

Repeatability of Hypoxia Tolerance of Individual Juvenile Striped Bass *Morone saxatilis* and Effects of Social Status

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ABSTRACT

Chesapeake Bay is the primary nursery for striped bass (*Morone saxatilis*), which are increasingly being exposed to hypoxic waters. Tolerance to hypoxia in fish is generally determined by a single exposure of an isolated individual or by exposing large groups of conspecifics to hypoxia without regard to social status. The importance of social context in determining physiological responses to stressors is being increasingly recognized. To determine whether social interactions influence hypoxia tolerance (HT) in striped bass, loss of equilibrium HT was assessed in the same fish while manipulating the social environment around it. Small group settings were used to be more representative of the normal sociality experienced by this species than the paired encounters typically used. After establishing the dominance hierarchy within a group of fish, HT was determined collectively for the individuals in that group, and then new groups were constructed from the same pool of fish. Individuals could then be followed across multiple settings for both repeatability of HT and hierarchy position ($\bar{X} = 4.2 \pm 0.91$ SD groups per individual). HT increased with repeated exposures to hypoxia ($P < 0.001$), with a significant increase by a third exposure ($P = 0.004$). Despite this changing HT, rank order of HT was significantly repeatable across trials for 6 mo ($P = 0.012$). Social status was significantly repeatable across trials of different group composition ($P = 0.02$) and unrelated to growth rate but affected HT weakly in a complex interaction with size. Final HT was significantly correlated with blood [hemoglobin] and hematocrit. The repeatability and large intraspecific variance of HT in juvenile striped bass suggest that HT is potentially an important determinant of Darwinian fitness in an increasingly hypoxic Chesapeake Bay.

Introduction

Hypoxia in Chesapeake Bay has dramatically increased in recent years as a result of cultural eutrophication and climate change (Diaz and Breitburg 2009; Breitburg et al. 2018). Unfortunately, Chesapeake Bay is the primary nursery for the culturally and commercially valuable striped bass *Morone saxatilis* (Walbaum, 1792), with up to 90% of the Atlantic Ocean's striped bass population originating there (Berggren and Lieberman 1978). Juveniles of this species frequent hypolimnetic regions of Chesapeake Bay that are hypoxia prone (Setzler-Hamilton et al. 1981; Breitburg 2002). These hypoxic zones are not static and can be driven into normoxic waters by winds and tidal currents that create seiches (Breitburg 1990, 1992); these can overwhelm a fish's first line of hypoxia defense, behavioral escape. Fish exposed to these rapid environmental O₂ depletions sometimes die (Rice et al. 2013) but otherwise will have to function in hypoxic water. Death results in an immediate loss of Darwinian fitness, but less severe hypoxia exposure can also have fitness consequences (Domenici et al. 2012). As the water [O₂] decreases, the difference between an animal's maximum metabolic rate and resting routine metabolic rate (metabolic scope) often decreases (Claireaux et al. 2000), thereby limiting an animal's capacity to engage aerobically in metabolically costly activities (Claireaux and Chabot 2016). If an animal exceeds its metabolic scope, energy demand must be met with some combination of metabolic arrest and/or anaerobic metabolism. Thus, oxygen scarcity has been associated with a number of potential fitness-reducing outcomes, such as diminished swimming capacity, reduced response to stimuli, behavioral abnormalities, slower growth, and immune system compromise (Burt et al. 2012; Domenici et al. 2012). Lapointe et al. (2014) showed that striped bass infected with a common bacterium have a higher critical oxygen tension (PO_{2crit}; the point at which they cannot meet their energy needs through aerobic metabolism; Claireaux and Chabot 2016) and greater loss of metabolic scope than uninfected fish. Such interactions between immune and respiratory systems in hypoxic waters could further affect fitness. If fitness depends on the ability to perform in hypoxic waters, measuring this ability will be important for predicting the future success of this species in its native range.

Since social status can be a chronic stressor with a multitude of physiological consequences for fish (Gilmour et al. 2005), understanding hypoxia's effects on striped bass will also require

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understanding how status and hypoxia tolerance (HT) interface in this social species. Compared to dominant fish, subordinates tend to be immunosuppressed, exhibiting increased susceptibility to infection (Peters et al. 1988) and toxicants (Sloman 2007). Subordinates also tend to have slower growth rates and increased levels of chronic stress biomarkers (Gilmour et al. 2005). In dominant fish, cortisol and catecholamine levels elevated by social encounters generally return to baseline after a social hierarchy is established (Thomas and Gilmour 2006). In contrast, cortisol levels in subordinate fish can remain elevated for up to 7 d following social encounters (Øverli et al. 1999; Sloman et al. 2000). While cortisol release is part of the animal's defense against environmental challenges and can be considered adaptive (Barton 2002), persistent elevation of cortisol can have negative consequences for metabolism, immune function, growth, and reproduction (Pickering and Pottinger 1995). Subordinate social status has also been shown to cause atypical mobilization of and abnormal response to catecholamines in rainbow trout (Thomas and Gilmour 2006, 2012). This chronic stress of being subordinate is thought to dampen a fish's ability to respond to an additional acute stressor such as hypoxia (Gilmour et al. 2005; Thomas and Gilmour 2012). In contrast, one of the more consistent findings is that dominant individuals have higher routine metabolic rates (e.g., Metcalfe et al. 1995, 2016; Kochhann et al. 2015), which could reduce their HT because of increased oxygen demand.

This study was designed to examine the null hypothesis that there is no effect of social status on HT of juvenile striped bass repetitively exposed to hypoxia. This was accomplished by following an individual's HT through multiple identical hypoxia exposures while manipulating the social environment around it. Social stress is typically studied with intraspecific pairs of fish in weakly structured environments (Sloman and Armstrong 2002). While this may force dominance hierarchy interactions, it is far from realistic for a shoaling species like striped bass (Setzler-Hamilton et al. 1981; Fay et al. 1983) and does not afford the opportunity to examine social positions other than dominant or subordinate. Wild juvenile striped bass are generally found in shoals (Westin and Rogers 1978), and shoaling species can be stressed by isolation (Nadler et al. 2016), which may mean that physiological responses to stress in striped bass would be less severe in group than in paired experiments. Interestingly, Ferrari et al. (2015) examined a number of behavioral traits in the co-familial European sea bass (*Dicentrarchus labrax*), which is also gregarious as a juvenile, and found that traits determined in group settings were more repeatable than traits determined individually or in pairs. HT is generally determined either on isolated fish (e.g., Snyder et al. 2016) or on large aggregations of fish at densities higher than would be found in nature, usually without time for social interactions to become established (e.g., Zambonino-Infante et al. 2013). Since sociality can modify oxygen preference in fish (Borowiec et al. 2018), it might also prove important in determining HT. To test this, we exposed the same juvenile striped bass (*Morone saxatilis*) to hypoxia while a member of variously constructed social groups (≥ 5 individuals per group) and report on repeatability of HT across social setting as well as repeatability of an individual's position in multiple dominance hierarchies.

Methods

Fish Handling

Wild young of the year striped bass were extremely rare at this point in time, so the experiment was limited to 20 individuals with an initial total length of 112–183 mm collected by the Maryland Department of Natural Resources trawl survey. The fish were captured at $\sim 4^{\circ}\text{C}$ and $\sim 10\text{‰}$ and brought to $20.1^{\circ} \pm 1.0^{\circ}\text{C}$ (mean \pm SD) by increasing the water temperature $\sim 2^{\circ}\text{C}$ per day and were also gradually changed over to an artificial brackish water solution of Crystal Sea Marine Mix in dechlorinated Baltimore City tap water ($9.2\text{‰} \pm 1.2\text{‰}$; mean \pm SD). The fish were not sexually dimorphic or sexed. When not being experimented on, the fish were held in a 355-L tank with a 12L:12D photoperiod and fed a mix of pellet (Hikari) and flake food (Aquarian) to satiation at least five times weekly.

After 4 mo of acclimation to the laboratory and recovery from capture, fish were anesthetized ($\text{MS-222 } 100 \text{ mg L}^{-1}$, buffered), weighed, measured, and then placed on an operating table with continuous anesthesia ($\text{MS-222 } 75 \text{ mg L}^{-1}$, buffered). There they were individually marked, with a passive integrated transponder (PIT; Biomark) injected into their abdominal cavity and a small external color bead sewn to the base of their dorsal fin to facilitate identification during behavioral observations. A cocktail of commercially available antibiotics (penicillin, cephalixin, amoxicillin, and ampicillin) was applied topically to the wound sites after tagging. All individuals were allowed a minimum 1-wk recovery from tagging before any hypoxia or dominance hierarchy testing began.

HT was tested on a complete group of 20 fish before any behavioral trials, under four different subgroup compositions after a dominance hierarchy had been established within that group (table 1), and on a complete group of 13 fish at the end of the experiment, 6 mo after the first hypoxia exposure. The subgroup compositions are described in table 1; however, the maximum size disparity group (MGT) requires some additional description. For this trial, the fish were ranked by size and divided into three size categories, and then two individuals were randomly selected from within each size category. Fish were weighed and measured after each HT trial. The order for subgroup trials was randomly determined by drawing numbers from a hat. All trials were conducted twice, with the exception of the maximum size disparity subgroup (no MGT II). Fish that did not appear healthy were removed from the experiment, reducing the number from 20 in the first group trial to 13 in the final group trial 6 mo later. The number of individuals in a subgroup trial was either five or six (table 1).

Determination of Social Status

Dominance trials were conducted in a 190-L opaque tank at an average temperature of $20.9^{\circ} \pm 0.9^{\circ}\text{C}$ and salinity of $9.4\text{‰} \pm 1.1\text{‰}$. Individuals were transferred to this tank, without air exposure, 5 d before the hypoxia challenge test (HCT). Dominance hierarchies in fish generally establish within a day (Kalyvas 2011). The group was then fasted the day following

Table 1: Summary of hypoxia tolerance trials

Trial	<i>n</i>	Days post 1st hypoxia	Mean mass (g)	Mean exposure time (min)
All fish I	20	0	25.46 ± 8.24	52.11 ± 44.61
RGT I	6	16	28.62 ± 12.55	243.99 ± 133.49
SGT I	6	29	20.13 ± 3.95	83.80 ± 63.53
MGT I	6	45	30.55 ± 6.44	176.74 ± 166.27
LGT I	6	63	42.28 ± 12.43	65.54 ± 40.50
SGT II	5	108	34.22 ± 12.01	222.62 ± 125.64
RGT II	5	137	43.98 ± 25.03	231.36 ± 119.62
LGT II	6	153	67.52 ± 13.91	384.11 ± 36.15
All fish II	13	174	62.53 ± 17.86	314.82 ± 96.84

Note. Values are means ± SD. The mean length of exposure was measured as time elapsed from 10% air saturation to when the average fish lost equilibrium. RGT = random group trial (group of five or six randomly selected individuals); SGT = small group trial (group of five or six smallest individuals); MGT = mixed group trial (group of two small, two large, and two medium-size fish); LGT = large group trial (group of the six largest individuals).

transfer to the experimental tank to ensure they would feed well during behavioral observations, which were then conducted on three consecutive days following the day of fasting. Fish were observed and video recorded for 35 min for each of the observational periods. During these periods, the first 5 min were used to record any territorial behavior before the introduction of food. Subsequently, a single pellet of food was introduced to the tank every minute for 30 min. Each time a fish took a pellet, they were assigned a point. These points and the behavioral observations were used to assign ranks to individuals within each subgroup (Metcalf et al. 1989). All video recordings were watched twice to ensure that points were properly assigned. HCTs were then conducted 24 h after the third feeding/dominance trial without further feeding in the same tank to avoid further disturbance to the animals.

Hypoxia Challenges

For all hypoxia challenges, the fish had been in the experimental tank at least 5 d and transferred there without air exposure. HCTs were initiated by lowering the oxygen concentration to 10% of air saturation (AS) over ~30 min (30.1 ± 1.1 min) by bubbling in nitrogen gas. Oxygen concentration decreased as an exponential function, with an average instantaneous slope of $-3.01\% \pm 0.38\% \text{ AS min}^{-1}$. Two galvanic oxygen-sensing probes were used to determine the level of AS in the experimental tank. The probes were calibrated before each trial. One probe was connected through a digital converter box to a solenoid valve attached to an air stone, which maintained dissolved oxygen saturation at the desired level (Oxy-Reg System, Loligo Systems). A transparent, one-way mirrored Plexiglas covered the hypoxia challenge tank during the trials to aid in maintaining 10% AS while allowing visibility of the fish and reducing visual disturbances for the fish. Dissolved oxygen saturation was maintained at 10% AS until an animal lost equilibrium (first overturn), signaling incipient mortality. If all animals had not lost equilibrium after 4 h at 10% AS, further decrements of 2% AS every hour were employed until all individuals had lost equilibrium. HT was analyzed as both the duration and severity of

hypoxia exposure or cumulative oxygen deficit (Nelson and Lipkey 2015). Briefly, if oxygen saturation is plotted as a function of time, cumulative oxygen deficit is the difference between the area under the curves of a hypothetical animal remaining at 100% AS throughout the experiment and the experimental animal's actual oxygen exposure until the time that it lost equilibrium, measured in units of time multiplied by percent saturation. Once an individual lost equilibrium, they were removed, measured, weighed, and transferred to a normoxic recovery tank as quickly as possible. No fish died as a result of an HCT, but several fish fell ill during the course of the 6 mo of regular hypoxia exposure, dominance determinations, and handling; they were removed from the experiment at the first overt sign of illness.

Blood Chemistry

Following the last HCT (second entire group trial), blood samples were taken from rapidly anesthetized ($0.42 \text{ g L}^{-1} \text{ MS-222}$) fish by cardiac puncture of exposed hearts using heparinized syringes that were immediately placed on ice until assayed. Hematocrit and hemoglobin concentrations were tested the same day. Hematocrit was determined after centrifugation at $13,000 \times g$ in capillary tubes. When sufficient blood was available, replicate hematocrit readings were taken and averaged. Hemoglobin concentration was measured using the cyanomethemoglobin method. Blood samples were added to Drabkin's reagent (Sigma-Aldrich, St. Louis) and compared to hemoglobin standards (Pointe Scientific, Canton, MI). Optical density was recorded at 540 nm in a Spectronic (Westbury, NY) Genesys 5 spectrophotometer. For erythrocyte counts, blood was diluted 1:50 in modified Dacie's solution and counted by eye in an improved Neubauer counting chamber. Replicate counts of 1 mm^2 were used to calculate the mean erythrocyte number for each individual.

For the determination of blood lactate concentration, aliquots of whole blood (0.1 mL) were deproteinized in 0.6 mol L^{-1} perchloric acid (0.9 mL), and the extracts were centrifuged at $12,700 \times g$ for 5 min at 4°C . Acidic supernatants were neutralized with the appropriate amount of KOH determined in a previous titration and then stored frozen until assayed for

lactate, according to Bergmeyer and Bernt (1974). All chemicals were purchased from Sigma-Aldrich, made fresh the day of the assay, and filtered before using.

Numerical and Statistical Analyses

Statistical analyses were done with SPSS, Statistica, or R, version 3.4.2, with a significance level of 0.05. Growth rate (G) was calculated for each individual at each hypoxia trial using the equation

$$G = ((M_f - M_i)(M_i^{-1}))d_f^{-1},$$

where M_f is the mass of the fish measured at the end of each trial, M_i is the initial mass of the fish measured at tagging, and d_f is the number of days between M_i and M_f . Because HT in these juvenile striped bass increased with repeated exposure, nonparametric analyses by rank were required for many relationships. Spearman's correlation analysis was used to examine the relationship between dominance rank and growth rate. Since individual trials had varying sample sizes ($n = 5$ or 6 ; table 1), ranks were scaled to the original sample size of 20 for nonparametric comparisons. For example, in trials of five individuals, the most resistant fish was ranked 1, second-most resistant 5.75, third-most resistant 10.5, fourth-most resistant 15.25, least resistant 20, and so forth. Kendall concordance was used to show repeatability of dominance rank and HT of individuals throughout the experiment. Cumulative link mixed models (CLMMs; `clmm` function in the R package `ordinal`; Christensen 2018) were used to test our null hypothesis that HT rank is not predicted by social dominance rank within each trial. The following models were constructed: HT rank as a function of (1) social dominance rank, (2) social dominance rank and fish mass, and (3) social dominance rank with a body mass interaction. Individual was included as a random effect in all models to account for the random repeated measures design across group trials. Models decreasing in complexity were pairwise compared with a likelihood ratio (LR) test using the χ^2 test statistic (table 2); this approach evaluated the significance of adding body weight as an explanatory variable and the interaction between social rank and body mass (i.e., analysis of deviance; Bolker et al. 2009; Christensen 2015; Mangiafico 2016). We also used CLMM to test for the probability that an individual's social rank is influenced by its HT rank or its size. The main effects of HT rank and body weight (g) on group social rank were tested separately because of their significant covaria-

tion (Pearson's $r = 0.56$, $P < 0.001$). We tested whether social group was a significant main factor as well as its interaction with mass (group \times mass). The LR test was again used here to estimate the significance of the main effects (table 2). Finally, least squares regression analysis was used to explore the relationship of HT in the final group hypoxia exposure with hematocrit, hemoglobin, lactate concentration, and other red blood cell parameters. This experiment complied with all Towson University animal care regulations and guidelines (Institutional Animal Care and Use Committee no. 102510 JN-11).

Results

Hypoxia Tolerance

HT was extremely variable in this group of juvenile striped bass and increased with repetitive hypoxia exposures. These results can be seen most dramatically by comparing the two HCTs for all fish at the beginning and end of the 6-mo experiment (fig. 1A). While substantial intraspecific variance remained, the average fish saw a more than seven-fold increase in its HT when it was assessed as both severity and time of exposure until loss of equilibrium (LOE). The average fish saw its HT increase from $3,927.5 \pm 864.9$ SE percent \times minutes at the first group hypoxia exposure to $29,969.8 \pm 2,367.5$ percent \times minutes at the final exposure (fig. 1A). The coefficient of variation in individual HT dropped from 101% in the first complete group trial ($n = 20$) to 29.6% in the last complete group trial ($n = 13$; fig. 1A). This large variance in HT was despite the fish being very similar in size and captured at the same time and place. Fish that completed the experiment had an average of 4.3 ± 0.24 SE hypoxia exposures over 6 mo that improved their average HT 7.6-fold and reduced variation in HT by 70%. Most fish followed a similar path of developing increased HT (fig. 1B). A Friedman test showed a statistically significant difference in HT depending on the number of times an individual was exposed to hypoxia ($\chi^2 = 24.7$, $df = 3$, $P < 0.001$). A post hoc Wilcoxon signed ranks test with Bonferroni correction (significance $\alpha = 0.008$) showed HT was significantly higher after a third exposure to low oxygen concentrations ($P = 0.004$).

Despite these large changes in absolute HT with repeated hypoxia exposure, the rank order of HT was significantly repeatable across trials for the individuals that participated in at least four trials (Kendall's coefficient of concordance; $W = 0.53$, $df = 12$, $P = 0.012$; fig. 2); that is, the most hypoxia-tolerant

Table 2: Constructed statistical models (cumulative link mixed models) to determine the significance of explanatory variables

Models	LR statistic	df	<i>P</i> value
Null model: social rank ~ 1			
Random effect model: social rank $\sim 1 + (1 ID)$	10.24	1	.001
Random effect model with weight: social rank $\sim W + (1 ID)$	1.86	1	.172
Random effect model with HT rank: social rank $\sim HT \text{ rank} + (1 ID)$.81	1	.195

Note. Presented are the results of likelihood ratio (LR) tests. Every two models with increased complexity were statistically compared (i.e., addition of an explanatory variable, random, or fixed effect). The term (1 | ID) is a random effect. It accounts for repeated measures at an individual fish level. Only addition of the random individual effect was significant (in boldface). The addition of fixed effects, weight (W), and hypoxia tolerance (HT) rank were evaluated separately, but both were insignificant variables in explaining the social rank position of an individual fish.

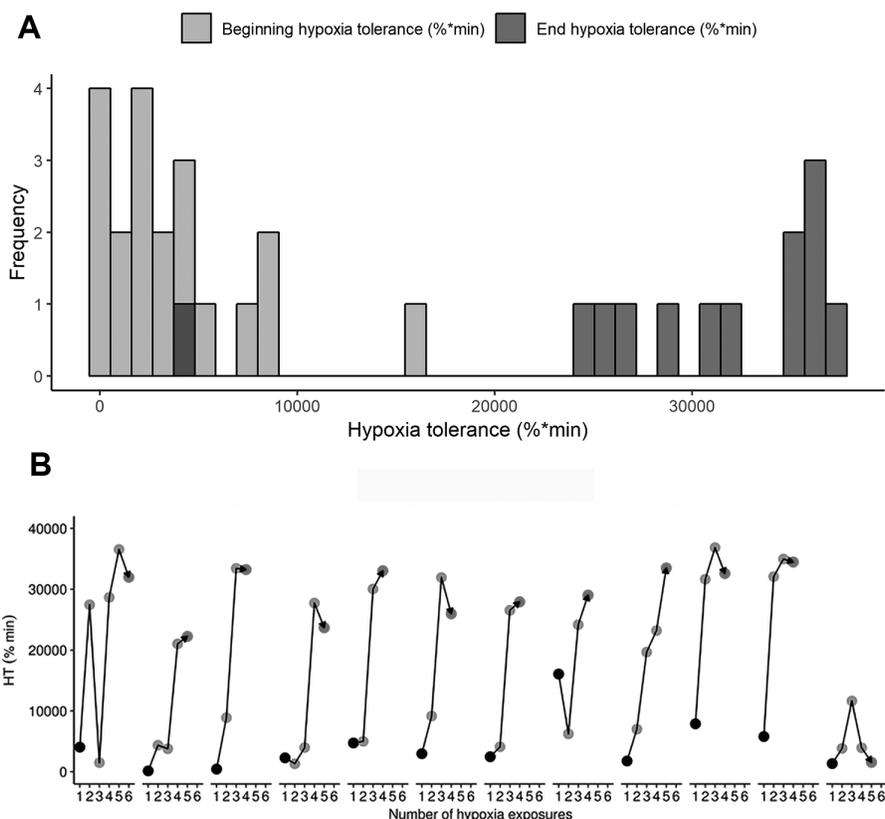


Figure 1. A, Histogram of individual hypoxia tolerances (HTs), measured as cumulative oxygen deficit, a measure of both length and severity of hypoxia exposure (see text). Results are shown for the two hypoxia challenge tests conducted with all of the fish together, at the beginning of the experiment ($n = 20$; light bars), and at the very end ($n = 13$; dark bars) 6 mo later. Hypoxia was established by decreasing oxygen saturation to 10% air saturation (AS) over ~ 30 min, where it was held for 4 h, followed by subsequent decrements of 2% AS per hour when necessary. The mean initial tolerance was a cumulative oxygen deficit of $3,927.5 \pm 864.9$ SE percent \times minutes, whereas the mean tolerance at the end of the experiment was $29,969.8 \pm 2,367.5$ percent \times minutes, or 7.6-fold greater. B, Changing HT of individual fish over time for those fish that had at least four hypoxia exposures. Cumulative number of exposures for each individual is plotted on the abscissa, and HT in percent \times minutes is plotted on the ordinate. Black circles indicate the first trial with all of the fish together, and medium gray circles indicate the final trial with all of the fish together. Intervening subgroup trials are plotted as light gray circles. The average time between hypoxia exposures was 46.5 ± 12.1 SD d. A color version of this figure is available online.

fish in the first trial tended to remain the most tolerant in subsequent trials despite all fish increasing in tolerance (this is readily observed in fig. 2). Traditional statistics returned no significant effect of size or growth rate on HT across the whole experiment or within any of the subgroup trials (Kruskal-Wallis, $P > 0.5$; Pearson's, $P > 0.05$ for all subgroups; fig. 3), but the CLMMs delivered a slightly more nuanced verdict (see below). Figure 3 presents mass, dominance rank, and HT for each of the subgroup trials.

Social Status

The dominance rank of an individual was consistent across trials for fish that participated in at least four trials regardless of group structure (Kendall's concordance coefficient; $W = 0.866$, $df = 5$, $P = 0.02$). This meant that an individual's dominance rank was generally repeatable despite a constantly changing cast of

conspecifics. The effect of social dominance on individual HT was complex and underwhelming. For a couple of the small group trials, both dominance and HT correlated positively but insignificantly with size ($n =$ only 5 or 6), but for the remainder of the trials, both appeared to vary independently of each other and size (fig. 3). When examining the effect of social hierarchy position on an individual's HT rank within a group statistically, including mass as a main effect in the CLMM improved the fit (mass as a main effect: LR statistic = 3.13, $P = 0.077$), and including its interaction with social rank significantly increased the fit further (social rank \times W : LR statistic = 19.08, $P = 0.0018$). The β coefficients of CLMM did not follow a consistent trend, for example, from the least to most dominant fish, but there was a trend for a higher-weight fish that was more socially dominant to then also have a higher HT. Overall, individuals with higher social status were not more likely to have a higher tolerance to hypoxia, and body size interacted in a complex way to determine

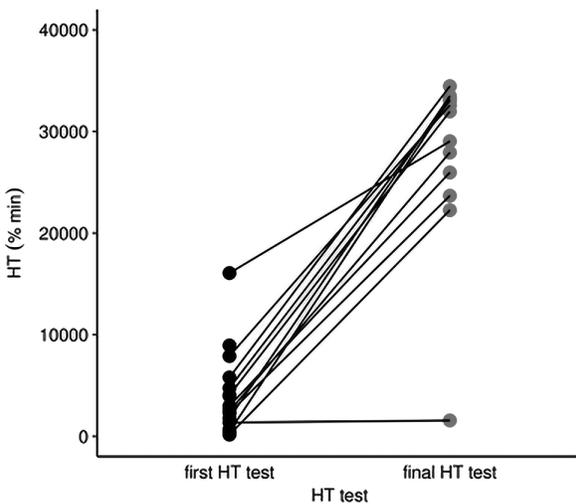


Figure 2. Individual hypoxia tolerance (HT) in the first and last hypoxia challenge tests (HCTs) for those fish that completed at least four HCTs demonstrating relative rank order preservation. For this small group of fish, HT was significantly repeatable across four trials (Kendall's coefficient of concordance; $W = 0.53$, $df = 12$, $P = 0.012$). A color version of this figure is available online.

that individual's HT at a given position in the social hierarchy (fig. 3). Most of the variation in HT was explained by the individual term in all of the models (table 2). When exploring the converse, that is, whether larger individuals or individuals with greater HT were more likely to place higher in the social hierarchy, CLMM indicated that neither was true (table 2). Individuals with higher relative HT or greater mass were not significantly more likely to have a higher social rank in a given group. CLMMs indicated that the social rank of each individual was not strongly predicted by either body mass (CLMM: $\hat{\beta}$ coefficient = -0.0265) or growth rate (Spearman's rank correlation; $\rho = -0.25$, $df = 14$, $P = 0.39$). Only the two small group trials (SGTs) had a significant correlation between size and dominance (SGT I: Pearson's $r = -0.860$, $P = 0.028$, $n = 6$; SGT II: $r = -0.909$, $P = 0.033$, $n = 5$), although these animals were of the same age and not that disparate in size (table 1).

Blood Chemistry

The results for the hematologic analyses are summarized in table 3. There was a significant positive relationship between both blood hematocrit ($P < 0.001$) and hemoglobin concentration ($P = 0.03$) at the end of the final HCT, with the HT measured in that final test as cumulative oxygen deficit (fig. 4). No other measured blood variable correlated significantly with HT in that test (table 3).

Discussion

HT was extremely variable in this cohort of juvenile striped bass but significantly repeatable despite an average seven-fold increase in HT over approximately four hypoxia exposures span-

ning 6 mo. Since juvenile striped bass in Chesapeake Bay are unlikely to always avoid hypoxia, they will have to respond to it with combinations of physiological regulation and phenotypic plasticity. The general initial physiological response to hypoxia is to defend arterial $[O_2]$ by increasing gill ventilation (Holeton 1980; Nelson et al. 2007) and to reduce heart rate while increasing cardiac stroke volume (Gamperl 2011). Since fishes expend $\sim 10\%$ of their resting metabolic rate in ventilating their gills (Glass and Rantin 2009), as the water PO_2 drops, this energy expenditure eventually costs more than the added metabolic efficiency that extracted O_2 brings, and the animal reaches its PO_{2crit} (Claireaux and Chabot 2016). At this point, the animal can no longer maintain its routine metabolic rate aerobically and must either use anaerobic metabolism, metabolic arrest, selective tissue perfusion, surface skimming, or some combination of these to survive. Therefore, oxygen consumption falls with further declines in environmental PO_2 , and if the fish cannot find more oxygenated waters, it will eventually be unable to maintain its equilibrium (LOE), which is indicative of incipient mortality. The actual water PO_2 at which PO_{2crit} or LOE happen is species dependent and can vary tremendously among individuals of a species, including striped bass (Lapointe et al. 2014; Nelson and Lipkey 2015). The PO_{2crit} can be a good indicator of HT as measured by LOE (Speers-Roesch et al. 2013; Rogers et al. 2016) but should not be used as a measure of HT (Wood 2018). As ours and others' results show, HT can be extremely variable on an intraspecific level (Nelson and Lipkey 2015; Rogers et al. 2016; Wood 2018). Since there are undoubtedly heritable components to this variability, it is likely to be a contributor to Darwinian fitness of fish in those many areas where hypoxia is on the rise.

Although this study did not try and assess heritability of HT, we did, for the first time, establish significant repeatability of HT across multiple hypoxia exposures and social situations in striped bass. Repeatability can be considered a potential indicator of heritability (Dohm 2002; Killen et al. 2016), and rank order of HT was significantly repeatable in those animals that completed at least four hypoxia challenges over a 6-mo period ($n = 13$, $P = 0.012$). Animals improved their HT along similar trajectories and thus maintained a rank similar to what they had at the beginning of the experiment (figs. 1B, 2). Many performance tests have been shown to be significantly repeatable over time in fish (e.g., Oufiero and Garland 2009; Nelson et al. 2015), but despite a recent surge in hypoxia studies, it is rare to find results from individuals that have undergone multiple tests. Claireaux et al. (2013) report significant repeatability of HT over 2 mo in the cofamiliar European sea bass (*Dicentrarchus labrax*), and Joyce et al. (2016) found significantly repeatable LOE HT in this species over 19 mo (four separate trials). Recently, the species list of fish shown to have repeatable HT has grown to include the gulf killifish (*Fundulus grandis*; Rees and Matute 2018) and the Atlantic croaker (*Micropogonias undulatus*; Pan et al. 2018). In addition to repeatability, a finding of epigenetic transfer of HT across a generation in fish (Ho and Burggren 2012) is suggestive of heritability of this trait.

Overlain on these results is the issue of whether metabolic rate itself is repeatable, since animals requiring more oxygen are

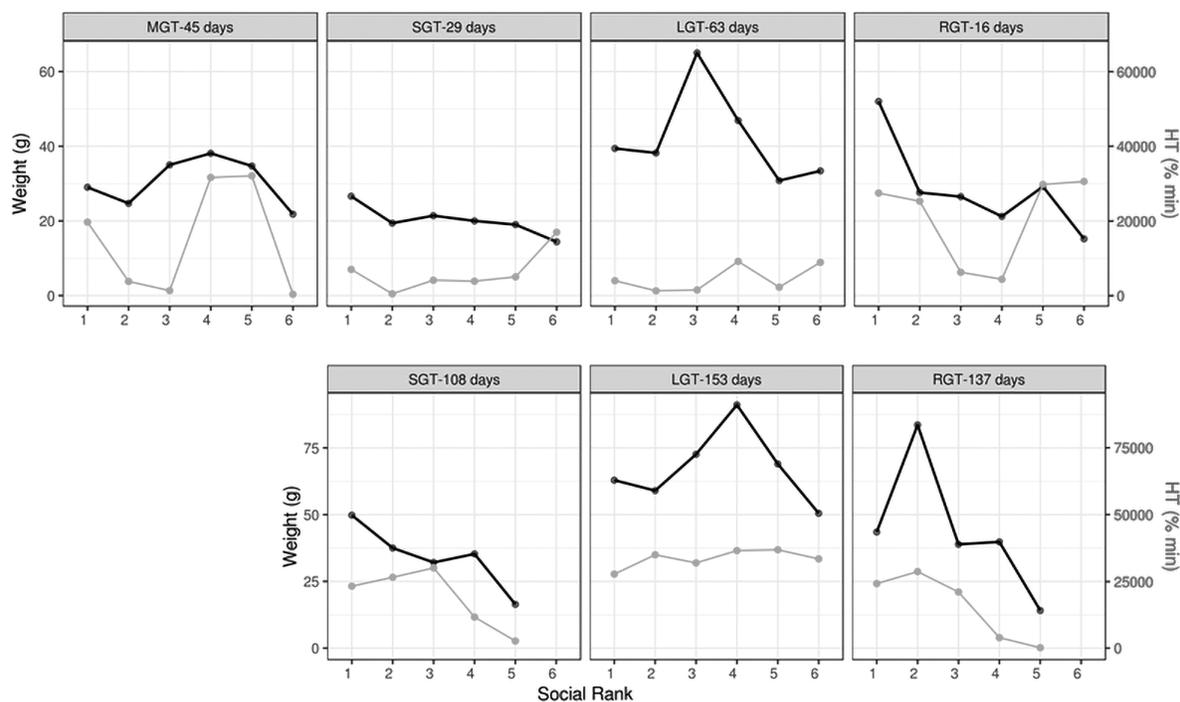


Figure 3. Mass and hypoxia tolerance as a function of social rank for each of the subgroup trials. Subgroup compositions are defined in the text and in the legend to table 1. For all plots, the social rank proceeds (left to right) from most (1) to least (5 or 6) dominant. A color version of this figure is available online.

predicted to be less tolerant of hypoxia. Norin et al. (2016) demonstrated repeatable standard and active oxygen consumption rates in barramundi (*Lates calcarifer*)—relevant to the present study because this repeatability was maintained across a hypoxia exposure. Reidy et al. (2000) reported significant 3-mo repeatability of oxygen consumption for Atlantic cod (*Gadus morhua*) swimming hard but not at lower levels of activity. Combining this result with those of Norin and Malte (2011) showing a decline in repeatability of both active and standard oxygen consumption rates from 5 to 15 wk in cultured brown trout (*Salmo trutta*) suggests that the individual metabolic response to human presence and/or laboratory residency may be important variables to consider when gauging HT and may contribute to the lack of reported measures of HT repeatability. In the present study, great care was taken to limit human contact leading up to the HT determinations. Since the significant repeatability of HT reported here was determined in static water with a small number of fish and HT in static water can be unrelated to HT while swimming (Nelson and Lipkey 2015), the repeatability of HT in swimming fish needs to be studied.

An average of approximately four hypoxia exposures over 6 mo increased individual HT in striped bass fairly uniformly (figs. 1B, 2). Phenotypically plastic responses to previous hypoxia exposure have been found in several other fish species and can change the value of both PO_{2crit} and HT (reviewed in Domenici et al. 2012). These changes in tolerance with previous

exposure and the lack of a phenotypically plastic response in some species (e.g., Brady and Targett 2010; Petersen and Gamperl 2010) may account for the few reports of HT repeatability. It is also possible that, unlike with striped bass, plastic responses to hypoxia vary by individual in some species and so are not repeatable. Of the factors considered here (individual, size, growth rate, and social status), individual was the statistically most important factor in determining HT, suggesting that individual

Table 3: Hematologic analysis following loss of equilibrium in the last hypoxia challenge test

	Mean	R^2	P value
Hct (%)	40.4 ± 13.1	.67	<.001*
[Hb] (g dL ⁻¹)	$9.5 \pm .4$.42	.031*
RBC count ($\times 10^6$ cells mm ⁻³)	$3.8 \pm .7$.19	.19
MCHC (kg cell $\times 10^{-14}$)	$2.6 \pm .5$.08	.39
[Lac] (mM L ⁻¹)	11.2 ± 4.6	.11	.311

Note. Hypoxia exposure consisted of 4 h at 10% air saturation (AS) followed by subsequent hourly decrements of 2% AS ($N = 11$). Values are mean \pm SD. P values indicate the significance level of the equation relating hypoxia tolerance to that variable. Hct = hematocrit (percent volume of a blood sample occupied by erythrocytes); [Hb] = hemoglobin concentration; RBC = erythrocyte count; MCHC = mean hemoglobin concentration per erythrocyte; [Lac] = plasma lactate concentration.

*Statistically significant linear regression.

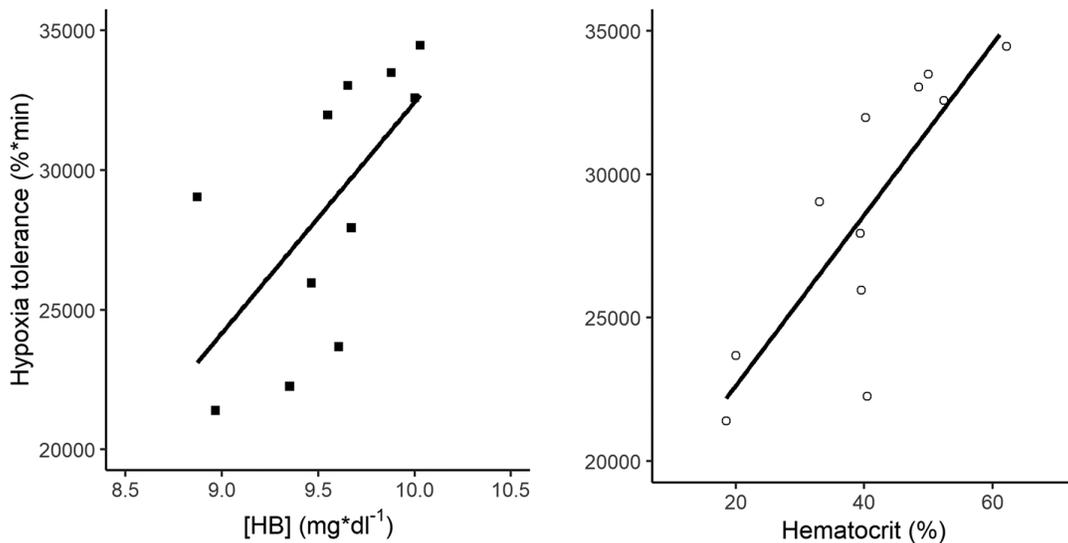


Figure 4. Hypoxia tolerance in the final hypoxia challenge test measured as cumulative oxygen deficit as a function of whole blood hemoglobin concentration ($P = 0.03$; *left*) and hematocrit ($P < 0.001$; *right*) in blood collected after loss of equilibrium (LOE) due to hypoxia exposure. Juvenile striped bass were exposed to 10% air saturation (AS) for the first 4 h, followed by subsequent hourly decrements of 2% AS until LOE occurred.

differences in physiology are the principal determinant of HT variance in striped bass, while phenotypic plasticity remains somewhat uniform.

Besides several blood chemistry variables (see below), the mechanism whereby juvenile striped bass increase their HT was not investigated. Studies from other species have implicated increased gill surface area as one of the key plastic traits that can improve HT (Timmerman and Chapman 2004; Fu et al. 2011). Cardiovascular changes in response to previous hypoxia exposure have also been reported, but whether they improve HT is debatable (Petersen and Gamperl 2010). Metabolically, increased anaerobic enzyme activity in the heart has been associated with increased tolerance in a tropical cichlid (Crocker et al. 2013) and a coastal sparid (Cook et al. 2013), as has increased anaerobic enzyme activity in type I skeletal muscle in the sparid (Cook et al. 2013). Interspecifically, comparisons have also implicated increased activity of anaerobic enzymes in the brain (Mandic et al. 2013), muscle (Davies et al. 2011), and heart (Borowiec et al. 2016) in differential HTs. Other studies have implicated increases in blood oxygen carrying capacity (Val et al. 1990) in the acclimation response. In the present study, blood samples were only taken once from animals at the end of the experiment under anesthesia. Nonetheless, the results reflect an advantage of having greater blood oxygen carrying capacity when challenged with hypoxia. The mean Hct of 40.4% and [Hb] of 9.5 g dL^{-1} following multiple hypoxia exposures were higher than the Hct of 33%–37% and [Hb] of 8 g dL^{-1} reported by Hopkins and Cech (1992) for hypoxia-naïve striped bass following net handling. Furthermore, both Hct and [Hb] correlated significantly with HT recorded moments before the blood sample was taken. The study of whether blood chemistry characteristics allow differential ex-

ploitation of hypoxic waters dates to a century ago (Krogh and Leitch 1919), yet the generality of this blood chemistry response to hypoxia remains unsettled. Numerous authors have reported either increased oxygen carrying capacity of the blood (e.g., Fu et al. 2011) or increased binding affinity of hemoglobin for oxygen (left shift; e.g., Rutjes et al. 2007). However, even within co-familars, one can find discrepancies. For example, one species of loricariid catfish (Siluriformes) exposed to hypoxia shows significantly higher [Hb] and smaller erythrocytes that contain more hemoglobin per erythrocyte than normoxic animals (Fernandes et al. 1999; Nelson et al. 2007), and Val et al. (1990) report a higher cell [Hb] in a second loricariid species exposed to hypoxia for 30 d or captured from hypoxia-prone habitats. Yet Weber et al. (1979) reported decreased cell [Hb] in other loricariids exposed to hypoxia for 4–7 d, suggesting that there may not be a generalized blood chemistry response to hypoxia even at the family level. Our results suggest that variance in blood oxygen transport is one determinant of HT in striped bass. This idea is also supported by experiments that show (1) hemoglobin oxygen affinity being correlated with HT in 13 sculpin species (Cottidae) that have evolved into habitats varying in oxygenation (Mandic et al. 2009) and (2) *in vivo* demonstration of greater erythrocyte recruitment in zebrafish (Cyprinidae) reared under hypoxic conditions (Schwerte et al. 2003). The lack of a significant relationship between erythrocyte number or mean erythrocyte [Hb] and HT in the present study is most likely indicative of the differential blood chemistry developing over the course of the 6-mo experiment and not simply being a differential response to the last HCT. Fish can respond to hypoxia by releasing immature erythrocytes from the spleen, but these tend to be smaller than mature erythrocytes (Murad et al. 1990), thus not concordant with our

data. The lack of a significant relationship between blood lactate concentration and HT in this small group of striped bass (table 3) would imply that anaerobic metabolic capacity and/or lactate processing are not related to whatever causes LOE and is concordant with other studies where LOE was used as an end point (e.g., Zambonino-Infante et al. 2013). Alternatively, since the more germane intracellular $[Lac^-]$ was not measured, our results may merely reflect differential lactate flux from tissues to the blood.

A key finding of this study was that significantly repeatable social status had only a weak influence on HT and that, conversely, HT did not influence social status. Social rank may cause physiological differences between individuals but could just as well result from those differences (Øverli et al. 2004). Current thought is that social status can be a strong predictor of physiological performance, but this idea has been largely developed from work done on pairs of primarily salmonid fishes (Sloman and Armstrong 2002). Low social status after paired interactions can have negative impacts on thermal (LeBlanc et al. 2011) and hypoxia (Thomas and Gilmour 2012) tolerance. Yet this study of striped bass in small groups found that an individual's social status only marginally influenced HT. This result emphasizes the importance of this experimental design focused on the individual. Two of the subgroups (SGT II and the second random group trial, RGT II) had correlations between social position and HT that may have led to different conclusions had fewer groupings been tested. One explanation for the weak dominance effect may be that the larger group size dampens the amount of aggression received by subordinates and increases the stress imposed on dominant fish to maintain rank, thus dispersing the consequences of social stress more evenly (Griffiths and Armstrong 1999). Supporting this interpretation is the fact that subordinates in the wild are rarely found to have chronically elevated stress hormone levels (Creel 2001), and in some cases, dominant organisms actually have higher stress hormone levels (Creel et al. 1996). Therefore, having dominance established and HT determined in groups rather than in pairs is one explanation for not finding the expected reduction in HT in subordinate striped bass (Gilmour et al. 2005). Interestingly, Ferrari et al. (2015) found several behavioral traits in the cofamilial European sea bass to be more repeatable when conducted in groups as opposed to individually, and Borowiec et al. (2018) found an African cichlid to be more likely to utilize hypoxic habitats if they contained conspecifics. Alternatively, the sequence of experimentation, the repeated hypoxia exposures, and the changing social situations may have habituated the fish to stress and blurred any effect of dominance. Reid et al. (1994) report desensitization of fish to stressors (i.e., chasing and fasting) after repeated exposure. In the present experiment, the average fish underwent four hypoxia exposures under at least three different social situations. Since the fish were exposed to hypoxia before any social experiments were carried out, it is entirely possible that the physiological remodeling initiated in response to the first hypoxia exposure obscured any dominance signal from subsequent experiments. Similarly, the striped bass could be exhibiting an attenuated neuroendocrine response (Reid et al. 1998) due to repetitive stress.

Dominance rank was significantly repeatable over multiple social situations in our study but was not unequivocally determined by size and did not correlate with growth rate. Individual was the most important factor determining both social status and HT (table 3). These results suggest that physiology and personality may be more determinant of dominance than size, albeit within a very narrow size range. This result may also further reflect the disparity between results generated from paired versus group social trials. Supporting this interpretation, Sloman et al. (2000) reported a higher growth rate in dominant fish from paired studies, yet Sloman and Armstrong (2002) report that relationship to be questionable when groups or more ecologically realistic conditions were studied.

In summary, HT in a small cohort of wild striped bass was a highly variable but significantly repeatable physiological trait despite being very plastic in response to hypoxia exposure. Individual differences in physiology determined the variance in HT, with differences in blood oxygen transport capacity being one of them. Position in multiple social hierarchies was a significantly repeatable trait, yet was marginally important in determining HT, as were both size and growth rate. Although HT while swimming needs to be measured, one can conclude from our results that HT is probably an important determinant of Darwinian fitness for striped bass in hypoxia-prone waters.

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