growth rates had a greater increase in HT (ΔHT) (Fig. 4A). The level of hypoxia tolerance varied substantially between individuals (CV (HCT1) = 14.14 %; CV (HCT2) = 18.98 %).

Respiration while swimming

The individual mean oxygen consumption rate was significantly different under different flow and oxygen level conditions (ANOVA: $F(4, 71.99) = 46.98$, $p < 0.001$; Fig. 5, Table 1). Tukey's Post hoc analysis results are summarized in Table 2. As expected, $\dot{M}O_2$ was significantly higher in high flow than in low flow conditions under both normoxia and hypoxia (N-LF*vs*N-HF: $p < 0.001$; H-LF*vs*H-HF: $p < 0.001$; Fig. 5,

Table 2), and there was no difference between metabolic rate in two low flow normoxia tests (N-LF*vs*N-LF2; $p = 0.1$; Table 2). Oxygen consumption rate was significantly lower under hypoxia than normoxia in high flow conditions (N-HF*vs*H-HF: $p < 0.001$; Table 2). And considerable inter-individual variance was found in all respirometry tests as indicated by CV % that ranged between 12.4 % - 24.8 % (Table 1). The mean oxygen consumption was significantly correlated with size (mass, g; Pearson's $r = 0.83$, $p < 0.001$; Fig. 2) and slightly, yet significantly-negatively correlated with growth rate (Pearson's $r = -0.24$, $p = 0.01$; Fig. 4B). Size also significantly positively correlated with $\Delta\dot{M}O_2$ (difference between respiration rate in LF and HF; Pearson's $r = 0.39$, $p = 0.017$, data not shown). An individual's relative rank position of respiration rate was significantly correlated between the low and high flow tests under the same oxygen levels (LF*vs*HF: $p < 0.05$ for both N and H, Table 2), in other words, the individuals with higher $\dot{M}O_2$ in low flow also had higher $\dot{M}O_2$ in high flow in hypoxic and normoxic conditions. But when comparing $\dot{M}O_2$ across the same flow conditions but at different oxygen levels, no significant relationship was identified (N-LF*vs*H-LF Spearman's $\rho = 0.33$, $p = 0.19$, Pearson's $r = 0.44$, $p = 0.07$; N-HF*vs*H-HF Spearman's $\rho = 0.42$, $p = 0.08$, Pearson's $r = 0.22$, $p = 0.38$; Table 2). Lastly, the relative magnitude that individuals increased their oxygen uptake rate while swimming in normoxic water ($\Delta\ \dot{M}O_2 = 85.79 \pm 12.17$, mean ± s.e.m.) was greater compared to that while swimming in hypoxia ($\Delta\ \dot{M}O_2 = 47.33 \pm 5.17$, mean ± s.e.m.) and the correlation between the two was not significant and individual rank order was not maintained (Spearman's $\rho = 0.33$, $p = 0.18$; Pearson's $r = 0.33$, $p = 0.19$; Fig. 6).

HT, swimming and respiration

There was no significant correlation between HT measured either before or after the respirometry tests and $\dot{M}O_2$ under any test conditions (H, N, high and low flow). There was also no relationship between ability to increase oxygen consumption while swimming (both H and N, $\Delta\dot{M}O_2$) and ability to increase HT over time (ΔHT) (Pearson's $r = 0.09$, $p = 0.6$; Fig. 7). However, there was a significant negative relationship between ΔHT and $\dot{M}O_2$ in low flow conditions under normoxia (Pearson's $r = -0.51$, $p < 0.001$; Fig. 4C), hypoxia (Pearson's $r = -0.29$, $p = 0.04$; Fig. 4C) and when combining normoxia and hypoxia tests (Pearson's $r = -0.32$, $p < 0.001$; Fig. 4C); this relationship was not significant at high flow conditions.

Discussion

Hypoxia tolerance (HT)

Juvenile striped bass are relatively hypoxia tolerant, yet their baseline HT levels vary substantially between individuals. Likewise, HT expressed as D_{CO} ranged between 10-40 % h (methods slightly different from those described here, see Claireaux and Chabot 2016) in co-familiar European sea bass, *Dicentrarchus labrax* (Claireaux and Chabot 2016, see also Claireaux et al. 2013), and Nelson and Lipkey (2015) reported CV of 33.3 % in HT (D_{CO}) of juvenile striped bass while swimming in minimal flow (for variation in other species see Mandic et al. 2009a; Mandic et al. 2012; Crans et al. 2015). Fish from this cohort were able to tolerate ≤ 10 % AS in 20ºC (equivalent to ~ 0.9 mg O_2 L^{-1}) between 3 to 7 hours before they lost equilibrium, which increased to 5 to 10 hours when tested again in approximately 11 weeks. This level of increase in HT is not

necessarily expected, because these fish were not hypoxia-acclimated and experienced only two acute hypoxia exposures while kept in the laboratory (including the first HCT) before they were re-tested for their HT. Furthermore, striped bass have not been recognized by investigators to be a particularly hypoxia tolerant species (*e.g.* Dixon et al. 2017). However, similar levels of HT and potential for plasticity were observed in juvenile striped bass before (Nelson and Lipkey, unpublished data). The underlying mechanisms of this plasticity are still unknown.

Generally, more HT fish are physiologically acclimated and/or adapted by having higher blood hemoglobin concentrations (Fu et al. 2011), higher hemoglobin-oxygen ($Hb\text{-}O_2$) affinity (low P_{50}, Richards 2011; Mandic et al. 2009a; but see Crans et al. 2015), increased capacity for anaerobic and decrease in aerobic metabolism while the overall whole-organism metabolism decreases (*i.e.* change in key enzyme activity; ATP turnover: Hochachka et al. 1996; Martínez et al. 2006; Richards 2011; Zhu et al. 2013; Crans et al. 2015). Morphologically hypoxia tolerant counterparts often have increased total gill surface area, TGSA (Nilsson 2007; Chapman et al. 2008; Mandic et al. 2009a; Richards 2011; Fu et al. 2011; Crans et al. 2015). Hypoxia acclimation can enhance one or more of these physiological and morphological parameters as it has been observed in several species of African cichlids (Chapman et al. 2008) in killifish, *Fundulus grandis* (Martínez et al. 2006; Borowiec et al. 2015), goldfish, *Carassius auratus* (Fu et al. 2011), Atlantic cod, *Gadus morhua* (Claireaux and Dutil 1992; Petersen and Gamperl 2010), and largemouth bass, *M. salmoides* (Gaulke et al. 2014). While these findings suggest that their HT also increases the actual increase in HT measures (P_{crit}, PO_2 at LOE, or time to LOE) are rarely re-tested (but see Fu et al. 2011; Borowiec et al. 2015).

On the other hand, during acute hypoxia exposure, fish exhibit a variety of defensive and protective responses, these include i) behavioral responses: ASR, observed personally but not well documented in striped bass (Dixon et al. 2017; Mandic et al. 2009b, but see Nelson and Lipkey 2015), boldness and avoidance (Domenici et al. 2013; Killen et al. 2013), ii) physiological responses: increased ventilation rate (Randall 1982), bradycardia (Petersen and Gamperl 2010), decreased cardiac volume (Petersen and Gamperl 2010), iii) biochemical responses: increased [Hb] (Wang et al. 2017), increased red blood cell count (Wang et al. 2017), increase in activity of anaerobic metabolic enzymes (Omlin and Weber 2010; Borowiec et al. 2015; Crans et al. 2015) and activity of aerobic metabolic enzymes (Crans et al. 2015), iv) and molecular responses: upregulation of major molecular gene expression regulator HIF-1 that regulates expression of many genes downstream (reviewed in Wu 2002), and differential protein expression profiles in muscle (Wulff et al. 2012). Note, that these responses that include all levels of biological organization, molecular, biochemical, physiological, morphological, and even organismal, are very diverse and not always parallel between studies involving different methodologies and species. Overall, the potential for phenotypic plasticity, underlying mechanisms, and the timescale at which changes will occur appear to be species and individual specific (Chapman et al. 2008; Borowiec et al. 2015; Joyce et al. 2016). Nevertheless, more research is necessary to explain the gain in hypoxia tolerance in fish and mechanisms in place to withstand hypoxia.

The hypoxia tolerance we report is comparable to that observed in juvenile striped bass previously (Lipkey 2012). Similarly, the co-familiar, European sea bass, *D. labrax*, are able to withstand < 20 % for up to ~ 9 h before LOE (Joyce et al. 2016), while

rainbow trout, *Oncorhynchus mykiss*, tolerated < 10 % AS for 5h (Roze et al. 2013). We also demonstrate a highly repeatable inter-individual variance of HT in juvenile striped bass. High between individual variation in numerous performance traits are often observed in fish (*e.g.* HT: Nelson and Lipkey, unpublished data; Roze et al. 2013; Borowiec et al. 2015; Joyce et al. 2016, metabolism: Metcalfe et al. 2016, locomotion: Nelson and Claireaux 2005; Marras et al. 2013), and is important and necessary if the trait is to be targeted by natural selection (Dohm 2002). Joyce at al. (2016) and Faust et al. (2004) suggest that the variance in whole-animal HT is directly associated with individual cardiac tolerance to hypoxia. Hearts from hypoxia-tolerant individuals of the same species produced significantly more force than hypoxia-sensitive counterparts (Joyce et al. 2016), and HT fish experienced lower reduction in cardiac function, *i.e.* lower decrease in cardiac output and stroke volume (note that authors in this study did not directly test individuals with the variant HT, but examined a suggested hypoxia tolerant subpopulation of the same species; Faust et al. 2004). Besides cardiac function, other physiological and morphological functions as discussed above can likely explain the existing variance in HT, but research addressing direct correlations is still underway (Davies et al. 2011; Borowiec et al. 2015). It also cannot be excluded that the method used to determine HT may affect the variation observed (*e.g.* Mandic et al. 2012). The responses to hypoxia and HT may differ depending on the nature of hypoxia encountered, *i.e.* the rate of oxygen depletion (Snyder et al. 2016), duration and severity of hypoxia (Plante et al. 1998), and even the time of the day (effect of circadian rhythm of fish) (Snyder et al. 2016).

Striped bass have been shown to actively avoid hypoxia (< 20 % AS; < 2 mg O_2 L^{-1}) in the Chesapeake Bay (Kraus et al. 2015). But we demonstrate here and in the previous studies in our lab that juvenile striped bass can tolerate < 20 % AS for a substantial period of time not only when minimally active, but also while swimming (Nelson and Lipkey, unpublished data; Nelson and Lipkey 2015). But the method used to quantify HT may create some uncertainty. Tolerance to hypoxia is often quantified as critical oxygen tension or concentration (P_{crit}; $[O_2]_{crit}$), an environmental PO_2 or $[O_2]$ at which animals begins oxyconforming (when resting oxygen consumption becomes dependent on environmental O_2, but this method excludes an individual's potential for anaerobic metabolism; Mandic et al. 2009a; Nilsson and Östlund-Nilsson 2003; Crans et al. 2015; Borowiec et al. 2016), or as the PO_2 and $[O_2]$ at which loss of equilibrium (LOE) occurs (Crans et al. 2015; Borowiec et al. 2016). Although, environmental PO_2 at P_{crit} and LOE are not always the same (Crans et al. 2015; Borowiec et al. 2016; Claireaux and Chabot 2016; but see Snyder et al. 2016), both methods are commonly used and proven to be valuable measures of HT.

More hypoxia tolerant individuals are likely to be more successful in environments like the Chesapeake Bay where hypoxia severity and persistence is predicted to keep increasing (Keeling et al. 2010). Generally, hypoxia tolerance can be acquired via two routes, evolution via natural selection and phenotypic plasticity, or a combination of both. As we demonstrated, the relative HT is a stable trait (repeatable rank order of HT) that varies between individuals, and fish from this cohort expressed a relatively uniform increase in HT with acute hypoxia re-exposure. Altogether, these findings suggest that both routes to increased HT are likely to occur in wild striped bass.

Respiration while swimming

As expected, oxygen consumption rate increased when juvenile striped bass swam at higher flow regardless of ambient oxygen levels (Petersen and Gamperl 2010; Pang et al. 2015). The $\dot{M}O_2$ was significantly lower at high flow when individual fish swum under hypoxic *vs.* normoxic conditions. The inability to increase oxygen consumption at high flow under hypoxia is likely due to limited oxygen uptake capacity at the gill and/or their limited ability to transport or use that oxygen resulting in a reduced scope for aerobic activity under hypoxia (Fry 1971; Petersen and Gamperl 2010; Claireaux and Chabot 2016). Aerobic scope sets the limit for the ability of the animal to increase aerobic metabolism (Norin and Clark 2016). Despite the decreased amount of oxygen consumed, all except one juvenile striped bass maintained their position in the swim tunnel-respirometer, so they were generally able to meet their energy demands over approximately 50 min to support swimming at selected speeds.

Metabolism is influenced by a combination of biotic and abiotic environmental factors (Fry 1971; Bushnell et al. 1984; Claireaux and Legardère 1999), physical and physiological activity (Webb 1971; Fu et al. 2011), lifestyle (Eliason and Farrell 2016), and is species and individual specific (Norin and Malte 2011; Norin and Malte 2012; Pang et al. 2015). We identified substantial levels of inter-individual variance in $\dot{M}O_2$ (CV 12.5-24.9 %) in juvenile striped bass, which is a common finding in metabolic parameters across many fish species, CV (AMR) of 15-20 % reported in young brown trout, *Salmo trutta* (Norin and Malte 2011), CV (AMR) 17.5 % in European sea bass, *D. labrax* (Marras et al. 2013), CV (AS) 32.5 % in Atlantic cod, *G. morhua* (Reidy et al. 2000) (for review see Metcalfe et al. 2016). Further, supporting our results Nelson and

Claireaux (2005) reported on increased $\dot{M}O_2$ CV % with increasing swimming activity on similar-size *D. labrax* tested at swim speeds of 0.45, 0.65, and 0.85 m s^{-1}. The likely contributors to the observed variation may be individual specific SMR (Norin and Malte 2011; Norin and Malte 2012; reviewed in Claireaux and Chabot 2016), metabolic enzyme activity (*e.g.* liver cytochrome c oxidase: Norin and Malte 2011), cardiac capacity (Farrell et al. 2009) or context-dependent individual life histories (developmental plasticity: Chapman et al. 2008; life history: Killen et al. 2016). Furthermore, hypoxia may reduce this variability due to metabolic constraints (Norin et al. 2016). It is suggested that heritable, genetic variation provides raw material for natural selection to act upon (Dohm 2002), thus these performances are currently a potential object for selection. But whether the source of this variation is environmental or genetic, or both is unknown.

The swim speeds selected here were not exhaustive to these fish as indicated by immediate recovery (the oxygen consumption returned to that during acclimation or the first respirometry test, Fig. 5; or no visible sign of exhaustion, *e.g.* resting against the back grid, were observed) from the HF test under normoxia. It further suggests that under optimal conditions these fish relied mostly, if not only, on aerobic respiration at the selected speeds (Domenici et al. 2013). On the other hand, under hypoxia, the high flow speed may be nearing their U_{crit} values. Previously reported results show substantial reduction in U_{crit} when fish were swum under similar or even milder levels of hypoxia as used here; U_{crit} reduced by approx. 30% in Altantic cod, *G. morhua* (Dutil et al. 2007; Petersen and Gamperl, 2010); by approx. 20 % in rainbow trout, *O. mykiss* (Bushnell et al. 1984), black carp, *Mylopharyngodon piceus* (Pang et al. 2015), coho salmon,

Oncorhynchus kisutch, and largemouth bass, *M. salmoides* (Dahlberg et al. 1968). Whether juvenile striped bass were approaching exhaustion and/or used anaerobic respiration during at H-HF swim is unclear but not unlikely (Domenici et al. 2013). Although, burst-and-coast swimming, indicative of anaerobic swimming (Dutil et al. 2007), was not observed at any of the trials. Future studies may incorporate the measurement of excess post exercise oxygen consumption (EPOC) or muscle and/or blood lactate level to investigate this further (Virani and Rees 2000, Omlin and Weber 2010; Weber et al. 2016, but the lactate levels may not be indicative of individual's HT in juvenile striped bass, J.A. Nelson and G.K. Lipkey, unpublished observations).

HT, swimming and respiration

We did not find any direct relationships or trade-offs between an individual's hypoxia tolerance measured in either of the HCTs and metabolic rate in any of the respirometry tests (N and H in high and low flow). Recently, several studies have addressed potentially existing trade-offs or complementarity between hypoxia tolerance and aerobic swimming ability in fish (both swimming speed and efficiency) (Fu et al. 2011; Fu et al. 2014; Crans et. al. 2015). However, there is no consensus how these traits trade-off in different species and individuals (Nilsson et al. 2007; Fu et al. 2011; Fu et al. 2014; Crans et al. 2015). Traits underlying both HT and swimming ability include increased ability for gas (O_2, CO_2) exchange at the gill (higher TGSA: Fu et al. 2011; Crans et al. 2015), more efficient O_2 transport to the tissue (*i.e.* increased oxygen carrying capacity: Fu et al. 2011; heightened pH sensitivity of Hb-O_2 binding affinity: Crans et al. 2015), and more efficient ATP use to complete a given task (*e.g.* basic metabolic needs, or active swimming; see Randall 1982; Hochachka et al. 1996; Mandic et al. 2009a). On

the other hand, several proposed trade-offs may exist. For example, HT is often associated with high Hb-O_2 affinity (Mandic et al. 2009a) but opposed to that, lower Hb-O_2 affinity facilitating O_2 unloading at the working tissue may be better in supporting high intensity swimming. Also, heavily sought after, is a trade-off between aerobic and anaerobic-glycolytic capacity (*e.g.* muscle types, muscle capillarity, metabolic enzyme activity, aerobic and anaerobic capacity of heart), which also may emerge as a trade-off between aerobic swimming ability and HT (Crans et al. 2015). For example, individuals with higher anaerobic capacity may survive severe hypoxia longer (reliance of glycolysis when ambient [O_2] drops below P_{crit} and/or anaerobic capacity of heart: Petersen and Gamperl 2010; Joyce et al. 2016), whereas those of higher aerobic capacity may be 'better swimmers' (Crans et al. 2015). Importantly, not all traits associated with high HT or aerobic swimming capacity can be gained via plasticity or acclimatization, some are genetically acquired through evolution (Kaufman et al. 1997; Mandic et al. 2009a). Based on the available literature, it seems probable that phenotypically gained traits (TGSA, [Hb], lower P_{crit}) are more likely to work in the same direction facilitating both HT and high intensity swimming capacity (Fu et al. 2011), whereas traits that have a genetic basis may trade-off to maximize or even specialize in one trait at the expense of reduction in the other trait (Fu et al. 2014, Crans et al. 2015; see also developmental plasticity, Nilsson et al. 2007). The cost of the plasticity *vs.* the potential gain also must be considered, for example, increased TGSA will likely increase HT, but it comes with its associated maintenance costs. Our results do not differentiate between the trade-off or complementation hypotheses. The relationship between HT and swimming efficiency in juvenile striped bass is still to be explored. There could be several reasons as to why we

did not identify any trends, for example, the flow levels selected did not test for individual maximum performance abilities (*e.g.* 'better swimmers' with higher U_{crit} shown to have lower HT; Crans et al. 2015) nor was it to an individual specific % of U_{max}. We also do not have data on individual's SMR and other metabolic parameters, thus physiologically relevant hypoxia tolerance measurements (*i.e.* P_{crit}). In the meantime, the lack of any signs of trade-offs indicate that more hypoxia tolerant individuals can be as good and possibly even 'better' swimmers than less hypoxia tolerant counterparts. Hypothetically, individuals that can gain hypoxia tolerance at minimal expense, and are simultaneously tolerant to hypoxia and efficient swimmers may be selected in hypoxic environments. In addition, the high variance and natural maintenance of this variation in all traits indicate that that different individuals may be better suited or at an advantage under differential environmental conditions (Killen et al. 2016).

Unlike HT as an independent measurement, we found an unexpected significant negative relationship between the increase in individual's hypoxia tolerance (ΔHT) and respiration rate in all low flow swim tests. This relationship was especially pronounced for normoxic swim tests, but still highly significant in hypoxic swim test and when all were combined. Not tested here, but if this relationship is related to individual SMR, our negative relationship may underlie that having lower SMR may enhance HT as found in other species (Mandic et al. 2009a). Also interesting, was a finding that growth rate positively significantly correlated with ΔHT, and negatively correlated with oxygen consumption rate under normoxia while swimming at low flow. This is unusual, because fish with higher metabolic demand typically eat and metabolize more food, thus have higher growth rates than their low-MR counterparts (Norin et al. 2016). Meanwhile,

energy from foodstuffs has to be budgeted or traded between growth, reproductive and physical activity and other physiological processes (Claireaux and Chabot 2016), likely including acclimatization-plasticity to hypoxia or potentially other conditions (*e.g.* McBryan et al. 2013). While the relationship between growth rate and swimming $\dot{M}O_2$ was significantly negatively only in low flow tests (significance was lost in high flow tests), it is not evident what trade-off may operate between swimming performance, respiration rate, and GR in this group of juvenile striped bass. Generally swimming capacity (burst type: Billerbeck et al. 2001; Killen et al. 2014, and sustained swimming: Billerbeck et al. 2001) is reduced in 'fast growers' (Handelsman et al. 2010). Furthermore, it is unexpected that individuals highly investing in growth, also have the highest increase in hypoxia tolerance. The reports on GR and body size on ability to environmentally acclimate and adapt are very scarce. In agreement with our findings, a fast-growing strain of rainbow trout was more hypoxia tolerant than was a slow growing strain (Roze et al. 2013). In reef fish, during larval to juvenile development when rapid growth and metabolism reorganization occur, metabolic demand (SMR) and their superior aerobic swimming performance dropped but simultaneously juveniles gained hypoxia tolerance (lower P_{crit}) (Nilsson et al. 2007). Although, only size, not growth rate was used to draw size-HT relationships, it demonstrated that higher GR can potentially lead to a higher increase in HT (Nilsson et al. 2007). In contrast, Sundt-Hansen et al. (2007) reports on transgenic enhanced-growth coho salmon larvae being less tolerant to hypoxia than slow growing wild type fish. Rapid growth, thus higher body size and possibly reproductive output, and high aerobic metabolic capacity are likely, but not necessarily, evolutionary favored traits in some fish species (Burton et al. 2011), but their

consequences or ability to acclimate to hypoxia are poorly explored. Also, because we don't know the underlying mechanism(s) of increase in HT, the energy costs of it are also unknown.

Other important factors to consider from our study are variation in performances within and across performances. As discussed above, inter-individual variation likely arises from varying SMR, MMR, AS, basline HT, other intrinsic and extrinsic life history related factors. But there is also a significant level of intra-individual variation, for example under the same flow conditions (LF and HF) the relative rank of the magnitude by how much individual increases its oxygen consumption under normoxic conditions ($\Delta \dot{M}O_2$ - N) is not the same as that under hypoxia ($\Delta \dot{M}O_2$ - H). This may be related to their AS, however, we don't know what fraction of their available AS each individual is using while swimming under hypoxic conditions, and whether this fraction remains the same across different oxygen conditions. Studying a different group of juvenile striped bass, Nelson and Lipkey (2015) demonstrated lower HT for swimming than resting fish, but they also reported the lack of relationship between an individual's rank order of HT between the swimming and resting states, which lines up with our results presenting the reordering of $\dot{M}O_2$ under two flow tests, and intra-individual variance. Everett and Crawford (2010) studied divergent populations of gulf killifish, *F. grandis*, occupying environments with different environmental oxygen patterns and found significant differences in routine oxygen consumption rates at low environmental [O_2] in individuals from different populations, while their HT measured as P_{crit} was uniform across populations. Also, $\dot{M}O_2$ of *F. grandis* under hypoxia was not related to the same individual $\dot{M}O_2$ in normoxia (Everett and Crawford 2010). Their results agree with ours

in that the metabolic rate and differences in metabolic rate when exposed to hypoxic or normoxic conditions may not explain an individual's HT, and it further suggest that plasticity of HT is species specific and may require different environmental HT-inducing stimuli (*e.g.* seasonal, daily, infrequent hypoxia exposures). Lastly, another source of variation may be differential scaling of metabolic rate between resting and swimming animals or fish exposed to hypoxia (Everett and Crawford 2010; Urbina and Glover 2013; Roze et al. 2013).

Summary, future perspectives and ecological relevance

Here we report significant rank order repeatability and plasticity in hypoxia tolerance of juvenile striped bass. While held in the lab conditions, 18 juvenile striped bass were acutely exposed to hypoxia three times, including hypoxia tolerance tests, and at the last exposure their HT was significantly higher than that tested approximately 11 weeks prior. What the underlying biochemical, physiological, and/or morphological mechanisms of this ability to increase HT are remains unknown and may be investigated in the future experiments. In between the HT tests, each individual was swum under low flow (10.2 cm s^{-1}) and high flow (their estimated 67 % U_{max}) in normoxic and hypoxic water, and their oxygen consumption rate measured. As expected, their aerobic metabolic capacity was suppressed under hypoxia, and oxygen consumption rate was higher when swimming in high flow. But more interestingly, relative individual hypoxia tolerance, whether measured before or after the respirometry tests, was not correlated with their respiration rate at any flow or oxygen level conditions. This finding is worth further investigation, because many physiological (*e.g.* oxygen carrying capacity, tissue oxygen demand, SMR, MMR, AS) and morphological (total gill surface area) traits are

associated with both aerobic swimming metabolic capacity and hypoxia tolerance performances and the trade-offs, if any, between them are still not clear in juvenile striped bass. Change in HT, however, significantly negatively correlated with an individual's $\dot{M}O_2$ while swimming in low flow under normoxia. Nevertheless, the identification and evaluation of trade-offs remain a current challenge in the field of fish physiology (Marras et al. 2013; Svendsen et al. 2015).

We report on substantial but repeatable inter-individual variation in all physiological performance parameters measured here. Variation in traits important to Darwinian fitness is maintained in the natural setting, it not only provides a raw material for natural selection to act upon, but also may be highly beneficial in rapidly changing environments. For example, it is very likely that different individuals will be selected under differential environmental conditions, with differential oxygen availability. The source of the variation we observed is uncertain. It is ecologically important to further investigate whether it has genetic and environmental basis and what role it plays in the environment.

Lastly, two limitations of this study include: i) the HF tests were estimated 67 % of each individuals U_{max} (Beamish 1970) and their actual U_{max} was unknown, and ii) a lack of measurement of SMR, MMR, and AS. Having these data available, may have helped to better explain some of the observed relationships or lack of them. There is a great potential for future research in juvenile striped bass and other fish species.

Table 1. The mean performance, variance, and mixed effect model output summary of respiration rate of 18 individual juvenile striped bass under two flow and two oxygen conditions.

LMM: oxygen consumption ~ test + (1| ID)

	Mean	d.f.	t-value	Lower CI	Upper CI	CV %†
H-LF	107.80	72	15.35	94	122	17.22
H-HF	155.13	72	22.09	141	169	19.28
N-LF	128.81	72	18.34	115	143	15.29
N-HF	214.60	72	30.56	201	228	24.78
N-LF2	125.86	72	17.92	112	140	12.39

Respiration rate expressed in $\dot{M}O_2$ (μmol min^{-1}), and data are corrected for fish size.

† Calculated separately, not and output of LMM

H = hypoxia (20 % air saturation, AS); N = normoxia (> 90 % AS); LF = low flow (10.2 cm s^{-1}); HF = high flow (estimated 67 % U_{max});

Table 2. Repeatability and Post Hoc-Tukey's analysis results of mixed effect model investigating the difference in respiration rate of individual striped bass swimming under low and high flow under normoxic and hypoxic conditions.

	Estimated difference	Repeatability: Spearman's ρ	Repeatability: Pearson's r
H-HF *vs.* H-LF	47.327 ***	0.59 *	0.68 *
N-LF *vs.* H-LF	21.006	0.33	0.44
N-HF *vs.* H-LF	106.799 ***	0.09	-0.046
N-LF2 *vs.* H-LF	18.061	0.20	0.25
N-LF *vs.* H-HF	-26.322 *	0.28	0.31
N-HF *vs.* H-HF	59.472 ***	0.42	0.22
N-LF2 *vs.* H-HF	-29.266 **	0.51 *	0.52 *
N-HF *vs.* N-LF	85.794 ***	0.38	0.26
N-LF2 *vs.* N-LF	-2.945	0.73 ***	0.62 ***
N-LF2 *vs.* N-HF	-88.739 ***	0.61 **	0.62 **

Significance levels indicated: * $p < 0.05$; ** $p < 0.01$, *** $p < 0.001$
H = hypoxia (20 % air saturation, AS); N = normoxia (> 90 % AS);
LF = low flow (10.2 cm s^{-1}); HF = high flow (estimated 67 % U_{max})

Figure Legends

Figure 1. Hypoxia challenge tests and respirometry tests schematic timeline. The first hypoxia challenge test (HCT1), was performed 4 weeks prior the first respirometry test, and HCT2 was performed 10 days after last fish was tested in the respirometer (HCTs indicated in light grey boxes). In between the HCTs, one at the time, randomly chosen fish were subjected to the respirometry test; i) an individual was acclimated for 24 h in low flow (10.2 cm s^{-1}) (dark grey), ii) oxygen consumption rate (measured as rate of oxygen decrease $\dot{V}O_2$, mmol s^{-1}, in the respirometer, expressed as $\dot{M}O_2$, see calculations in text) was measured under normoxia (97-100 % AS) indicated in green boxes and hypoxia (20 % AS) in red boxes. Tests in low flow, 10.2 cm s^{-1}, are in light colored boxes, and dark colored boxes = tests in high flow, or at flow of estimated 67% U_{max}. All tests consisted of three cycles of 10 min of measuring and a 5 min flush.

The numbers in figure represent the following: 1 and 5) velocity increased to 67% U_{max} at a rate 6 cm s^{-1} min^{-1} while flushing from previous respirometry measurement cycle/test; 2 and 6) followed by a 10 min acclimation to increased water velocity; 3) velocity was decreased at the same rate as it was increased also while flushing from respirometry; 4) oxygen concentration was decreased to 20 % AS over a period of an hour. All fish were weighed and measured after HCT1, immediately after the respirometry test and after HCT2.

Figure 2. Body size (mass, g) and oxygen consumption rate relationship of 18 juvenile striped bass. Data from respirometry tests under low flow in normoxic water (N-LF and N-LF2, three measurements per fish in each test) represented. Metabolic rate significantly increased with increasing body mass (solid line represents regression line; Pearson's $r = 0.85$, $p < 0.001$).

Figure 3. Hypoxia tolerance (HT) over time. Panel A: Rank order repeatability of each individual striped bass' hypoxia tolerance; HT expressed as cumulative oxygen deficit (D_{CO}; Nelson and Lipkey 2015), and was significantly repeatable over period of 11 weeks and 4 days (solid line is regression line, dashed line represents the line of identity; Spearman's $\rho = 0.59$, $p = 0.012$; Kendall's $\tau = 0.45$; $p = 0.009$). Panel B: Histogram of HT (D_{CO}) in a group of 18 juvenile striped bass in the first hypoxia challenge test (HCT1) and the second test (HCT2); the average increase of HT (D_{CO}) of each individual was 127.44 ± 24.58 (%h, mean ± s.e.m.).

Figure 4. The relationships between increase in hypoxia tolerance (ΔHT), respiration rate, and growth rate of juvenile striped bass. Panel A: A plot representing significant positive relationship between individual growth rate and ΔHT (solid line is a regression line; Pearson's $r = 0.25$, $p = 0.02$). Panel B: A plot showing a significant negative relationship between $\dot{M}O_2$ in low flow normoxic conditions (respiration rate is size adjusted; n = 3 for each fish in two low flow tests) and their growth rate, g day^{-1} (solid line is a regression line; Pearson's $r = -0.24$, $p = 0.01$). Panel C: A plot showing significant negative relationship between ΔHT and average (n=3) $\dot{M}O_2$ in low flow conditions under normoxia (empty triangles, dashed regression line; Pearson's $r = -0.51$,

$p < 0.001$), hypoxia (filled triangles, solid regression line; Pearson's $r = -0.29$, $p = 0.04$) and when combining normoxia and hypoxia tests (Pearson's $r = -0.32$, $p < 0.001$).

Figure 5. Respiration rate under different environmental oxygen and flow conditions: H-LF (hypoxia low flow), H-HF (hypoxia high flow), N-LF (normoxia low flow), N-HF (normoxia high flow), N-LF2 (normoxia low flow, recovery). In the boxplot the horizontal solid lines are median values of all individual respiration rate measurements (n = 3 in each test, diamonds for hypoxia, and circles for normoxia tests, darker color for HF tests, empty for LF tests), boxes are interquartile ranges (IQR), and whiskers comprise the full range of data excluding outliers defined as the values more than ± 1.5 IQR outside of the box. Oxygen consumption rate was significantly different between the tests (ANOVA: F (4, 71.99) = 46.98, $p < 0.001$).

Figure 6. The difference between respiration rate of in each juvenile striped bass (n=18) while swimming against low flow (10.2 cm s^{-1}) and high flow (estimated 67 % U_{max}) in normoxia *vs*. hypoxia. The increase in oxygen consumption rate ($\Delta\dot{M}O_2$) in normoxia (N, >90 % air saturation, AS) was not statistically correlated to that in hypoxia (H, ~ 20 % AS) (Spearman's $\rho = 0.33$, $p = 0.18$; Pearson's $r = 0.33$, $p = 0.19$).

Figure 7. The relationship between the change in hypoxia tolerance of 18 juvenile striped bass and their measured increase in oxygen consumption rate while swimming in low and high flow under two conditions: hypoxia (20 % air saturation, AS) and normoxia > 90 % AS. Metabolic rate, $\dot{M}O_2$, was measured of all individuals under hypoxia and normoxia while swimming against low flow (10.2 cm s^{-1}) and high flow (their estimated 67 % U_{max}) and change, $\Delta\dot{M}O_2$, calculated for each condition. Each individuals relative hypoxia tolerance, HT, was measure twice ~ 11 weeks apart, and change in HT

calculated. The relationship was insignificant regardless of oxygen levels. Black triangles and solid line correspond to hypoxia respirometry tests; empty triangles and dashed line show results of normoxia tests.

Figure 1.

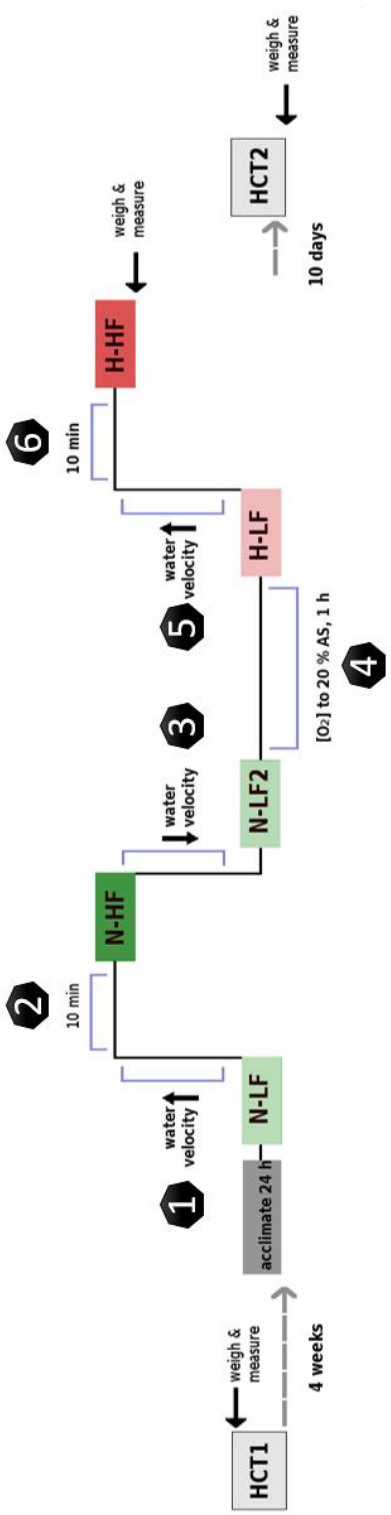

weigh & measure
HCT2
10 days
weigh & measure
H-HF
6
10 min
water velocity
5
H-LF
3
4
[O2] to 20 % AS, 1 h
water velocity
N-LF2
N-HF
2
10 min
water velocity
1
N-LF
acclimate 24 h
weigh & measure
HCT1
4 weeks

Figure 2.

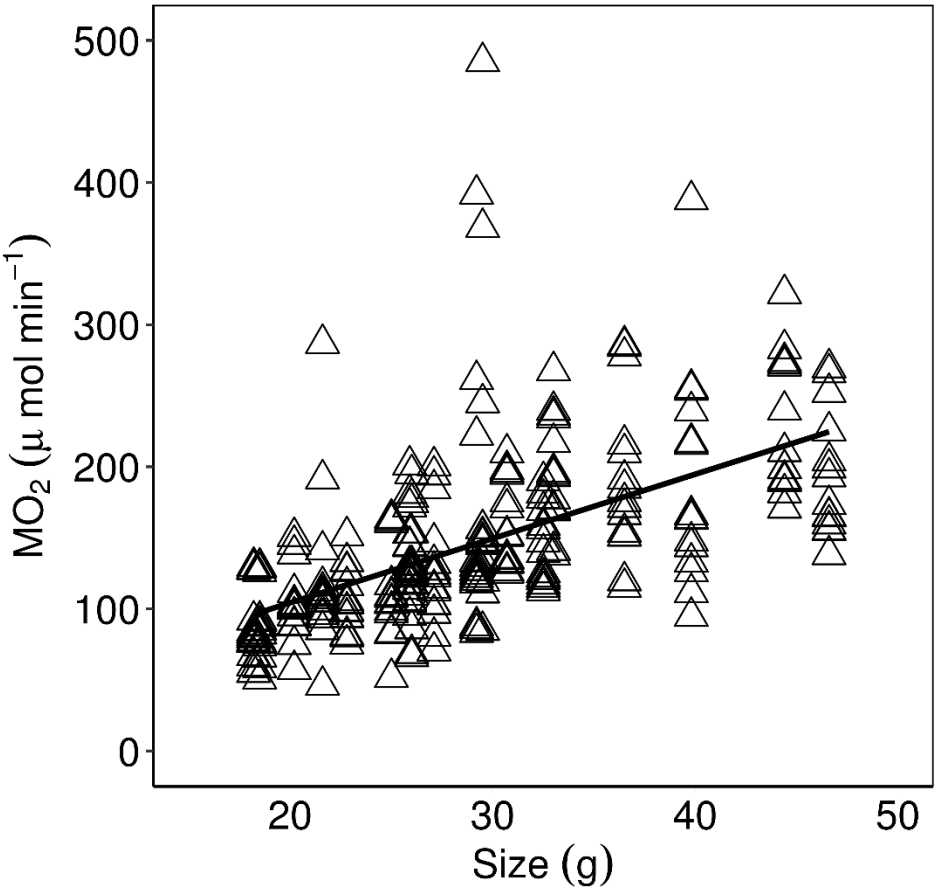
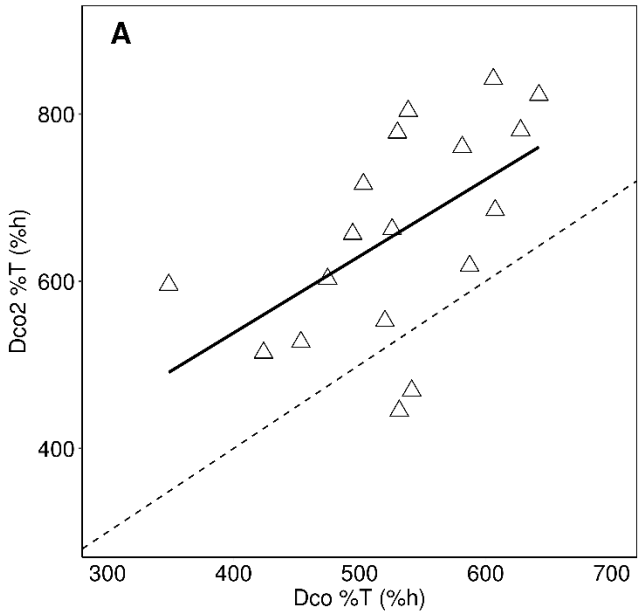


Figure 3.


A
800
600
400
Dco2 %T (%h)
300
400
500
600
700
Dco %T (%h)

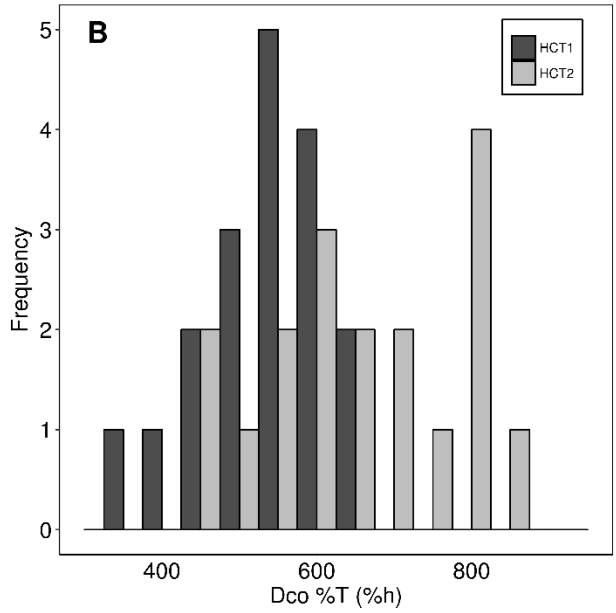

B
HCT1
HCT2
5
4
3
2
1
0
Frequency
400
600
800
Dco %T (%h)

Figure 4.

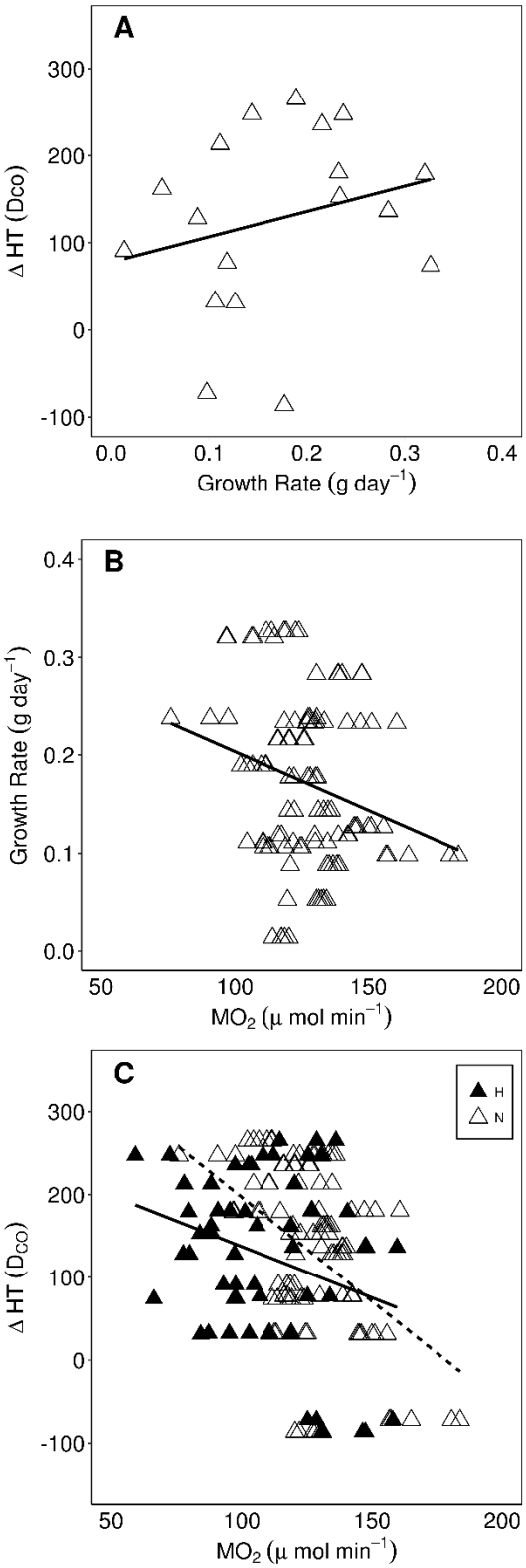

A
300
200
100
0
-100
Δ HT (Dco)
0.0
0.1
0.2
0.3
0.4
Growth Rate (g day^{-1})
B
0.4
0.3
0.2
0.1
0.0
Growth Rate (g day^{-1})
50
100
150
200
MO$_2$ (μ mol min^{-1})
C
H
N
300
200
100
0
-100
Δ HT (D$_{CO}$)
50
100
150
200
MO$_2$ (μ mol min^{-1})

Figure 5.

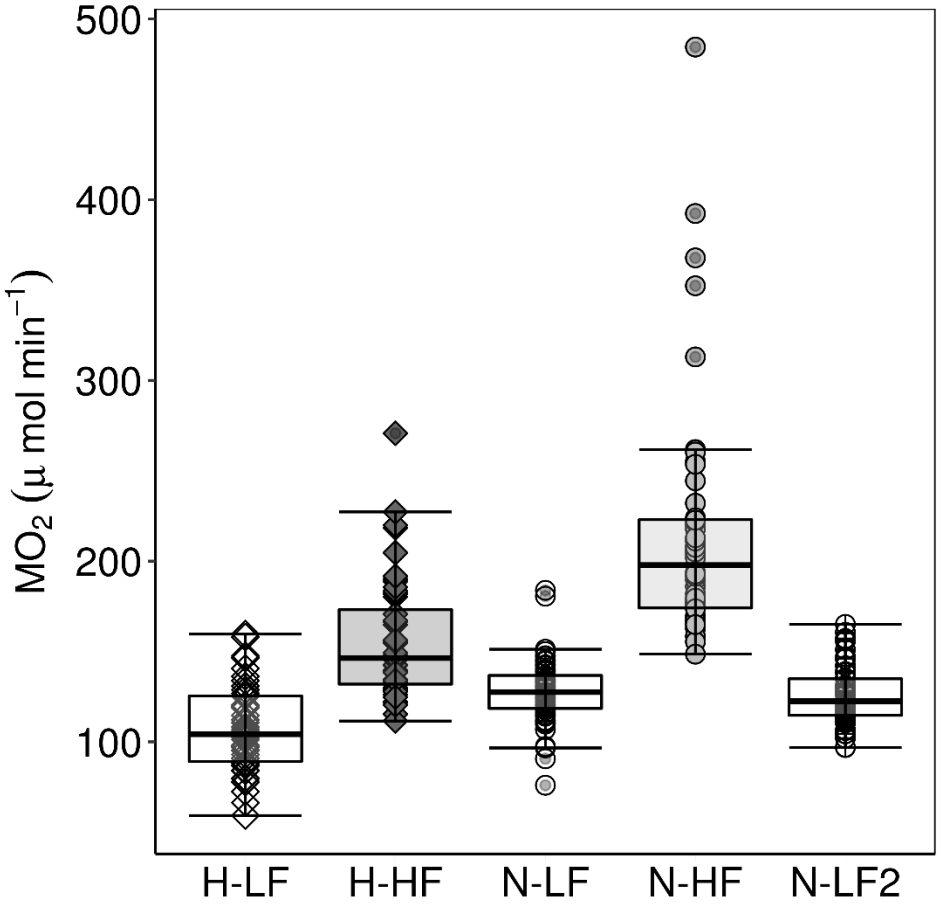


Figure 6.

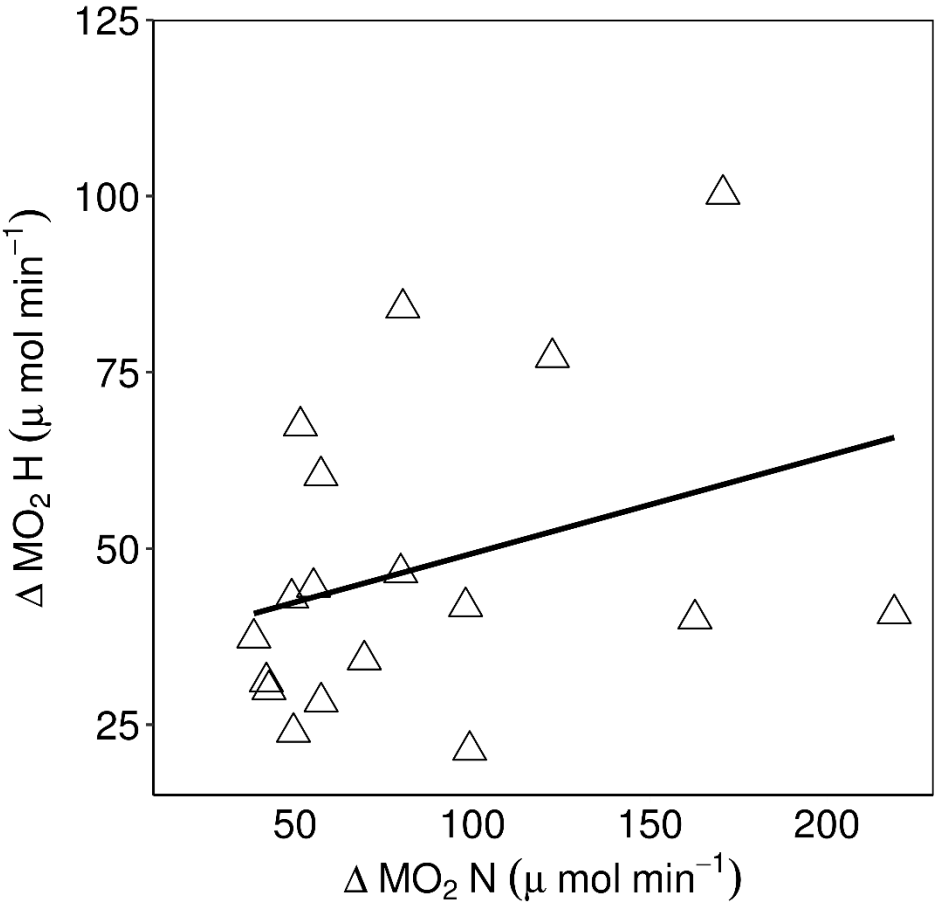
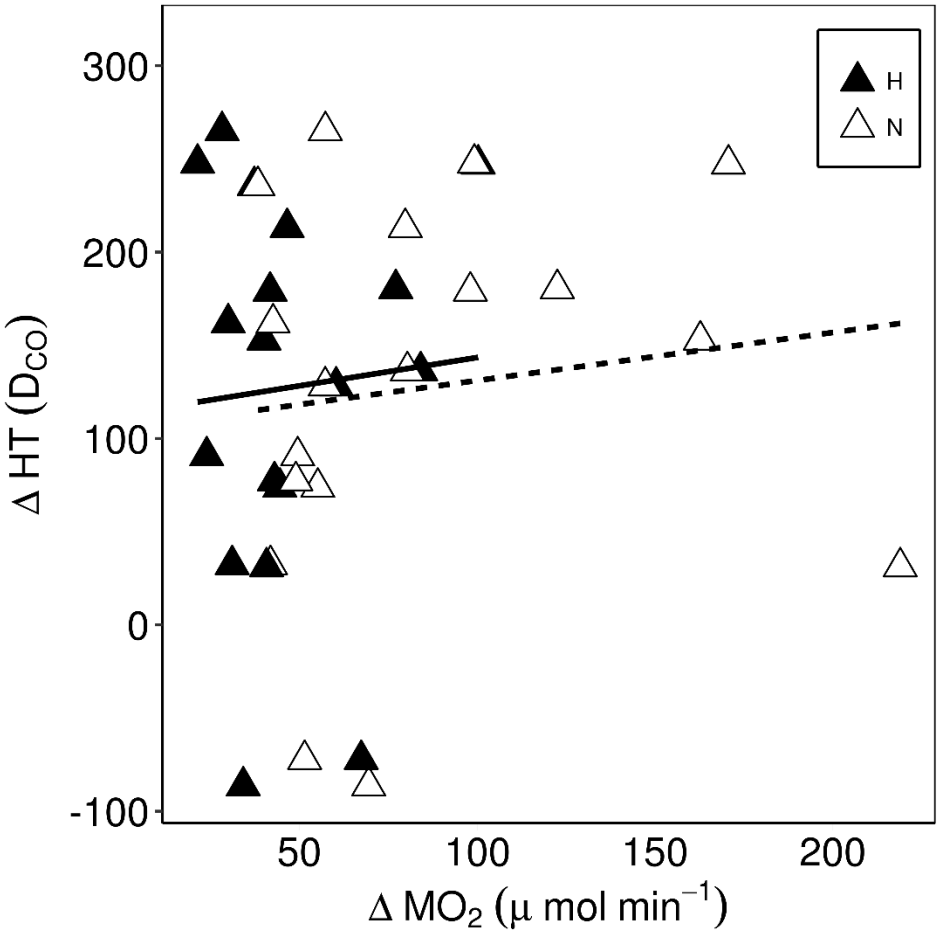


Figure 7.



Appendices

IACUC approval number: 12042013JN-02

New IACUC protocol # 1611000152 approved (approval # pending):

TOWSON UNIVERSITY

December 13, 2016

Office of Sponsored Programs and Research

Towson University
8000 York Road
Towson, MD 21252-0001

t. 410 704-2236
f. 410 704-4494

To: Dr. Jay Nelson

From: Towson University Institutional Animal Care and Use Committee
Dr. Jack Shepard, Chairperson

RE: IACUC PROTOCOL# 1611000152
Intraspecific Variance in Hypoxia Tolerance in Juvenile Striped Bass (Morone saxafilis)

This is to certify that the Institutional Animal Care and Use Committee has reviewed your protocol and granted FULL APPROVAL. The approval date for this protocol is December 12, 2016.

The protocol is approved for a period of 3 years and will expire on December 11, 2019.

If you need to extend the protocol beyond this date, you must submit an updated Animal Care and Use form at least three months prior to the expiration.

Your protocol can now be viewed in MyOSPR. For more information, please visit: http://www.towson.edu/academics/research/sponsored/myospr.html

If you have any questions, please do not hesitate to contact the OSPR Compliance Administrator by phone (410.704.2236) or email (OSPR@towson.edu).

Jack Shepard/ah

Dr. Jack Shepard, Chairperson
Towson IACUC

IACUC approval number: 03312014CO-02

TOWSON UNIVERSITY

March 30, 2017

To: Christopher Oufiero

From: Towson University Institutional Animal Care and Use Committee
Jack Shepard, Chairperson

RE: IACUC PROTOCOL RENEWAL# 03312014CO-02/1606000080
Investigations into the diversity of swimming performance among fish: examining ecological, morphological, and physiological correlates

Office of Sponsored Programs and Research

Towson University
8000 York Road
Towson, MD 21252-0001

t. 410 704-2236
f. 410 704-4494

This is to certify that the Institutional Animal Care and Use Committee has reviewed and renewed your protocol. The renewal date for this protocol is March 31st, 2017.

Your protocol is renewed for an additional period of 3 years; an annual report must be submitted to the IACUC six weeks before each anniversary date of the protocol.

Please note your protocol will expire March 30th, 2020. If you need to extend the protocol beyond this date, you must submit an Animal Care and Use form at least three months prior to the expiration.

If you have any questions, please do not hesitate to contact the Office of Sponsored Programs and Research by phone (410-704-2236) or email (OSPR@towson.edu).

Jack Shepard, PhD
Chairperson, Towson University IACUC

References

Abrahams M.V, M. Mangel, and K. Hedges. 2007. Predator – prey interactions and changing environments: who benefits? Philos Trans R Soc B Biol Sci 362:2095–2104.

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

Allan B.J.M., P. Domenici, S.A. Watson, P.L. Munday, and M.I. McCormick. 2017. Warming has a greater effect than elevated CO2 on predator – prey interactions in coral reef fish. Proc Natl Acad Sci 284. DOI: http://dx.doi.org/10.1098/rspb.2017.0784

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.

Beamish F.W.H. 1970. Oxygen consumption of largemouth bass, *Micropterus salmoides*, in relation to swimming speed and temperature. Can J Zool 48:1221–1228.

Beddow T.A., J.L.V. Leeuwen, and I.A. Johnston. 1995. Swimming kinematics of fast start are altered by temperature acclimation in the marine fish *Myoxocephalus scorpius*. J Exp Biol 198:203–208.

Bell G.W. and D.B. Eggleston. 2005. Species-specific avoidance responses by blue crabs and fish to chronic and episodic hypoxia. Mar Biol 146:761–770.

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.

Bickler P.E. and L.T. Buck. 2007. Hypoxia tolerance in reptiles, amphibians, and fishes: life with variable oxygen availability. Annu Rev Physiol 69:145–170.)

Billerbeck J.M., T.E. Lankford, and D.O. Conover. 2001. Evolution of intrinsic growth and energy acquisition rates. I. trade-offs with swimming performance in *Menidia menidia*. Evolution55:1863–1872.

Blake R.W. 2004. Fish functional design and swimming performance. J Fish Biol 65:1193–1222.

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 -Part A Mol Integr Physiol 198:59–71.

Borowiec B.G., K.L. Darcy, D.M. Gillette, and G.R. Scott. 2015. Distinct physiological strategies are used to cope with constant hypoxia and intermittent hypoxia in killifish (*Fundulus heteroclitus*). J Exp Biol 218:1198–1211.

Brandt S.B., M. Gerken, K.J. Hartman, and E. Demers. 2009. Effects of hypoxia on food consumption and growth of juvenile striped bass (*Morone saxatilis*). J Exp Mar Bio Ecol 381:S142–S149.

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. 2002. Effects of hypoxia, and the balance between hypoxia and enrichment, on coastal fishes and fisheries. Estuaries 25:767–781.

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. Ann Rev Mar Sci 1:329–349.

Breitburg D.L., N. Steingerg, S. DuBeau, C. Cooksey, and E.D. Houde. 1994. Effects of low dissolved oxygen on predation on estuarine fish larvae. Mar Ecol Prog Ser 104:235–246.

Brokordt K., H. Pérez, and F. Campos. 2013. Environmental hypoxia reduces the escape response capcity of juvenile and adult scallops *Argopecten purpuratus*. J Shellfish Res 32:369–376.

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.

Burton T., S.S. Killen, J.D. Armstrong, and N.B. Metcalfe. 2011. What causes intraspecific variation in resting metabolic rate and what are its ecological consequences? Proc R Soc B 3465–3473.

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.

Camp A.L., T.J. Roberts, and E.L. Brainerd. 2015. Swimming muscles power suction feeding in largemouth bass. Proc Natl Acad Sci 112:8690–8695.

Carroll A.M. 2004. Morphology predicts suction feeding performance in centrarchid fishes. J Exp Biol 207:3873–3881.

Chapman L., J. Albert, and F. Galis. 2008. Developmental plasticity, genetic differentiation, and hypoxia-induced trade-offs in an African cichlid fish. Open Evol J 2:75–88.

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.

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., L.S. Kaufman, C.A. Chapman, and F.E. McKenzie. 1995. Hypoxia tolerance in twelve species of East African cichlids: Potential for low oxygen refugia in Lake Victoria. Conserv Biol 9:1274–1288.

Claireaux G. and C. Lefrançois. 2007. Linking environmental variability and fish performance: integration through the concept of scope for activity. Philos Trans R Soc B Biol Sci 362:2031–2041.

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. and J.-D. Dutil. 1992. Physiological response of the Atlantic cod (*Gadus morhua*) to hypoxia at various environmental salinities. J Exp Biol 163:97–118.

Claireaux G. and J.P. Lagardère. 1999. Influence of temperature, oxygen and salinity on the metabolism of the European sea bass. J Sea Res 42:157–168.

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.

Claireaux G., M. Théron, M. Prineau, M. Dussauze, F.-X. Merlin, and S. Le Floch. 2013. Effects of oil exposure and dispersant use upon environmental adaptation performance and fitness in the European sea bass, *Dicentrarchus labrax*. Aquat Toxicol 130:160–170.

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.

Cook A. 1996. Ontogeny of feeding morphology and kinematics in juvenile fishes: a case study of the cottid fish *Clinocottus analis*. J Exp Biol 199:1961–1971.

Cooper J.E., R.A. Rulifson, J.J. Isely, and S.E. Winslow. 1998. Food habits and growth of juvenile striped bass, *Morone saxatilis*, in Albemarle Sound, North Carolina. Estuaries 21:307–317.

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

Crans K.D., N.A. Pranckevicius, and G.R. Scott. 2015. Physiological tradeoffs may underlie the evolution of hypoxia tolerance and exercise performance in sunfish (Centrarchidae). J Exp Biol 218:3264–3275.

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.

Davies R., C.D. Moyes, and Y.S. Wang. 2011. Intra- and inter-specific variation in metabolic gene expression in relationship to environmental hypoxia. Comp Biochem Physiol Part A 159:25–31.

Day S.W., T.E. Higham, R. Holzman, and S. Van Wassenbergh. 2015. Morphology, kinematics, and dynamics: The mechanics of suction feeding in fishes. Integr Comp Biol 1–15.

deVries M.S. and P.C. Wainwright. 2006. The effects of acute temperature change on prey capture kinematics in largemouth bass, *Micropterus salmoides*. Copeia 3:437–444.

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.

Diaz R.J. and R. Rosenberg. 2008. Spreading dead zones and consequences for marine ecosystems. Science 321:926–929.

Diaz R.J. and R. Rosenberg. 2011. Introduction to environmental and economic consequences of hypoxia. Water Resour Dev 21:71–82.

Dixon R.L., P.A. Grecay, and T.E. Targett. 2017. Responses of juvenile Atlantic silverside, striped killifish, mummichog, and striped bass to acute hypoxia and acidification: Aquatic surface respiration and survival. J Exp Mar Bio Ecol 493:20–30.

Dohm M.R. 2002. estimates do not always set an upper limit repeatability to heritability. Funct Ecol 16:273–280.

Domenici P. 2010. Context-dependent variability in the components of fish escape response: Integrating locomotor performance and behavior. J Exp Zool Part A Ecol Genet Physiol 313 A:59–79.

Domenici P. and R.W. Blake. 1993. Escape Trajectories in Angelfish (*Pterophyllum Eimekei*). J Exp Biol 177:253–272.

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

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

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-Verlag, Berlin Heidelberg.

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.

Eaton R.C. and D.S. Emberley. 1991. How stimulus direction determines the trajectory of the Mauthner-initiated escape response in a teleost fish. J Exp Biol 161:469–487.

Eliason E.J. and A.P. Farrell. 2016. Oxygen uptake in Pacific salmon *Oncorhynchus* spp.: when ecology and physiology meet. J Fish Biol 88:359–388.

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

Farrell A.P., E.J. Eliason, E. Sandblom, and T.D. Clark. 2009. Fish cardiorespiratory physiology in an era of climate change. Can J Zool 87:835–851.

Faust H.A., A.K. Gamperl, and J.K. Rodnick. 2004. All rainbow trout (*Oncorhynchus mykiss*) are not created equal: intra-specific variation in cardiac hypoxia tolerance. J Exp Biol 207:1005–1015.

Fay C.W., R.J. Neves, and G.B. Pardue. 1983. *Species profiles: life histories and environmental requirements of coastal fishes and invertebrates (Mid-Atlantic). Striped bass*. Fish and Wildife Service, U.S. Department of the Interior, Washington, DC.

Ferry-Graham L.A., P.C. Wainwright, M.W. Westneat, and D.R. Bellwood. 2001. Modulation of prey capture kinematics in the cheeklined wrasse *Oxycheilinus digrammus* (Teleostei: Labridae). J Exp Zool 290:88–100.

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 Physiol. Academic Press, New York.

Fu S.-J., C. Fu, G.-J. Yan, Z.-D. Cao, A.-J. Zhang, and X. Pang. 2014. Interspecific variation in hypoxia tolerance, swimming performance and plasticity in cyprinids that prefer different habitats. J Exp Biol 217:590–597.

Fu S.-J., C.J. Brauner, Z.-D. Cao, J.G. Richards, J.-L. Peng, R. Dhillon, and Y.-X. Wang. 2011. The effect of acclimation to hypoxia and sustained exercise on subsequent hypoxia tolerance and swimming performance in goldfish (*Carassius auratus*). J Exp Biol 214:2080–2088.

Gaulke G.L., C.E. Dennis, D.H. Wahl, and C.D. Suski. 2014. Acclimation to a low oxygen environment alters the hematology of largemouth bass (*Micropterus salmoides*). Fish Physiol Biochem 40:129–140.

Gerry S.P., A. Robbins, and D.J. Ellerby. 2012. Variation in fast-start performance within a population of polyphenic bluegill (*Lepomis macrochirus*). Physiol Biochem Zool 85:694–703.

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.

Gibb A.C. and L. Ferry-Graham. 2005. Cranial movements during suction feeding in teleost fishes: Are they modified to enhance suction production? Zoology 108:141–53.

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.

Grubich J.R. 2001. Prey Capture in Actinopterygian Fishes: A review of suction feeding motor patterns with new evidence from an elopomorph fish, *Megalops atlanticus*. Am Zool 41:1258–1265.

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.

Higham T.E., S.W. Day, and P.C. Wainwright. 2006. Multidimensional analysis of suction feeding performance in fishes: fluid speed, acceleration, strike accuracy and the ingested volume of water. J Exp Biol 209:2713–2725.

Hochachka P.W., L.T. Buck, C.J. Doll, and S.C. Land. 1996. Unifying theory of hypoxia tolerance: molecular/metabolic defense and rescue mechanisms for surviving oxygen lack. Proc Natl Acad Sci USA 93:9493–9498.

Holt R.E. and C. Jørgensen. 2015. Climate change in fish: effects of respiratory constraints on optimal life history and behaviour. Biol Lett 11:20141032.

Jornod M. and D.G. Roche. 2015. Inter- vs intra-individual variation and temporal repeatability of escape responses in the coral reef fish *Amblyglyphidodon curacao*. Biol Open 4:1395–1399.

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.

Joyce W., K. Ozolina, F. Mauduit, H. Ollivier, G. Claireaux, and H.A. Shiels. 2016. Individual variation in whole-animal hypoxia tolerance is associated with cardiac hypoxia tolerance in a marine teleost. Biol Lett 12:20150708.

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.

Kaufman L.S., L.J. Chapman, and C.A. Chapman. 1997. Evolution fast forward: haplochromine fishes of the Lake Victoria region. Endeavour 21:23–30.

Keeling R.F., A. Körtzinger, and N. Gruber. 2010. Oxygen deoxygenation in a warming world. Ann Rev Mar Sci 2:199–229.

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.

Killen S.S., D. Atkinson, and D.S. Glazier. 2010. The intraspecific scaling of metabolic rate with body mass in fishes depends on lifestyle and temperature. Ecol Lett 13:184–193.

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 217:859–65.

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.

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.

Kraus R.T., D.H. Secor, and R.L. Wingate. 2015. Testing the thermal-niche oxygen-squeeze hypothesis for estuarine striped bass. Environ Biol Fishes 98:2083–2092.

Langerhans B.R. and D.N. Reznick. 2010. Swimming Performance in Fishes: Predicting Evolution with Biomechanics. Pp. 200–248 in P. Domenici and B.G. Kapoor eds. Fish Locomot An Eco-ethological Perspect. Science Publishers.

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.

Liem K.F. 1980. Acquisition of energy by teleosts: adaptive mechanisms and evolutionary patterns. Pp. 299–334 in M.A. Ali ed. Environ Physiol fishes. Plenum Press, New York.

Lipkey G. 2012. Factors contributing to the intraspecific variation of hypoxia tolerance in juvenile striped bass (*Morone saxatilis*). MSc Thesis. Towson University, Towson.

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.

Lundvall D., R. Svanbäck, L. Persson, and P. Byström. 1999. Size-dependent predation in piscivores: interactions between predator foraging and prey avoidance abilities. Can J Fish Aquat Sci 56:1285–1292.

Lyon J.P., T.J. Ryan, and M.P. Scroggie. 2007. Effects of temperature on the fast-start swimming performance of an Australian freshwater fish. Ecol Freshw Fish 17:184–188.

Mandic M., A.E. Todgham, and J.G. Richards. 2009a. Mechanisms and evolution of hypoxia tolerance in fish. Proc Biol Sci 276:735–744.

Mandic M., B. Speers-Roesch, and J.G. Richards. 2012. Hypoxia tolerance in sculpins is associated with high anaerobic enzyme activity in brain but not in liver or muscle. Physiol Biochem Zool 86:92–105.

Mandic M., K.A. Sloman, and J.G. Richards. 2009b. Escaping to the surface: a phylogenetically independent analysis of hypoxia- induced respiratory behaviors in sculpins. Physiol Biochem Zool 82:730–738.

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, G. Claireaux, P. Domenici, and D.J. McKenzie. 2011. Behavioural and kinematic components of the fast-start escape response in fish: individual variation and temporal repeatability. J Exp Biol 214:3102–3110.

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.

Martínez M.L., C. Landry, R. Boehm, S. Manning, A.O. Cheek, and B.B. Rees. 2006. Effects of long-term hypoxia on enzymes of carbohydrate metabolism in the Gulf killifish, *Fundulus grandis*. J Exp Biol 209:3851–3861.

McBryan T.L., K. Anttila, T.M. Healy, and P.M. Schulte. 2013. Integrative and comparative biology responses to temperature and hypoxia as interacting stressors in fish: implications for adaptation to environmental change. Integr Comp Biol 53:648–659.

McKenzie D.J. 2011. Energetics of Fish Swimming | Energetics of Fish Swimming. Pp. 1636–1644 in A.P. Farrell ed. Encycl Fish Physiol From Genome to Environ. Academic Press; Elsevier Inc., San Diego.

McKenzie D.J., J.F. Steffensen, K. Korsmeyer, N.M. Whiteley, P. Bronzi, and E.W. Taylor. 2007. Swimming alters responses to hypoxia in the Adriatic sturgeon *Acipenser naccarii*. J Fish Biol 70:651–658.

Meager J.J., T. Solbakken, A.C. Utne-Palm, and T. Oen. 2005. Effects of turbidity on the reaction distance, search time, and foraging success of juvenile Atlantic cod (*Gadus morhua*). Can J Fish Aquat Sci 62:1978–1984.

Metcalfe N.B., A.C. Taylor, and J.E. Thorpe. 1995. Metabolic rate, social status and life-history strategies in Atlantic salmon. Anim Behav 49:431–436.

Metcalfe N.B., T.E. Van Leeuwen, and S.S. Killen. 2016. Does individual variation in metabolic phenotype predict fish behaviour and performance? J Fish Biol 88:298–321.

Najjar R.G., C.R. Pyke, M.B. Adams, D. Breitburg, C. Hershner, M. Kemp, R. Howarth, et al. 2010. Potential climate-change impacts on the Chesapeake Bay. Estuar Coast Shelf Sci 86:1–20.

Nasuchon N., M. Yagi, Y. Kawabata, K. Gao, and A. Ishimatsu. 2016. Escape responses of the Japanese anchovy *Engraulis japonicus* under elevated temperature and CO2 conditions. Fish Sci 82:435–444.

Nelson J.A. 2016. Oxygen consumption rate v. rate of energy utilization of fishes: a comparison and brief history of the two measurements. J Fish Biol 88:10–25.

Nelson J.A. and D. Chabot. 2011. General Energy Metabolism. Pp. 1566–1572 in A.P. Farrell ed. Encycl Fish Physiol From Genome to Environ. Academic Press; Elsevier Inc., San Diego.

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

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., 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., 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.

Nemeth D.H. 1997. Modulation of attack behavior and its effect on feeding performance in a trophic generalist fish, *Hexagrammos decagrammus*. J Exp Biol 200:2155–2164.

Nikinmaa M. 2001. Haemoglobin function in vertebrates: Evolutionary changes in cellular regulation in hypoxia. Respir Physiol 128:317–329.

Nilsson G.E. 2007. Gill remodeling in fish - a new fashion or an ancient secret? J Exp Biol 210:2403–2409.

Nilsson G.E. and S. Östlund-Nilsson. 2003. Hypoxia in paradise: widespread hypoxia tolerance in coral reef fishes. Proc Biol Sci 271 Suppl:S30-3.

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

Nilsson G.E., S. Ostlund-Nilsson, R. Penfold, and A.S. Grutter. 2007. From record performance to hypoxia tolerance: respiratory transition in damselfish larvae settling on a coral reef. Proc R Soc B 274:79–85.

Norin T. and H. Malte. 2011. Repeatability of standard metabolic rate, active metabolic rate and aerobic scope in young brown trout during a period of moderate food availability. J Exp Biol 214:1668–1675.

Norin T. and H. Malte. 2012. Intraspecific variation in aerobic metabolic rate of fish: relations with organ size and enzyme activity in brown trout. Physiol Biochem Zool 85:645–656.

Norin T. and T.D. Clark. 2016. Measurement and relevance of maximum metabolic rate. J Fish Biol 88:122–151.

Norin T., H. Malte, and T.D. Clark. 2016. Differential plasticity of metabolic rate phenotypes in a tropical fish facing environmental change. Funct Ecol 30:369–378.

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.

Nowroozi B.N. and E.L. Brainerd. 2013. X-ray motion analysis of the vertebral column during the startle response in striped bass, *Morone saxatilis*. J Exp Biol 216:2833–2842.

Omlin T. and J.-M. Weber. 2010. Hypoxia stimulates lactate disposal in rainbow trout. J Exp Biol 213:3802–3809.

Osse J.W.M., F.A. Sibbing, and J.G.M. Van Den Boogaart. 1997. Intra-oral food manipulation of carp and other cyprinids: adaptations and limitations. Acta Physiol Scand 161:47–57.

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.

Oufiero C.E., R.A. Holzman, F.A. Young, and P.C. Wainwright. 2012. New insights from serranid fishes on the role of trade-offs in suction-feeding diversification. J Exp Biol 215:3845–3855.

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.

Pang X., S.-J. Fu, and Y.-G. Zhang. 2015. Individual variation in metabolism and swimming performance in juvenile black carp (*Mylopharyngodon piceus*) and the effects of hypoxia. Mar Freshw Behav Physiol 48:431–443.

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.

Pichavant K., J. Person-Le-Ruyet, N. Le Bayon, A. Severe, A. Le Roux, and G. Boeuf. 2001. Comparative effects of long-term hypoxia on growth, feeding and oxygen consumption in juvenile turbot and European sea bass. J Fish Biol Oct 59:875–883.

Pihl L., S.P. Baden, and R.J. Diaz. 1991. Effects of periodic hypoxia on distribution of demersal fish and crustaceans. Mar Biol 108:349–360.

Plante S., D. Chabot, and J.-D.D. Dutil. 1998. Hypoxia tolerance in Atlantic cod. J Fish Biol 53:1342–1356.

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.

Poulin R., N.G. Wolf, and D.L. Kramer. 1987. The effect of hypoxia on the vulnerability of guppies (*Poecilia reticulata*, Poeciliidae) to an aquatic predator (*Astronotus ocektus*, Cichlidae). Environ Biol Fishes 20:285–292.

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

Ramasamy R.A., B.J.M. Allan, and M.I. McCormick. 2015. Plasticity of escape responses: prior predator experience enhances escape performance in a coral reef fish. PLoS ONE 10:e0132790.

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

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.

Richard B.A. and P.C. Wainwright. 1995. Scaling the feeding mechanisms of largemouth bass (*Micropterus salmoides*): kinematics of prey capture. J Exp Biol 198:419–433.

Richards J.G. 2011. Physiological, behavioral and biochemical adaptations of intertidal fishes to hypoxia. J Exp Biol 214:191–199.

Robb T. and M. V. Abrahams. 2003. Variation in tolerance to hypoxia in a predator and prey species: an ecological advantage of being small? J Fish Biol 62:1067–1081.

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.

Roze T., F. Christen, A. Amerand, and G. Claireaux. 2013. Trade-off between thermal sensitivity, hypoxia tolerance and growth in fish. J Therm Biol 38:98–106.

Saloom M.E. and R.S. Duncan. 2005. Low dissolved oxygen levels reduce anti - predation behaviours of the freshwater clam *Corbicula fluminea*. Freshw Biol 50:1233–1238.

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 Regul Integr Comp Physiol 291:R711–R718.

Sass G.G. and P.J. Motta. 2002. The effects of satiation on strike mode and prey capture kinematics in the largemouth bass, *Micropterus salmoides*. Environ Biol Fishes 441–454.

Scully M.E. 2016a. The contribution of physical processes to inter-annual variations of hypoxia in Chesapeake Bay: A 30-yr modeling study. Limnol Oceanogr 61:2243–2260.

Scully M.E. 2016b. 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.

Sloan T.J. and R.G. Turingan. 2012. Invariant feeding kinematics of two trophically distinct nonnative Florida fishes, *Belonesox belizanus* and *Cichlasoma urophthalmus* across environmental temperature regimes. Int J Biol 4:117–126.

Snyder S., L.E. Nadler, J.S. Bayley, M.B.S. Svendsen, J.L. Johansen, P. Domenici, and J.F. Steffensen. 2016. Effect of closed *v.* intermittent-flow respirometry on hypoxia tolerance in the shiner perch *Cymatogaster aggregata*. J Fish Biol 88:252–264.

Sundt-Hansen L., L.F. Sundstrom, S. Einum, K. Hindar, I.A. Fleming, and R.H. Devlin. 2007. Genetically enhanced growth causes increased mortality in hypoxic environments. Biol Lett 3:165–168.

Svendsen J.C., B. Tirsgaard, G.A. Cordero, and J.F. Steffensen. 2015. Intraspecific variation in aerobic and anaerobic locomotion: gilthead sea bream (*Sparus aurata*) and Trinidadian guppy (*Poecilia reticulata*) do not exhibit a trade-off between maximum sustained swimming speed and minimum cost of transport. Front Physiol 6:1–12.

Townhill B.L., J.K. Pinnegar, D.A. Righton, and J.D. Metcalfe. 2017. Fisheries, low oxygen and climate change: how much do we really know? J Fish Biol 90:723–750.

Tran H.Q., R.S. Mehta, and P.C. Wainwright. 2010. Effects of ram speed on prey capture kinematics of juvenile Indo-Pacific tarpon, *Megalops cyprinoides*. Zoology 113:75–84.

Turesson H., A. Satta, and P. Domenici. 2009. Preparing for escape: anti-predator posture and fast-start performance in gobies. J Exp Biol 212:2925–2933.

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.

Virani N.A. and B.B. Rees. 2000. Oxygen consumption, blood lactate and inter-individual variation in the gulf killifish, *Fundulus grandis*, during hypoxia and recovery. Comp Biochem Physiol Part A 126:397–405.

Wainwright P.C. 2002. The evolution of feeding motor patterns in vertebrates. Curr Opin Neurobiol 12:691–695.

Wainwright P.C. and J.P. Friel. 2000. Effects of prey type on motor pattern variance in tetraodontiform fishes. J Exp Zool 286:563–571.

Wakeling J., K. Kemp, and I. Johnston. 1999. The biomechanics of fast-starts during ontogeny in the common carp *Cyprinus carpio*. J Exp Biol 202:3057–67.

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.

Walter J.F., A.S. Overton, K.H. Ferry, and M.E. Mather. 2003. Atlantic coast feeding habits of striped bass: A synthesis supporting a coast-wide understanding of trophic biology. Fish Manag Ecol 10:349–360.

Wang Q.-F., W.-L. Shen, C.-C. Hou, C. Liu, X.-F. Wu, and J.-Q. Zhu. 2017. Physiological responses and changes in gene expression in the large yellow croaker *Larimichthys crocea* following exposure to hypoxia. Chemosphere 169:418–427.

Webb P.W. 1971. The swimming energetics of trout. II. Oxygen consumption and swimming efficiency. J Exp Biol 55:521–540.

Webb P.W. 1978. Fast-start Performance and Body Form in Seven Species of Teleost Fish. J Exp Biol 74:211–226.

Webb P.W. 1984. Form and Function in Fish Swimming. Sci Am 251:72–82.

Webb P.W. 1986. Effects of body form and response threshold on the vulnerability of four species of teleost prey attacked by largemouth bass (*Miropterus salmoides*). Can J Fish Aquat Sci 43:763–771.

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

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

Westneat M.W. and A.M. Olsen. 2015. How fish power suction feeding. Proc Natl Acad Sci 112:8525–8526.

Wintzer A.P. and P.J. Motta. 2004. The effects of temperature on prey-capture kinematics of the bluegill (*Lepomis macrochirus*): implications for feeding studies. Can J Zool 82:794–799.

Wu R.S.S. 2002. Hypoxia: From molecular responses to ecosystem responses. Mar Pollut Bull 45:35–45.

Wulff T., A. Jokumsen, P. Højrup, and F. Jessen. 2012. Time-dependent changes in protein expression in rainbow trout muscle following hypoxia. J Proteomics 75:2342–2351.

Zhu C.D., Z.H. Wang, and B. Yan. 2013. Strategies for hypoxia adaptation in fish species: A review. J Comp Physiol B 183:1005–1013.

Curriculum Vitae

KRISTA KRASKURA
Dept. of Biological Sciences
Towson University
Towson, MD 21252, USA
[redacted]

EDUCATION

MS Biological Sciences, Towson University – Towson, MD
Expected Thesis Defense date: July 2017 current GPA 3.9
Advisor: Dr. Jay A. Nelson

BS Marine and Environmental Sciences, Hampton University – Hampton, VA
Graduation Date: May 2015 GPA 3.8/4.1 (3.9 major GPA)
Advisors: Dr. Benjamin Cuker, Dr. Deidre Gibson, Dr. Andrij Z. Horodysky.

High School Degree, Murjani Sport Gymnasium - Murjani, Latvia
Graduation date: May 2011

COURSEWORK

Biometry + Lab
Biology + Lab
Botany + Lab
Zoology + Lab
Geology + Lab
Env. Sciences + Lab
Oceanography + Lab
Organic Chemistry + Lab
General Chemistry + Lab
Physics + Lab
Environmental Ecol.
Advanced Physiology
Gen. and Cell. Physiology
Mechanisms in Physiology
Ecol. and Evol. Physiology
Marine and Env. Biotech.
Gene Expr. and Regul.

TECHNICAL SKILLS

-Fish physiology lab (fish rearing, tagging, fish physiological & behavioral studies)
-Biometry (biological statistics/data analysis)
-Computer skills (R-CRAN statistics programming language)
-Environmental Science (YSI, photometer, fluorometer, forestry, energy conservation)
-General Chemistry and Qualitative analysis (titration, calorimetry, quantum mechanics)
-Organic Chemistry (distillation, filtration, recrystallization, spectroscopy)
-Oceanography research experience (pelagic plankton study)

	-Molecular techniques (DNA cloning, PCR, gel electrophoresis)	
PERSONAL SKILLS	-Leadership/ Teamwork/ Communication -Time Management/ Planning and Organizing -Analytical Thinking and Investigation -Flexibility	
HONORS/ AWARDS	Wilfred B. Hathaway Award for the Outstanding Graduate Students in the Biological Sciences, Towson University	04/2017
	Towson Graduate Student Association (GSA) Research award ($500)	2016/17 acad. yr
	Towson Graduate Student Association (GSA) Travel award ($500)	2016/17 acad. yr
	Student travel award participating 12th International Congress on the Biology of Fish (ICBF)	06/2016
	Towson GSA Travel award ($500)	2015/16 acad. yr
	Towson GSA Research award ($500)	2015/16 acad. yr
	Member of Chi Alpha Sigma, The National College Athlete Honor Society	2014 – 2015
	Hampton Univ. Volleyball Team Captain	2013 – 2015
	Hampton Univ. Athletic Academic achievement awards	2012 – 2015
	Hampton Univ. Dean's List	2011 – 2015
	Hampton Univ. Athletic Scholarship	2011 – 2015
	Murjani Sport Gymnasium Alumni Society Scholarship	2011 – 2012
EXPERIENCE	TA at Towson University; Human Anatomy and Physiology Lab (BIOL 221L)	08/2015 – 05/2017
	Intern, Institute of Marine Environmental Technology (IMET) Crustacean endocrinology lab under Dr. Chung. J. Sook	05/2014 – 08/2014

	NOAA – Sea Grant Sustainable Fisheries and Aquaculture Ambassador. Built and maintained sustainable aquaponics systems. Hosted hands-on workshops educating and organizing local communities (Hampton, VA)	10/2013 – 05/2015
	Intern at the Virginia Seafood Agriculture Research and Extension Center (VSAREC). Performed aquaculture system maintenance and regulated method compliance regarding scientific research. Under supervision of Dr. Michael L. Jahncke and Dr. Michael H. Schwarz	05/2013 – 08/2013
	Manuscript reviewer for journal "Fish Physiology and Biochemistry"	11/2016
PUBLICATIONS	Kraskura, K. and J. A. Nelson. Hypoxia and sprint swimming performance of juvenile striped bass, *Morone saxatilis.* In review.	
PRESENTATIONS	Kraskura, K. * and J. A. Nelson. Sprint, fast start and prey capture performance of juvenile striped bass under levels of hypoxia encountered in nature. Society of Integrative and Comparative Biology (SICB) Annual Meeting New Orleans, Louisiana. Oral presentation.	01/2017
	Respiratory system. Guest lecture at Towson University in Animal Physiology (BIOL 325)	10/2016
	Kraskura, K.* Hypoxia diminishes sprint swimming performance in juvenile striped bass. 12th International Congress on the Biology of Fish (ICBF), San Marcos, Texas. Oral presentation.	06/2016
	Bennington, R., K. Kraskura*, J. A. Nelson, C. E. Oufiero, and K. Ricci. Repeatability and kinematics of gymnotiform swimming performance in the black ghost knifefish. International Congress on the Biology of Fish (ICBF), San Marcos, Texas. Poster.	06/2016

Kraskura, K.*, J. A. Nelson, C. E. Oufiero, and K. Ricci. Allometry and repeatability of gymnotiform swimming performance in black ghost knifefish (*Apteronotus albifrons*). Society of Integrative and Comparative Biology (SICB) Annual Meeting Portland, Oregon. Poster. 01/2016

Kraskura, K.*, M. H. Schwarz, S. Urick, M. L. Jahncke, and Z. A. Horodysky. Effects of feeding rate and feeding frequency on growth of juvenile tilapia (*Oreochromis niloticus/O.aureus*). Ocean Sciences Meeting Honolulu, Hawaii. Poster. 02/2014

REFERENCES

Jay A. Nelson, PhD
Professor
Department of Biological Sciences
Towson University
Towson, MD 21252
Phone: (410) 704-3945
Email: jnelson@towson.edu

Christopher E. Oufiero, PhD
Assistant Professor
Department of Biological Sciences
Towson University
Towson, MD 21252
Phone: (410) 704-3743
Email: coufiero@towson.edu

Andrij Z. Horodysky, PhD
Assistant Professor
Department of Marine Science and Environmental Sciences
Hampton University
Hampton, VA 23668
Phone: (757) 728-6655
Email: andrij.horodysky@hamptonu.edu