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FACTORS CONTRIBUTING TO THE INTRASPECIFIC VARIATION OF HYPOXIA TOLERANCE IN JUVENILE STRIPED BASS *(MORONE SAXATILIS)*

by
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THESIS APPROVAL PAGE

This is to certify that the thesis prepared by Genine Lipkey entitled Factors contributing to intraspecific variation of hypoxia tolerance in juvenile striped bass (*Morone saxatilis*), has been approved by her committee as satisfactory completion of the requirement for the degree of Master of Science.

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ABSTRACT

FACTORS THAT CONTRIBUTE TO THE INTRASPECIFIC VARIATION OF HYPOXIA TOLERANCE IN JUVENILE STRIPED BASS *(MORONE SAXATILIS)*

By Genine Lipkey

Hypoxia in coastal waters is a growing concern. Hypoxic zones in the Chesapeake Bay may pose a threat to the striped bass (*Morone saxatilis*) population. Hypoxia tolerance can be extremely variable among individual fish of similar size. How well an individual copes with hypoxia is determined by many factors. The purpose of this study is to investigate two factors thought to influence intraspecific variation in hypoxia tolerance: (1) locomotion and (2) social status. Individual striped bass were exposed to hypoxia individually and in groups. When exposed individually they were tested in both static flow (< 3 cm s^{-1}) and moderate flow conditions (50% estimated U_{crit}). When exposed in groups, behavioral observations were made before exposure to determine social status of individuals. Individuals were found to be much less tolerant of hypoxia when forced to swim in moderate flow conditions, and social status did not have an effect on hypoxia tolerance.

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CHAPTER 1: LOCOMOTION

Hypoxia tolerance of swimming juvenile striped bass *Morone saxatilis* (Walbum 1792)

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RH: HYPOXIA TOLERANCE OF SWIMMING *M. SAXATILIS*

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ABSTRACT

Juvenile striped bass *Morone saxatilis* were individually exposed to aquatic hypoxia (10% air saturation) under two flow regimes: static flow and moderate flow, adjusted to body size. Individuals were always less tolerant of hypoxia when swimming (Wilcoxon; $S = 45.5$, N = 13, $P < 0.05$), reducing the mean hypoxia tolerance from approximately 5 h in static conditions to 46 min in flow conditions. There was no relationship between an individual's hypoxia tolerance in static conditions and flow conditions (Spearman's Rank; $\rho = -0.29$, N = 13, $P = 0.34$), suggesting there are different factor(s) that determine an individual's hypoxia tolerance while at rest and swimming. For an active pelagic fish like *M. saxatilis*, assessing hypoxia tolerance in flow conditions may give a more realistic understanding of the species' ability to cope with and respond to hypoxia, and give environmental managers more realistic information to assess the impact of future dead zone expansions.

Key words: behaviour; escape response; energy saving response; anaerobic metabolic capacity

INTRODUCTION

The availability of oxygen in the environment determines the upper bound to metabolic scope for fish. Metabolic scope is the difference between maximum metabolic rate (MMR) and resting metabolic rate (RMR), and determines an animal's capacity to engage in metabolically expensive activities such as swimming and digestion (Claireaux et al, 2000). Consequently, oxygen is a limiting resource for fish (Fry, 1971). As environmental oxygen concentration decreases, metabolic scope diminishes until it is depleted, at which point a fish must transition to anaerobic metabolism to meet its metabolic demands. Anaerobic metabolism only captures about 8% of the energy from food that can be extracted aerobically (Gnaiger, 1993) and is not sustainable due to finite fuel stores and accumulation of deleterious end products. Thus the scarcity of oxygen can have direct (i.e. mortality) and indirect (i.e. reducing feeding, locomotion, and growth) effects on fish, and commonly determines the spatial distribution of fish in the environment (e.g. Pihl et al., 1991; D'Amours, 1993), at times forcing fish to occupy suboptimal habitats (e.g. Coutant, 1985; Eby & Crowder, 2002; Thompson et al., 2010).

In regions where dissolved oxygen is limited (i.e. hypoxia), the fitness of an organism may depend on their hypoxia tolerance (HT), which is known to vary among (e.g. Pihl, et al., 1991; Wannamaker & Rice, 2000; Vanquer-Sunyer & Duarte, 2008) and within species (e.g. Claireaux & Largardere, 1999; Mandic et al., 2009; Killen et al., 2011). This variance is also contextually dependent upon the physiology, behaviour, and environment of the fish (Richards, 2011). For example, under moderate hypoxia of 50% air saturation (AS), the European sea bass *Dicentrarchus labrax* (L. 1758) showed disorientation and reduced ability to respond to stimuli (Lefrancois & Domenici, 2006),

which may have reduced its prey capture and predator avoidance abilities. This species is also known to increase aquatic surface respiration in hypoxic water, which could make them more susceptible to predation (Killen et al., 2011). Additionally, Vagner et al., (2008) found that grey flathead mullet *Mugil cephalus* L. 1758, swimming at optimal speeds typical of habitat exploration and routine activity, fatigued quicker under severe hypoxic conditions (15% AS). Thus differential survival and ability to carry on routine biological functions in waters that experience frequent hypoxic episodes may be a function of an individual's HT.

Hypoxia tolerance has generally been tested under static flow conditions, either with large groups of fish together or with individual fish in a respirometer or "fish box" (e.g. Robb & Abrahams, 2003; Farwell et al., 2007; McKenzie et al., 2008). Loss of equilibrium (LOE) is the most commonly used endpoint in these tests. Such experiments usually result in a wide range of HTs for individuals of similar size and from the same population (e.g. Miller et al., 2002; Davies et al., 2011; Killen et al., 2011). While this type of trial may accurately assess HT in relatively inactive demersal species, or very small ones unable to escape hypoxic zones, pelagic schooling species are more likely to encounter hypoxia while moving through the environment.

One pelagic species likely to encounter hypoxic zones while swimming is the striped bass *Morone saxatilis* (Walbum 1792), a semi-anadromous fish that naturally ranges along the western Atlantic Coast of North America from Canada to Florida, and into the Gulf of Mexico (Setzler et al., 1981). Approximately 70% of individuals from stocks North of Cape Hatteras, North Carolina originate from the Chesapeake Bay (Waldman et al. 1997), where hypoxic zones occur annually during summer (Hagy et al.,

2004; Kemp et al., 2005). Juveniles of this species are commonly found in hypolimnetic regions of the Bay that are particularly susceptible to summer hypoxia because the stratified water retards oxygenation from flow, wind, and wave action, while the turbid epilimnion represses hypolimnetic photosynthesis (Cloern, 2001; Breitburg, 2002; Costantini et al., 2008). After being spawned in tidal rivers, juveniles migrate into the estuary proper (Setzler-Hamilton et al., 1981), where they aggregate in large schools in regions with strong current (Woolcott, 1962). This energetic lifestyle of juvenile *M. saxatilis* makes oxygen concentration an important determinant of suitable habitat.

Due to the commercial and recreational importance of *M. saxatilis* in the Chesapeake region, it is imperative to have a realistic understanding of this species' ability to cope with and respond to hypoxia, as hypoxic regions are predicted to expand with climate change (Keeling et al., 2010). Thus this study was conducted to determine how swimming activity impacts HT of individual juvenile *M. saxatilis*. Hypoxia challenge tests were conducted in Brett-type swim tunnels (Nelson, 1989) under either static or moderate flow conditions. Specifically, we tested the null hypothesis that juvenile *M. saxatilis* have the same hypoxia tolerance regardless of swimming activity.

MATERIALS AND METHODS

FISH MAINTENANCE

Thirteen juvenile *M. saxatilis*, 123 – 183 mm total length (TL), were collected by the Maryland Department of Natural Resources trawl survey from the main channel of the Chesapeake Bay on 3 February 2011, and transported back to Towson University. Fish were captured at approximately 4°C and brought up to 20°C by increasing the water

temperature 2°C per day. Throughout the duration of the study, fish were held at an average temperature of 20.1 ± 1.0 °C (mean ± S.D.) and salinity of 9.2 ± 1.2 in a 355 L tank under a 12 h daily light cycle, and fed a mix of pellet (Hikari) and flake food (Aquarian®) to satiation at least five times weekly.

All fish were allowed to acclimate to laboratory conditions for four months prior to initiation of the experiments. Fish were initially weighed, measured and individually marked with Passive Integrated Transponders (PIT) (Biomark, Idaho, USA) injected into the abdominal cavity just anterior to the pelvic girdle under anesthesia (MS-222 1.2 mg L^{-1}, buffered). Antibiotics were applied topically to the wound sites after tagging. All individuals were allowed a minimum of two weeks recovery from tagging before hypoxia testing began.

ACCLIMATION PROTOCOL

Hypoxia challenge trials were conducted in Brett-type swim tunnels as described in Nelson (1989) at 20.4 ± 0.9°C and salinity of 8.4 ± 0.8 in both (i) static and (ii) flow conditions. Individuals were fasted for 24 h prior to being transferred to the swim tunnel, and were not fed during the subsequent 24 h acclimation to the swim tunnel. Individuals were exposed to a minimal water speed (< 3 cm s^{-1}) during the 24 h acclimation period to allow the fish to adjust to the direction of the flow. Approximately half of the individuals were tested in static conditions (N = 7) prior to being tested under flow conditions, while the others were tested in the reverse order to eliminate the effect of trial sequence.

HYPOXIA CHALLENGE TRIALS

Trials were initiated by lowering the oxygen concentration to 10% AS over approximately 30 min (29.9 ± 1.26 min) by bubbling in nitrogen gas. Oxygen concentration decreased as an exponential function with an average instantaneous slope of 3.12 ± 0.38% AS min^{-1} (N = 22 trials). Two galvanic oxygen-sensing probes were used to determine the level of AS in the swim tunnel (one anterior and one posterior to the swimming section). 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, Denmark). When 10% AS was achieved, a timer was started, and HT was recorded as time to loss of equilibrium (LOE), in minutes. Once an individual lost equilibrium, they were removed, measured, weighed and transferred to a recovery tank as quickly as possible. The 13 fish reported on here survived both trials.

Static conditions had minimal current (< 3 cm s^{-1}) driven by an air stone and small pump to allow mixing of the water and homogenous distribution of dissolved gases, but did not require the fish to swim to maintain station. During flow trials, fish were swum at 50% of their estimated critical swimming speed (U_{crit}). The U_{crit} of different-sized fish was estimated using data from Beamish (1970) for largemouth bass *Micropterus salmoides* (Lacepede 1802) of similar size (19.7 ± 3.7 cm) at 20°C. The equation used to predict 50% U_{crit} in *M. saxatilis* was

$$v = (10^{1.4465}) (10^{0.0137\,L}) (0.5)$$

Where v is 50% of the estimated critical swimming speed in cm s^{-1} that the fish were swum at, and L is the most current TL of the fish, in cm. Flow was manually controlled

by a rheostat. The relationship between the rheostat and water speed was established prior to the experiments through a regression of flow meter velocity readings measured at various rheostat settings at 18 locations throughout the swimming chamber at each setting with a Marsh-McBirney model 2000 Flo-mate®.

A flow trial was initiated by increasing the velocity of water flow by approximately 2.5 cm s^{-1} every 30 s until 50% U_{crit} was achieved. Fish then remained at that speed for the rest of 5 min, at which point oxygen tension was reduced. A light was placed at the back of the swimming chamber to deter the fish from resting. If a fish proceeded to rest, it was gently touched with a blunt probe. If the individual was still unable to return to swimming in the water column after three attempts, the trial was stopped and the time was noted as the HT, in min.

Some individuals in the static trials showed no sign of losing equilibrium after 6 h at 10% AS. Thus, further decrements of oxygen saturation were employed to ensure LOE under static conditions. After 4 h at 10% AS, the level was reduced to 8% AS, and subsequently decreased by 2% AS every hour beyond that. Behaviour of the fish (i.e. resting on bottom, escape response, aquatic surface respiration) was noted throughout all trials. Escape response behaviour was defined as any agitated or burst swimming, coupled with a change in orientation in the swimming chamber.

STATISTICAL ANALYSIS

All statistical analyses were conducted with an alpha level < 0.05 in SPSS (www.ibm.com/software/analytics/spss/). A Wilcoxon signed rank test was conducted to determine if HT of individuals was significantly different in static versus flow conditions,

and Spearman's rank correlation analysis was used to explore correlations between HT, body size, and growth of the individual in both static and flow conditions (Dytham, 2011). Relative mass gain per day (G) was calculated for each individual for each trial using the equation

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

Where M_f is the mass of the fish at the time of the trial, M_i is the initial mass of the fish, and d_f is the number of days between when M_i was taken and M_f was taken (mean = 96.9 ± 43.8 days). A Kruskal-Wallis test was used to determine if the order in which individuals were exposed to the two conditions had an effect on HT, and a Spearman's rank correlation was also used to explore if there was a relationship between an individual's HT in static and flow conditions (Dytham, 2011). This experiment complied with all Towson University animal care regulations and guidelines. IACUC #102510 JN-11.

RESULTS

HYPOXIA CHALLENGE TRIALS

Hypoxia tolerance of juvenile *M. saxatilis* was significantly higher in static conditions (Wilcoxon; $S = 45.5$, N = 13, $P < 0.05$) (Fig. 1). Each individual was more tolerant of hypoxia under static versus flow conditions (Fig. 2). The average HT for static trials was 312.09 min, ranging from 97.02 - 424.93 min, while the average HT for flow trials was only 46.54 min, ranging from 0 – 154.37 min. Under static conditions, ten individuals had oxygen concentrations reduced below 10% AS before LOE occurred (6% N = 4, 4% N = 4, 2% N = 2), whereas no individuals lasted the whole 4 h at 10% AS

under flow conditions. Two individuals survived to 2% AS under static conditions before losing equilibrium. Under flow conditions, four individuals were unable to swim at oxygen tensions above 10% AS, losing equilibrium as the oxygen concentration reached 17, 15, 12, and 11% AS. In contrast, three individuals were able to swim at 50% U_{crit} for more than 2 h at 10% AS. Interestingly, these three individuals were not the best performing individuals in the static trials (e.g. Individuals 4 and 13, Fig. 2). Similarly, there was no relationship between an individual's HT in static conditions and their HT in flow conditions (Spearman's Rank; $\rho = -0.29$, N = 13, $P = 0.34$). Despite the smaller range of times until LOE under flow conditions, there was greater variation in HT among fish in the flow (CV = 125.7%) versus static (CV = 34.3%) conditions.

There was no correlation between an individual's mass at the time of the trial and HT in either static (Spearman's Rank; $\rho = -0.27$, N = 13, $P = 0.37$) or flow (Spearman's Rank; $\rho = 0.51$, N = 13, $P = 0.07$) trials (Table I). There was also no correlation between growth rate and HT in static (Spearman's Rank; $\rho = -0.044$, N = 13, $P = 0.89$) or flow (Spearman's Rank; $\rho = 0.078$, N = 13, $P = 0.80$) conditions (Table I). The mean HT of the individuals exposed to static conditions first was not significantly different than those exposed to static conditions second (Kruskal-Wallis; Z = 0.36, DF = 1, $P = 0.72$), nor did the order affect the HT under flow conditions (Kruskal-Wallis; Z = 0.65, DF = 1, $P = 0.51$).

BEHAVIOURAL OBSERVATIONS

During both static and flow trials, fish commonly exhibited escape response behaviour, and aquatic surface respiration (ASR). Escape response behaviour was most common when oxygen concentration was decreasing as opposed to being held at a constant level, and was observed at oxygen concentrations ranging from 50 – 4% AS. ASR often began between 15 - 12% AS (N=5), but was observed at concentrations ranging from 22 – 4% AS. Three individuals performed ASR for the entire duration at oxygen concentrations below 10% AS. In static conditions, once 10% AS was reached, approximately 70% of individuals remained quiescent, resting calmly on the bottom of the swim tunnel (N=9) for the duration of the trial.

DISCUSSION

The principal findings of the present study were that swimming activity decreases HT and that different factor(s) determine HT at rest than while swimming. The juvenile *M. saxatilis* from the present study were relatively tolerant to hypoxia, having the ability to withstand an oxygen level of 10% AS for over 5 h on average when resting. This result is consistent with Chittenden (1971) who reduced oxygen levels in a static tank to an average of 0.95 mg L^{-1} (~ 10% AS) before LOE was observed in young of the year *M. saxatilis* (70 – 110 mm TL). In contrast, Atlantic cod *Gadus morhua* L. 1758 are more sensitive to hypoxia, with a lethal oxygen concentration estimated at 20% AS (Chabot & Claireaux, 2008), whereas Crucian carp *Carassius carassius* (L. 1758) are extremely tolerant to oxygen stress surviving one to two days in anoxia at room temperature (Nilsson & Renshaw, 2004).

The first response of animals to environmental perturbations is generally behavioural (Kramer, 1987). In the present study, behavioural strategies included an early escape response in most fish as oxygen tension was lowered, followed by ASR in many, and concluding with quiescence in most. The initiation of an escape response before inactivity is consistent with results of Chittenden (1971), who observed restlessness (i.e. escape response) ranging from 1.3 - 3.7 mg L^{-1} (~14 – 40% AS), followed by inactivity ranging from 0.9 – 3.2 mg L^{-1} (~10 – 35% AS) in *M. saxatilis*. Additionally, *D. labrax,* a co-familiar to *M. saxatilis*, also exhibit an increase in activity in response to mild and severe hypoxia (Killen et al., 2011). An escape response may be an effective strategy under certain conditions (see below), but avoidance of hypoxic water may not always be possible in the wild, a condition mimicked by our experimental design.

For instance, hypoxia can develop quickly in shallow coastal waters in the Chesapeake Bay, when bottom water is driven up by winds and tidal currents (seiches), plummeting dissolved oxygen (Breitburg, 1990). The magnitude of the hypoxic water intrusion can exceed many animals' escape capacity, and can last up to 10 h, leading to fish kills (Breitburg, 2002). Under these conditions, a fish can cope by balancing oxygen demand with the available supply by selectively depressing metabolic rate through a reduction of some energy-demanding processes (Hochachka & Guppy, 1987; Dalla via et al., 1994; Almeida-Val et al., 2000). The processes that are generally sacrificed are energetically costly (e.g. digestion, locomotion and growth) and allow the animal to route available oxygen and energy to maintenance functions necessary for survival, but will have obvious tradeoffs for long-term fitness (Wu, 2002). *M. saxatilis* from the present study were capable of existing in such a state for an average of 5 h.

In contrast, when swimming, *M. saxatilis* were always less tolerant of hypoxia, lasting only, on average, 46 min. This may seem like an intuitively obvious result, but a loss of equilibrium due to other environmental stressors is unaffected by swimming activity. For example, critical thermal maximum (CT_{max}) of blacknose dace *Rhinichthys atratulus* (Hermann 1804) was not affected by swimming (JAN, unpubl. data) despite the fact that dissolved oxygen was also decreasing with the rising temperature. An animal engaged in an energetically costly activity, will require more oxygen to sustain the increase in energy demand. For example, during prolonged swimming, oxygen uptake is 12 – 15 times more than at rest, and of that increase in uptake, 93% is directed to the muscles (Randall & Daxboeck, 1982; Webber et al., 1998). Thus, swimming performance in some species may be limited by the ability to take up and deliver oxygen to the muscles, and will decrease as environmental oxygen decreases (Jones, 1971). Therefore, hypoxia tolerance has a metabolic origin, clearly being affected by activity level. Whereas, thermal tolerance, being unaffected by activity level, likely indicates a neural origin (Rajaguru, 2002).

The maximum oxygen that can be supplied by the cardio-respiratory system is much less when a fish is swimming in hypoxic conditions compared with normoxia. This is evidenced by significant reductions in U_{crit} (Jones, 1971; Bushnell et al., 1984; Dutil at al., 2007; Petersen & Gamperl, 2010) and metabolic scope in fish swimming in hypoxic water (Chabot & Claireaux, 2008). Little is known about adjustments made by fish to cope with hypoxia while swimming. When the maximum capacity of the cardio-respiratory system is reached, fish can continue to power locomotion by anaerobic metabolism (Reidy et al., 1995; Rahel & Nutzman, 1994; Vagner et al., 2008), albeit not

indefinitely due to lactic acidosis and a limited supply of glucose stored as glycogen. Thus, it is proposed that the variation of HT in swimming individual juvenile *M. saxatilis* is primarily due to differences in anaerobic metabolic capacity. This idea is supported by the larger coefficient of variation in HT of these fish when swimming versus resting, coupled with the results of Marras et al. (2010) who showed much larger variation in the anaerobic capacity of a cohort of *D. labrax* undergoing repetitive constant acceleration tests than in their aerobic capacity.

An important finding of this study was that there was no relationship between an individual's HT in static conditions and their HT under flow conditions. Since these were the same individuals, it strongly suggests different mechanisms whereby equilibrium is lost due to hypoxic conditions encountered while swimming, rather than at rest, as in traditional laboratory studies. This finding also supports the multiple processes theory to account for different metabolic scaling coefficients between resting and exercising animals (Darveau et al., 2002). This theory posits that the organismal metabolic scaling coefficient is a summation of the individual processes cycling ATP at the cellular level, each process' unique scaling coefficient and its fractional contribution to total metabolic rate (Darveau et al., 2002; Hochachka et al., 2003). Thus, since different processes such as protein synthesis and Na^{+}/K^{+} ATPase activity are the dominant consumers of energy at rest, and processes such as Myosin and Ca^{++} ATPases dominate during exercise, there is no reason to expect metabolic scaling coefficients to be the same in these two states. Likewise, if different processes are accounting for a majority of the oxygen utilization in the two different states, there is no reason to *a priori* expect an animal to have equal HT at each of the two activity levels. Since a reduction in oxygen consumption is observed in

fish in hypoxia both at rest (Speers-Roesch et al., 2010) and during exercise (Fu et al., 2011), there may be further intraspecific variation in how they selectively arrest metabolic processes, producing further shuffling of their relative HTs (i.e. a hypoxia response-swimming interaction term). Therefore, it is proposed that two separate pathways account for LOE in fish exposed to hypoxia while at rest and while swimming (Fig. 3).

Hypoxia tolerance at rest will be a function of an animal's ability to depress aerobic metabolic rate, to extract oxygen from the environment, and to supply that oxygen to only those tissues essential for life maintenance. Therefore, there is a negative relationship between resting metabolic rate (RMR) and hypoxia tolerance, whereby individuals with higher RMR have increased sensitivity to low oxygen (Claireaux and Lagaradere, 1999; McKenzie et al., 2008), resulting in a shorter time to LOE under static conditions.

In hypoxic flow conditions, individuals are forced to incorporate anaerobic metabolism as a result of the increased metabolic demands of locomotion. Under these circumstances, HT may be primarily controlled by factors such as the amount of anaerobic fuel stored in the muscles or the ability to tolerate or remove harmful end products, which would increase anaerobic metabolic capacity. For example, greater anaerobic fuel stores may allow the individual to sustain locomotion and maintenance metabolism longer, increasing time to LOE. There is some evidence that anaerobic fuel stores increase HT. McKenzie at al. (2008) found juvenile Dover sole *Solea solea* (L. 1758) fed *Artemia* enriched with essential fatty acids had greater HT than those fed un-enriched *Artemia*. Additionally, preliminary data showed individual *D. labrax* with

greater glycogen stores, also had increased HT (Guy Claireaux, personal communication). Future research should focus efforts on discerning the influence these factors have on determining the HT of swimming fish.

The significant reduction in HT observed in flow conditions coupled with the animal's initial escape response has important implications for estimating HT in active pelagic species. Our results showed escape response behaviour was most common as oxygen concentrations were decreasing, as opposed to being held at a stable level. Herbert & Steffensen (2005) found similar results in *G. morhua*, which exhibited escape response by increasing their swimming speed 18% as oxygen was reduced from 19.9 to 13.2 kPa (~100 - 66% AS). An escape response may be an advantageous strategy when fish are capable of avoiding hypoxic regions, which are often patchy and unpredictable in the aquatic environment (Breitburg, 2002). It may be particularly important for juvenile *M. saxatilis* to escape hypoxic zones, as it is known to hinder growth (Cech, 1984; Brandt et al., 2009), which in turn increases time exposed to size-dependent predation (Sogard, 1997). In fact, *M. saxatilis* have been observed avoiding water with low oxygen concentration in the laboratory (Hill et al., 1981) and in the field (Matthews et al., 1985; Thompson et al., 2010). However, if the hypoxic zone is too large to escape, our results suggest that this escape response could actually exacerbate the effects of the hypoxic water on *M. saxatilis*.

Since the rank hypoxia tolerance of individuals at rest and swimming were unrelated in these wild-caught fish, alternative selective regimes with respect to hypoxia may be operating in Chesapeake Bay. For example, although *M. saxatilis* HT is significantly reduced when swimming, some individuals were capable of lasting up to 2

h, which could allow those individuals to utilize hypoxic regions to forage, giving them a fitness advantage at times. In fact, Thompson et al. (2010) observed some adult *M. saxatilis* making short excursions into deeper hypoxic water during the day in Narrows Reservoir (NC, USA), which may indicate foraging behaviour. However, depending on the size and unpredictability of the dead zones, selection may sometimes favor those animals that do better in the quiescent phase of HT. For instance, Chapman et al. (2002) observed native cichlids utilizing hypoxic regions of Lake Victoria as a refuge from predation by the Nile perch. Similarly, juvenile *M. saxatilis*, are extremely susceptible to predation by larger species in the main channel of the Chesapeake Bay and could escape predation by utilizing hypoxic regions (Coutant, 1985; Robb and Abrahams, 2003). Both strategies have contextual superiority over the other, allowing alternative selective regimes to excise, potentially dependent on life stage.

For a trait to be subjected to natural selection it must first (i) be variable within the population, (ii) affect the fitness of individuals, (iii) and be heritable (Endler, 1986). As shown in Fig. 2, HT is extremely variable, even in individuals of similar size. Hypoxia tolerance, as mentioned above, could also affect fitness, especially since juveniles of this species exhibit reduced growth and predation efficiency under hypoxic conditions (Breitburg, 1994). It is also important to note that HT measurements appear to be repeatable over time (7 months) (GKL, unpubl. data). The heritability of HT has yet to be determined, but should be further explored, as HT may be under selection in nature (Nilsson & Ostlund-Nilsson, 2008).

This study demonstrates that swimming activity and behaviour can greatly affect a fish's ability to cope with hypoxia, and potentially overall fitness, making it imperative

that future studies incorporate species-specific behaviour and lifestyle when determining the protocol for hypoxia challenge trials. Without consideration for activity level and behavioural responses, researchers are likely to over or under estimate HT, which can impact planners' decisions for the future occurrence and expansion of hypoxia.

In the literature there is some discrepancy regarding whether body size affects HT. Some studies, such as Alemdia-Val et al., (2000), found a positive correlation between HT and body size in *Astronotus ocellatus* (Agassiz 1831). However, the present study found no effect of body size on HT whether swimming or inactive. These results are in accordance with Plante et al. (1998) who found no effect of body size on HT in *G. morhua,* and Chittenden (1971) who found no effect of fish size on oxygen levels at LOE or death in *M. saxatilis*. Nilsson & Ostlund-Nilsson (2008) found similar results with various species of damselfish (Perciformes: Pomacentridae), but concluded that in severe hypoxia when a fish must transition to anaerobic metabolism, larger fish have an advantage due to their lower mass-specific metabolic rate. With a lower mass-specific metabolic rate, it will take longer for lethal levels of end products to be reached and allows glycogen fuel stores to last longer in the individual. This may explain the marginal *P* value for the mass-HT correlation in flow conditions ($P = 0.07$), since swimming individuals were forced to transition to anaerobic metabolism.

A tradeoff between growth rate and HT was one concern of this study. When an organism is shuttling energy into somatic growth, it reduces available energy for other activities. The energy that is used for growth could otherwise be allocated to make physiological and morphological adjustments that would increase the individual's metabolic capacity, such as increasing gill surface area, blood hemoglobin

concentrations, and energy fuel supplies (Richards, 2011). Yet no relationship was found between growth rate and HT in the present study, albeit with a small sample size (N=13).

In summary, HT of juvenile *M. saxatilis* is significantly lower when swimming at 50% U_{crit}. Different rank orders of tolerance among swimming and resting fish suggest different determinant causes of hypoxia tolerance in the two physiological states. Due to the energetic lifestyle of juvenile *M. saxatilis*, HT estimates from flow trials may be more relevant for management decisions of this species and may merit consideration, when estimating the HT of other species.

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Table I. Mass, growth rate, and hypoxia tolerance (HT) of juvenile striped bass *Morone saxatilis* in different flow regimes

	Static			Flow		
Fish	Mass (g)	G[a]	HT[b]	Mass (g)	G[a]	HT[b]
1	74.8	0.512	116.97	80.3	0.532	7.92
3	31.1	0.723	97.02	42.0	0.770	26.67
4	35.3	0.107	393.88	37.5	0.221	0.00
8	57.9	0.633	302.23	42.5	0.645	11.45
9	19.0	0.221	361.23	37.1	0.739	64.73
10	39.5	0.409	364.72	57.0	0.597	149.35
11	27.3	0.864	380.65	46.4	0.939	0.00
12	59.5	0.798	315.77	38.6	0.861	5.05
13	31.4	0.617	424.93	36.6	0.618	0.00
14	62.7	1.209	344.82	51.4	1.114	122.62
15	35.0	0.533	211.13	44.7	0.794	154.37
16	54.4	0.863	422.48	51.3	0.888	62.82
18	44.2	1.102	321.30	31.7	1.056	0.00

(a) G, relative mass gain per day (%); (b) HT, hypoxia tolerance measured as time to loss of equilibrium (min). Hypoxic exposure consisted of 4 h at 10 % air saturation followed by subsequent hourly decrements of 2% air saturation. Static velocity was < 3 cm s^{-1} and Flow velocity was 50% of an estimated *Ucrit.*

FIGURE CAPTIONS

FIG. 1. Mean hypoxia tolerance (HT), measured as time to loss of equilibrium, of juvenile *M. saxatilis* at 10% air saturation for the first 4 h, followed by subsequent hourly decrements of 2% air saturation when necessary. Hypoxia tolerance was statistically significantly different (Wilcoxon; S=45.5, N=13, P<<0.05) when individual fish were subjected to different flow regimes, either static (< 3 cm s^{-1}) or flow (50% U_{crit}). Data are means ± 2 S.E.

FIG. 2. Hypoxia tolerance, measured as time to loss of equilibrium, of 13 individual juvenile *M. saxatilis* at 10% air saturation for the first 4 h, followed by subsequent hourly decrements of 2% air saturation (indicated by the dashed lines) when individual fish were subjected to different flow regimes, either static (< 3 cm s^{-1}) or flow (50% U_{crit}). Individual numbers along the x-axis indicate the identification number for the fish throughout the experiments.

FIG. 3. Different pathways for loss of equilibrium (LOE) when a fish is swimming versus inactive. The example processes consuming the most energy during quiescence and locomotor activity are reported from Darveau et al. (2002).

Fig 1

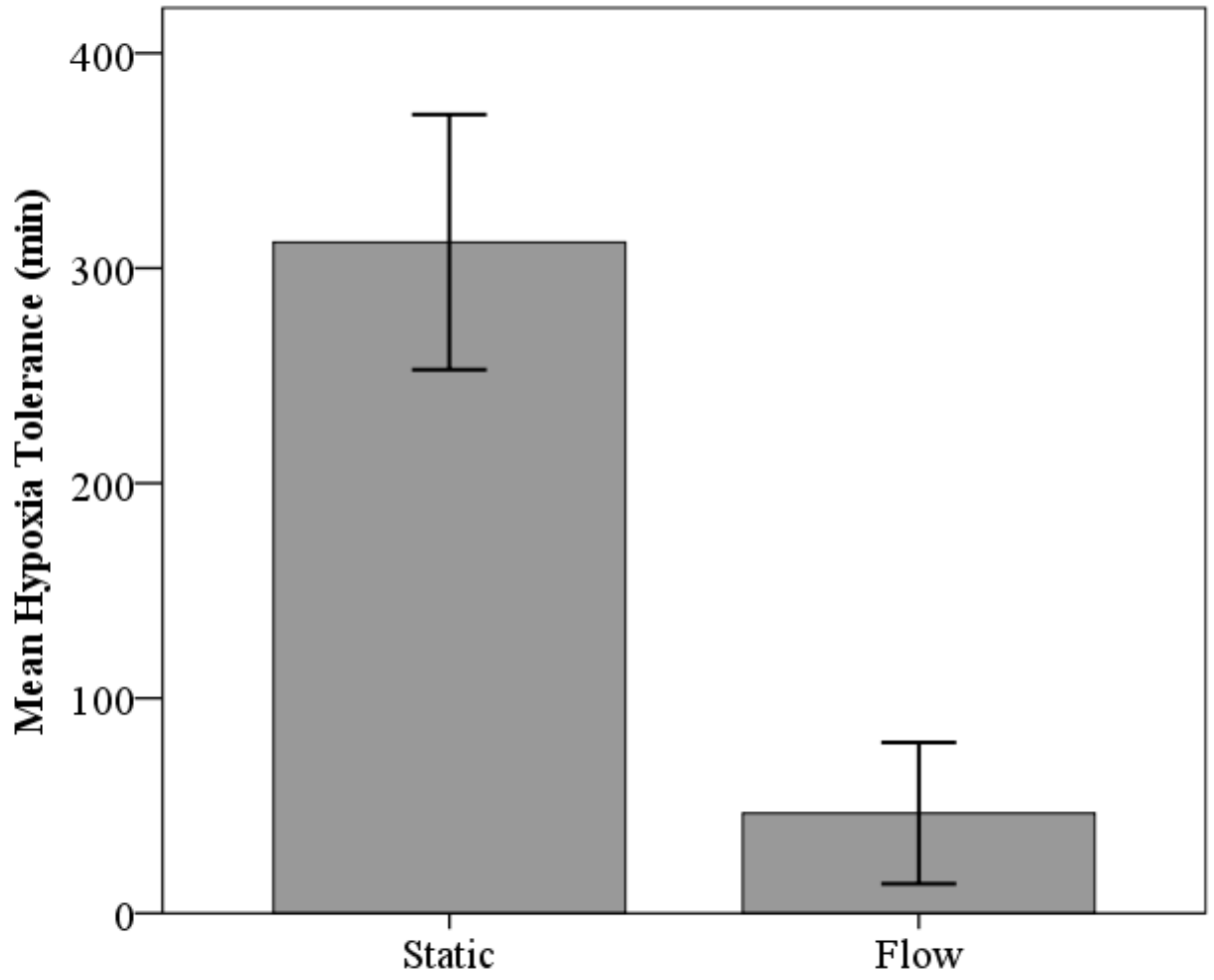


Fig 2

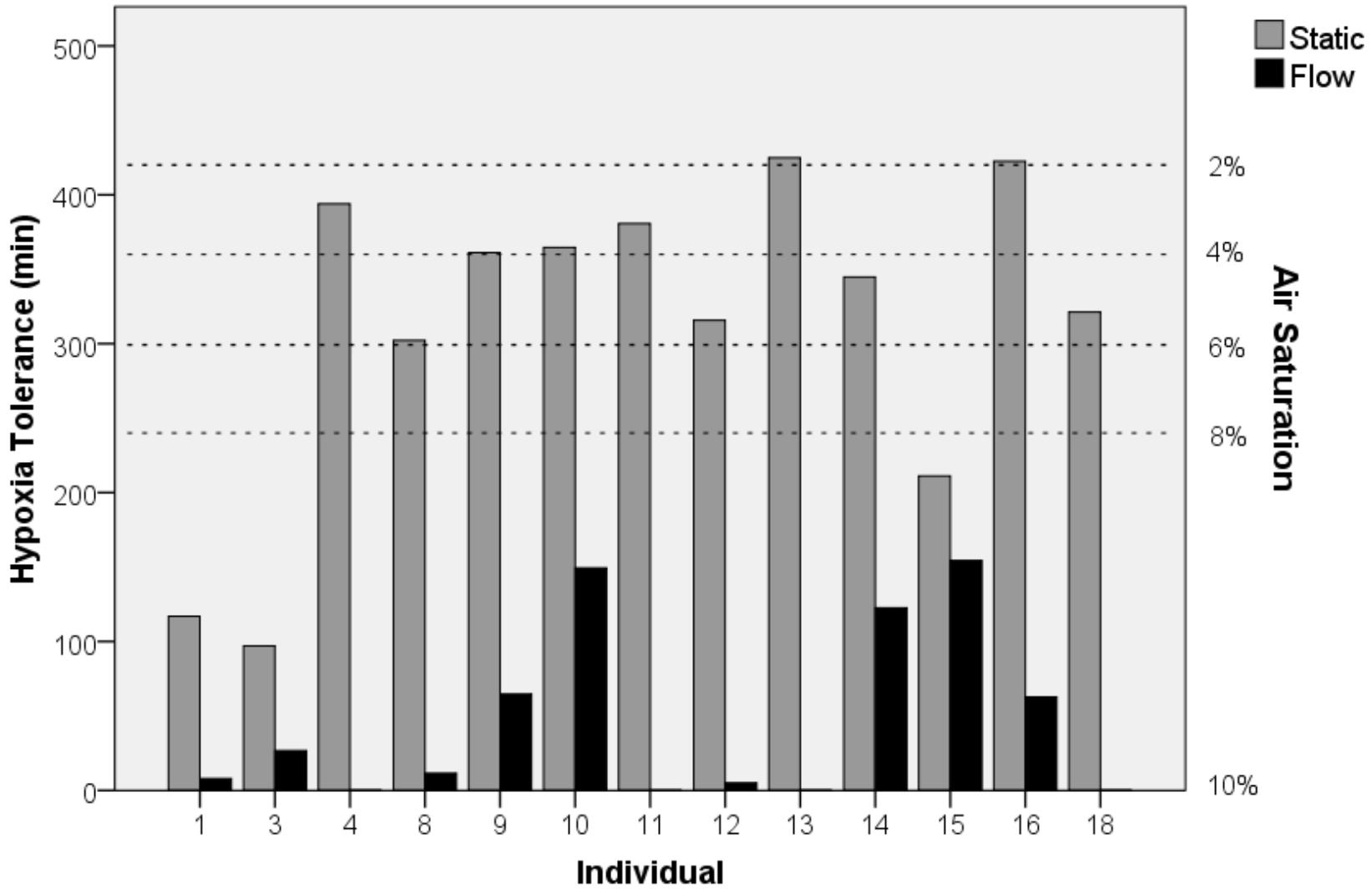
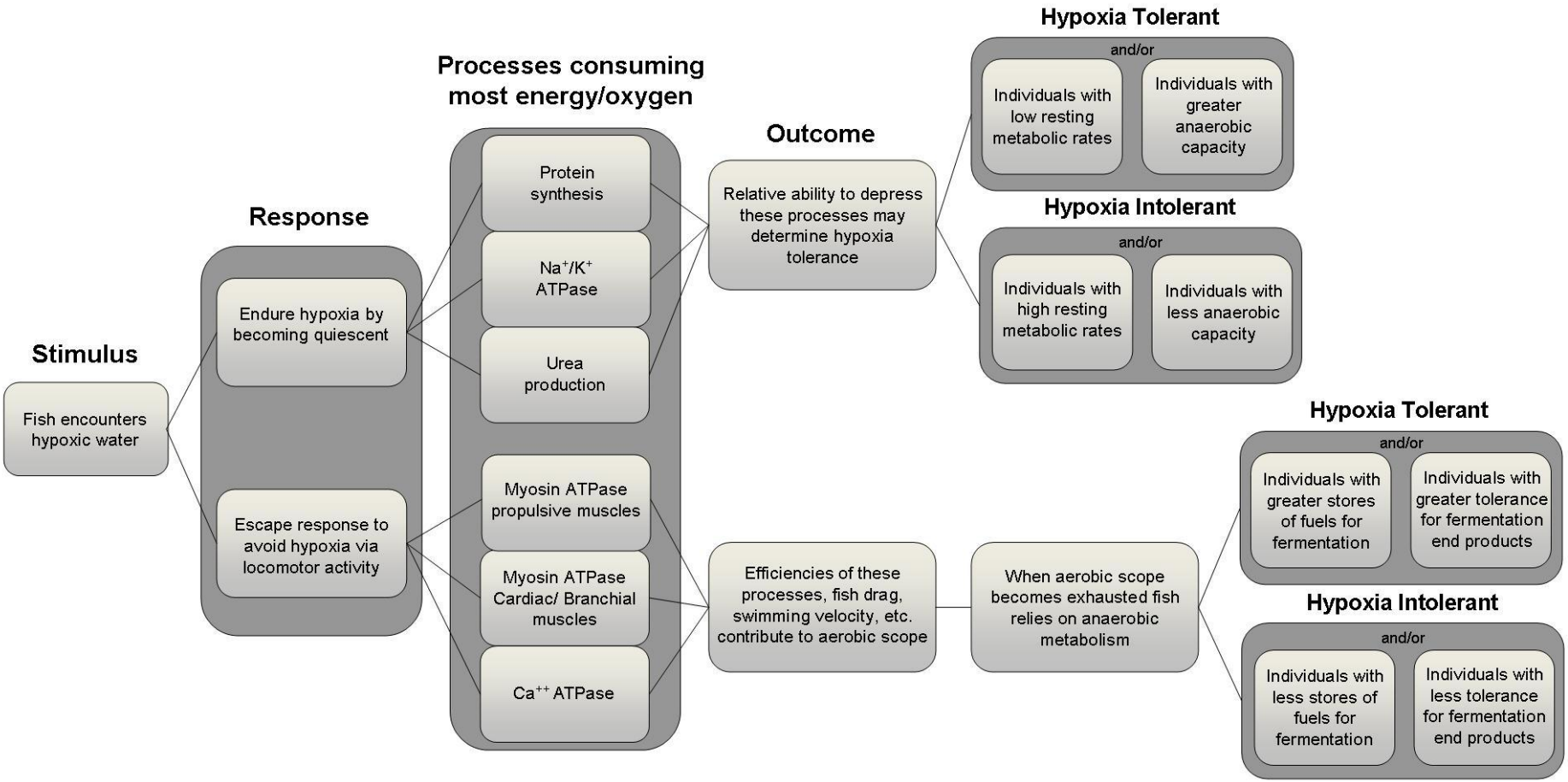

Static
Flow
Hypoxia Tolerance (min)
500
400
300
200
100
0
2%
4%
6%
8%
10%
Air Saturation
1
3
4
8
9
10
11
12
13
14
15
16
18
Individual

Fig 3


Stimulus
Fish encounters hypoxic water
Response
Endure hypoxia by becoming quiescent
Escape response to avoid hypoxia via locomotor activity
Processes consuming most energy/oxygen
Protein synthesis
Na+/K+ ATPase
Urea production
Myosin ATPase propulsive muscles
Myosin ATPase Cardiac/ Branchial muscles
Ca++ ATPase
Outcome
Relative ability to depress these processes may determine hypoxia tolerance
Efficiencies of these processes, fish drag, swimming velocity, etc. contribute to aerobic scope
When aerobic scope becomes exhausted fish relies on anaerobic metabolism
Hypoxia Tolerant
and/or
Individuals with low resting metabolic rates
Individuals with greater anaerobic capacity
Hypoxia Intolerant
and/or
Individuals with high resting metabolic rates
Individuals with less anaerobic capacity
Hypoxia Tolerant
and/or
Individuals with greater stores of fuels for fermentation
Individuals with greater tolerance for fermentation end products
Hypoxia Intolerant
and/or
Individuals with less stores of fuels for fermentation
Individuals with less tolerance for fermentation end products

CHAPTER 2: SOCIAL STATUS

The effect of social status on the hypoxia tolerance of juvenile striped bass *Morone saxatilis*

GENINE. K. LIPKEY

ABSTRACT

Hypoxia tolerance of individual juvenile striped bass was assessed in varying social environments to determine if social status affects an individual's ability to tolerate hypoxia. Social status was determined by assigning points to individuals as they fed prior to being challenged with hypoxia. An individual's social status was repeatable across trials regardless of the social context ($W = 0.866$, $df = 5$, $p = 0.02$), but did not affect hypoxia tolerance ($X^2 = 3.6$ $df = 5, 17$, $p = 0.61$). Hypoxia tolerance increased with repeated exposures to hypoxia ($X^2 = 24.7$, $df = 3, 11$, $p < 0.001$), with a significant increase by the third exposure ($p = 0.004$), and an individual's hypoxia tolerance rank was repeatable across trials ($W = 0.53$, $df = 12$, $p < 0.01$). Blood analysis from the final complete group trial revealed that both hemoglobin concentration and hematocrit positively correlated with hypoxia tolerance ($r^2 = 0.42$, $N = 11$, $p = 0.03$; $r^2 = 0.11$, $N = 11$, $p = 0.002$; respectively). The group setting used in these experiments may be more comparable to nature, where social stress is dispersed among individuals rather than solely endured by a subordinate, as seen in paired fish. The increase in hypoxia tolerance with repeated exposure may be due to persistent changes in blood parameters that increase blood oxygen carrying capacity, and therefore hypoxia tolerance.

Keywords: Dominance, blood oxygen carrying capacity, intermittent hypoxia, training

INTRODUCTION

Social status is a chronic stressor that has a multitude of physiological consequences for fish (Gilmour et al., 2005). Compared to dominant fish, subordinates tend to be immuno-suppressed, exhibiting increased susceptibility to infection (Peters et al., 1998) and toxicants (Sloman, 2007). Subordinates generally also have slower growth rates (Metcalfe, 1986; Pottinger and Pickering, 1992; Sloman et al., 2000), which may result from competition for food (Metcalfe, 1989), diminished appetite (Overli et al., 1998), or increased metabolic costs (Sloman and Armstrong, 2002). Subordinate fish will also generally have increased plasma glucose concentrations and decreased hepatic glycogen stores (Buchner et al., 2004), which are both well-known indicators of chronic stress (Lankford et al, 2005). These negative physiological effects are thought to originate from higher plasma levels of the primary stress hormone cortisol (Gilmour et al., 2005), part of the neuroendocrine response to stress in vertebrates.

In fish, the primary neuroendocrine response to stress involves activation of the hypothalamo-pituitary-interrenal (HPI) axis, which is responsible for the mobilization of cortisol (Mommsen et al., 1999). Simultaneously, but acting more short-term, the sympathetico-chromaffin pathway is activated, resulting in the release of catecholamines (adrenaline and noradrenaline) to the plasma (Reid et al., 1998). In dominant fish, cortisol and catecholamine levels elevated by social encounters will return to baseline after the social hierarchy is established (Thomas and Gilmour, 2006). In contrast, cortisol levels in subordinate fish remain elevated for up to seven days following social encounters (Overli et al., 1999; Sloman et al., 2000). While cortisol release is part of the animal's defense against environmental challenges and is considered adaptive (Barton, 2002), persistent

elevation of cortisol can have negative consequences for metabolism, immune function, growth, and reproduction of the organism (Pickering and Pottinger, 1995). Subordinate social status has also been shown to affect red blood cell (RBC) responsiveness to catecholamines, and to cause atypical mobilization of 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 additional acute stressors (Gilmour et al., 2005). Support for this idea may be drawn from the increased susceptibility to infection and toxicants (Peters et al., 1998; Sloman, 2007) and reduced capacity to cope with thermal stress, both at the cellular and whole organism level in subordinates (LeBlanc et al., 2011). Presently, little is known about the contribution of social status to an animal's tolerance of low environmental oxygen concentration.

Hypoxia (< 2 mg O_2 L^{-1}), is a common stressor encountered by aquatic organisms. The occurrence of hypoxia in shallow coastal and estuarine environments has dramatically increased since the beginning of the 19^{th} century as a result of human activities (Diaz, 2001). Further expansion of global hypoxic zones is predicted from global climate change with expectant warming of surface water temperatures and increased water column stratification (Keeling, 2011). Hypoxia occurs annually in the Chesapeake Bay (Kemp et al., 2005), which is an important nursery and spawning habitat for striped bass (*Morone saxatilis*).

The striped bass is a semi-anadromous species that naturally ranges along the western Atlantic coast from Canada to Florida, and into the Gulf of Mexico (Setzler et al., 1980). Juveniles of this species are commonly found in hypolimnetic regions of Chesapeake Bay (Setzler et al., 1981) that are particularly susceptible to summer hypoxia

(Cloern, 2001; Breitburg, 2002). In the estuary, bottom water can be driven up by winds and tidal currents (seiches) plummeting dissolved oxygen of adjacent waters (Breitburg, 1990). The magnitude of the hypoxic water intrusion can exceed many animals' escape capacity, and last up to 10 h, leading to fish kills (Breitburg, 2002). Since the striped bass supports important recreational and commercial fisheries, it is important to have an understanding of the environmental factors affecting their hypoxia tolerance.

Hypoxia is known to elicit some of the same physiological changes as social stress including, dampened growth (Stierhoff et al., 2006), appetite (Thetmeyer et al., 1999), and immune system functionality (Bowden, 2008). Specifically, fish can show lower antibody responses after mild hypoxic exposure (Cecchini and Saroglia, 2002).

The primary neuroendocrine response to hypoxic stress, when oxygen levels drop below 50% of normoxia, is mobilization of catecholamines (Tetens and Christensen, 1987), which causes the release of red blood cells (RBC) from the spleen thereby increasing RBC concentration to increase oxygen uptake and transport (Randall and Perry, 1992). Circulating catecholamines also enhance hemoglobin-oxygen ($Hb\text{-}O_2$) binding affinity by stimulating the RBC Na^+/H^+ exchanger, resulting in a net outflow of H^+ (Claireaux et al., 1988; Nikinimaa, 1992). The net outflow of H^+ raises the intracellular pH of the RBC stimulating a reverse Bohr shift which increases $Hb\text{-}O_2$ affinity (Tufts and Randall, 1989). Simultaneously, Na^+ accumulates inside the erythrocyte eliciting osmotic swelling of the RBC. Thus hypoxia is known to induce an increase in hematocrit in many teleost fishes as a result of the recruitment of RBCs from the spleen accompanied by RBC swelling (Randall, 1982). A greater ability to tolerate hypoxia has been associated with increased $Hb\text{-}O_2$ binding affinity (left shift) (Brix et al.,

1999) and hematocrit (Chapman et al., 2002), which enhances the ability of the organism to take up oxygen from the environment presumably allowing the organism to maintain oxygen delivery to the tissues.

When oxygen uptake from the environment can no longer sustain maintenance metabolism, the fish must transition to anaerobic metabolism to meet its metabolic demands. The oxygen concentration where this transition occurs is termed the critical oxygen tension (P_{crit}). Therefore, individuals that are less hypoxia tolerant have higher P_{crit}. Anaerobic metabolism is not sustainable due to finite fuel stores, inefficiency, and accumulation of end products. When an individual has a high P_{crit}, that individual will transition to anaerobic metabolism at higher environmental oxygen concentrations and begin producing lactate. Thus lactate concentrations can be indicative of exposure to hypoxia below P_{crit}, and the fish's ability to cope with hypoxic stress. Increased blood lactate concentration in response to severe hypoxia has been observed in both teleosts (e.g. Maxime et al., 2000) and sturgeons (e.g. Baker et al., 2005), indicating the level of hypoxia to which they were exposed to in those studies was below the P_{crit} for those species or the animals were otherwise stressed.

Since similar endocrine pathways and physiological changes are activated by social and hypoxic stress, this study was undertaken to examine the null hypothesis that there is no effect of social status on hypoxia tolerance of juvenile striped bass. Social stress is generally 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 (Griffiths and Armstrong, 1999). In nature, animals would have access to refugia, could flee, and could also interact with others of their

population, which may mean that physiological responses to social stress are less severe than these experiments would predict. Therefore, the present study exposed juvenile striped bass (*Morone saxatilis*) to hypoxia (10% air saturation) in various social groups (i.e. greater than five individuals) after a dominance hierarchy had been established.

MATERIALS & METHODS

FISH MAINTENAINCE

Twenty juvenile striped bass, 112 – 183 mm total length (TL), were collected by the Maryland Department of Natural Resources trawl survey from the main channel of the Chesapeake Bay on 3 February 2011, and transported back to Towson University in Chesapeake Bay water. Fish were captured at approximately 4°C and brought up to 20°C by increasing the water temperature approximately 2°C per day and gradually changed over to an artificial 9.2 ± 1.2 (mean ± S.D.) salinity solution of Marine Mix Bio-crystals® in Baltimore City tap water. Throughout the duration of the study, fish were held at an average temperature of 20.1 ± 1.0°C in a 355 L tank under a 12:12 h daily light cycle, and fed a mix of pellet (Hikari®) and flake food (Aquarian®) to satiation (when feeding commenced) at least five times weekly.

All fish were allowed to acclimate to laboratory conditions for four months prior to initiation of the experiments. Fish were initially weighed, measured, and individually marked with Passive Integrated Transponders (PIT) (Biomark®, Idaho, USA) injected into the abdominal cavity just anterior to the pelvic girdle under anesthesia (MS-222 1.2 mg L^{-1}, buffered). Also while under anesthesia, a small external color bead was sewn at the base of the dorsal fin to allow for identification during behavioral observations.

Antibiotics were applied topically to the wound sites after tagging. All individuals were allowed a minimum of one week recovery from tagging before any hypoxia or dominance hierarchy testing began.

Hypoxia tolerance was tested both under complete group and four different sub-group conditions. The sub-group conditions were: (1) random groups of six individuals, (2) mixed groups of six differently sized individuals (see description below), (3) the six smallest individuals, and (4) the six largest fish. Individuals were allotted a minimum of one week recovery time between trials. Two complete group hypoxia challenge tests were conducted with all healthy striped bass together in a single tank. The first was conducted prior to (N=20) and the second following (N=13) all dominance trials.

To determine which individuals to use for a mixed sub-group trial, I ranked the fish by size, and divided the list into three groups of six. Then I randomly selected two individuals from each group by drawing numbers from a hat, so that the mixed trial had a gradient of sizes. Fish were weighed and measured after each hypoxia tolerance trial. The order for group trials was randomly determined by drawing numbers from a hat. All group trials were conducted twice with the exception of the mixed group trial. Due to moralities during the sixth month experiment, the number of individuals in dominance trials varied between five and six. Due to the randomization of individuals in trials, some individuals participated in more trials than others (See Table I.)

DOMINANCE TRIALS

Dominance trials were conducted in a 190 L opaque tank at an average temperature of 20.9 ± 0.9 °C and salinity of 9.4 ± 1.1. Individuals were transferred to this

tank, without air exposure, five days prior to the hypoxia challenge test, which allowed dominance hierarchies to form and reduced residual stress from transfer between tanks. The group was fasted the day following transfer to the experimental tank to ensure they would feed well during behavioral observations, which were conducted on the three consecutive days following fasting (Fig 1.). A point system based on the number of food items taken was used to assign ranks to individuals with one being the most dominant, and six being the most subordinate (Metcalfe et al. 1989). Fish were observed and video recorded for 35 minutes for each of three behavioral observation periods. During these periods, the first 5 min were used to observe any territorial behavior prior to the introduction of food. Subsequently, a single pellet of food was introduced to the tank every min for 30 min. Each time a fish took a pellet they received a point. All video recordings were watched twice to ensure points were properly assigned. The individual that took the most pellets after three days of behavioral observations was assigned a rank of one, the next most captured pellets assigned a rank of two, and so on until all individuals in the trial were assigned ranks. For ties, half ranks were assigned. For instance, if two fish both took the most pellets, they would each receive a rank of 1.5. A hypoxia challenge test was conducted on the sixth day in the same tank used for dominance hierarchy establishment (Fig. 1).

HYPOXIA CHALLENGE TEST

Hypoxia challenge tests were initiated by lowering the oxygen concentration to 10% of air saturation (AS) over approximately 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 ± 0.38% 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®, Denmark). When 10% AS was achieved, a timer was started, and HT was recorded as the time to loss of equilibrium (LOE), in minutes. Once an individual lost equilibrium, they were removed, measured, weighed, and transferred to a normoxic recovery tank as quickly as possible. A transparent one-way mirrored plexiglass covered the hypoxia challenge tank during the trials to aid in maintaining 10% AS, allow visibility of the fish, and reduce external stimuli. Pre-testing revealed that some individuals showed no sign of losing equilibrium after 6 h at 10% AS. Thus, further decrements of oxygen saturation were employed to ensure LOE in all individuals. After 4 h at 10% AS, the level was reduced to 8% AS, and subsequently decreased by 2% AS every hour beyond that.

BLOOD ANALYSIS

Following the Complete II hypoxia challenge test, blood samples were taken from rapidly anaesthetized (0.42g L^{-1} MS-222) fish by cardiac puncture of exposed hearts using heparinized syringes which were immediately placed on ice until assayed. Hematocrit and hemoglobin concentration were tested the same day.

Hematocrit was determined after centrifugation at 13,000 x *g* in capillary tubes. When sufficient blood was available, duplicate hematocrit readings were taken and averaged for more accurate results. Hemoglobin concentration was measured using the cyanomethemoglobin method. Blood samples were added to Drabkin's reagent (Sigma-

Aldrich Co.®) and compared to hemoglobin standards (Pointe Scientific, Inc.®). Optical density was recorded at 540 nm in a Spectronic^{TM} GENESYS 5^{TM} spectrophotometer. For erythrocyte counts, blood was diluted 1:50 in modified Dacie's solution, and counted by eye in an improved Neubauer counting chamber. Due to some clotting, samples were sonicated for 2 s at 10% amplitude in a Branson Digital Sonifier, to get more accurate RBC counts. 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), the extracts were centrifuged at 12,700 x *g* for 5 min at 4°C. Acidic supernatants were neutralized with the appropriate amount of KOH determined by a previous titration, and then stored frozen until assayed for lactate according to Bergmeyer and Bernt (1974). All chemicals were purchased from Sigma® and made fresh the day of the assay and filtered before using.

STATISTICAL ANALYSIS

All statistical analyses were done with SPSS with a significance level of 0.05. Relative mass gain per day (*G*) was calculated for each individual for each 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 a trial (i.e. Day 6; after loss of equilibrium), M_i is the initial mass of the fish measured approximately one week before it was transferred to the experimental tank, and d_f is the number of days between when M_i was taken and M_f was taken. Spearman's correlation analysis was used to examine the

relationship between dominance rank and both weight rank and growth rate. Kendall concordance was used to show repeatability of dominance rank and HT of individuals throughout the experiments. A Friedman's test was used to explore if HT was affected by repeated exposures to low oxygen concentrations followed by a *post hoc* Wilcoxon signed ranks test with Bonferonni correction. A Kruskal-Wallis test was used to determine if HT was affected by dominance rank. Lastly, least squares regression analysis was used to explore the relationship of HT with hematocrit, hemoglobin, lactate concentration, and RBC counts. This experiment complied with all Towson University animal care regulations and guidelines. IACUC #102510 JN-11.

RESULTS

DOMINANCE

Individuals did not show position holding or territoriality during behavioral observations. However, individuals often chased one another, typically immediately after one individual captured a food item. It was also common for more than one individual to go after a single food item. The dominance rank of an individual was consistent across trials for fish that participated in at least three trials (Kendall's Concordance Coefficient; $W = 0.866$, $df = 5$, $p = 0.02$). As such, an individual's dominance rank was repeatable regardless of the social context. The dominance rank of an individual did not have an effect on growth rate (Spearman's rank correlation; $\rho = -0.25$, $df = 14$, $p = 0.39$), and an individual's weight rank within this group of similar sized individuals was not a predictor of dominance rank (Spearman's rank correlation; $\rho = 0.49$, $df = 14$, $p = 0.07$) (Table II).

GROUP TRIALS

Summaries of the replicate complete and sub-group trials can be seen in Table III. Among the Complete I & II, SGT I & II, and LGT I & II trials, a trend can be seen where the second trial has a greater mean HT than the first. This trend becomes even clearer when analyzed on an individual basis. Individual HT increases as the individual is repeatedly exposed to hypoxia (Fig 2). A Friedman test showed a statistically significant difference in HT depending on the number of times an individual was exposed to hypoxia ($X^2 = 24.7$, df = 3, $p < 0.001$). A *post hoc* Wilcoxon signed ranks test with Bonferonni correction (sig $\alpha = 0.008$) showed HT was significantly higher after the third exposure to low oxygen concentrations ($p = 0.004$) (Fig.3).

Rank analysis was imperative for further analysis because HT increased with repeated exposure. Since trials had varying sample sizes, ranks were scaled to a standard sample size of 20 for comparative purposes. HT from this point forward will be reported as ranked values (HT_{corr}). For example, in trials of six, an individual with the fourth highest HT is given a HT_{corr} of 13.33.

Interestingly, HT_{corr} is repeatable across trials for individuals that participated in at least four trials (Kendall's coefficient of concordance; $W = 0.53$, df = 12, $p < 0.01$). Therefore the most hypoxia tolerant fish in the first trial tended to remain the most tolerant in subsequent trials (Fig. 4), similar to dominance rank repeatability.

Finally, the dominance rank assigned to an individual had no effect on the value of HT_{corr} (Kruskal-Wallis; $X^2 = 3.6$ df = 5, 17, $p = 0.61$). Fig. 5 shows that no specific rank consistently contributed to the cumulative group time in hypoxia. The mean time to loss of equilibrium from an individual's most consistent rank was used in this analysis to

correct for pseudoreplication, and is justified by the finding of dominance rank to be repeatable.

BLOOD ANALYSIS

The results for the hematological analysis are summarized in Table IV. The mean hematocrit (Hct) was 40.4 ± 13.1%. The mean hemoglobin concentration ([Hb]) was 9.5 ± 0.4 g dL-1. RBC count mean was 3.8 x 10^{6} ± 0.7 cells mm^{-3}. The group mean hemoglobin concentration per erythrocyte (MCHC) was 2.6 ± 0.5 kg cell x 10^{-14}. The mean lactate concentration ([lac]) was 11.2 ± 4.6 mM L^{-1}. There was a significant positive relationship between Hct and HT (Least squares regression analysis; $r^2 = 0.67$, N = 11, p = 0.002) (Fig. 6a). Similarly, [Hb] increased with increasing HT (Least squares regression analysis; $r^2 = 0.42$, N = 11, p = 0.03) (Fig. 6b). Neither RBC count (Least squares regression analysis; $r^2 = 0.19$, N = 11, p = 0.19), nor MCHC (Least squares regression analysis; $r^2 = 0.08$, N = 11, p = 0.39) had significant relationships with HT. There was no relationship between [lac] and HT (Least squares regression analysis; $r^2 = 0.11$, N = 11, p = 0.32).

DISCUSSION

The present study found that hypoxia tolerance (HT) increased with repeated exposure to hypoxia, and an individual's relative HT rank (i.e. HT_{corr}) and dominance rank were repeatable across trials. Additionally, hemoglobin concentration ([Hb]) and hematocrit (Hct) correlated with increasing HT. This study also showed that in juvenile

striped bass social status had no affect on an individual's ability to cope with hypoxia in groups (i.e. greater than five individuals).

When a fish is repeatedly exercised (i.e. trained) by being forced to swim, an activity that, like hypoxia, challenges the cardiorespiratory system, many physiological variables are altered (Davison, 1997). For instance, during training, [Hb] (Hochachka, 1961), RBC count (Young and Cech, 1993), and Hct increase (Thorarensen et al., 1987) resulting in greater blood oxygen carrying capacity. Exercise training has also been shown to reduce cortisol and catecholamine release to onset of exercise (Woodward and Smith, 1985). Since hypoxia similarly challenges the cardiorespiratory system, the observed increase in HT with repeated exposure observed in this study may be the result of a training effect. Repeated exposure to hypoxia in mammals causes persistent physiological changes that increase blood oxygen transport to the tissues, which allows for greater endurance capacity (Neubauer, 2001). For example, intermittent episodes of hypoxia in humans caused an increase in erythrocyte number and [Hb] (Rodriguez et al., 1999; Casas et al., 2000). Therefore, repeated exposure of fish to hypoxia might be eliciting similar physiological changes which caused the observed increase in HT.

While blood samples were only taken from a single trial in the present study (i.e. Complete II), the results do reflect an advantage of having greater oxygen carrying capacity when challenged with hypoxia. The mean Hct of 40.4% and [Hb] of 9.5 g dL^{-1} observed in this study are similar to results of striped bass stressed by net handling, where Hct was reported as 33-37% and [Hb] was reported as 8 g dL^{-1} (Hopkins and Cech, 1992). The blood analysis also revealed that both Hct and [Hb] correlated with increasing HT. Similar relationships have been found in other fish exposed to hypoxia including,

rainbow trout (Claireaux et al., 1988) and yellowtail (*Seriola quinwueradiata*) (Yamamoto et al., 1985), which showed increased Hb-O_2 affinity and increasing hemoglobin concentration. Complementary to those findings, Cook et al. (2011) showed experimentally induced anemia in snapper (*Pagrus auratus*) caused them to have lower hypoxia tolerance. Increasing HT with repeated exposure, along with positive correlations of Hct and [Hb] with HT, suggest that an individual's environmental history with regard to hypoxia may determine their HT at a given time by modulating blood oxygen carrying capacity. Thus, it might be expected for individuals from well oxygenated environments to have a lower HT because of fewer exposures than those that encounter hypoxic regions annually.

In this study, individuals with a high RBC count did not necessarily exhibit a high HT. Many studies have shown that when exposed to hypoxia fish exhibit an increase in the number of RBC per volume of plasma (e.g. Claireaux et al., 1988; Randall and Perry, 1992). While increasing RBC per volume of plasma increases oxygen loading at the gills (Claireaux et al., 1988), it also increases the viscosity of the plasma (Gustafsson et al., 1981), causing more difficulty in pumping the blood (Richardson and Guyton, 1959). Therefore, there is a physiological limit to the number of RBC per volume of plasma. This limit also depends on the volume of the RBC themselves, where an inverse relationship between volume of RBC and concentration exists. Since RBC swelling is associated with an increase in blood oxygen affinity (Claireaux et al., 1988), variations in RBC swelling and RBC number among individuals might explain why no trend was found between RBC count and HT.

The present study found considerable variability of HT, and repeatability of HT rank ($HT_{corr)}$ throughout the 7 month experiment. Similar observations have been in other performance traits in fish, such as swimming performance (Kolok, 1992; Nelson et al, 1994; Kieffer, 1995;), where some individuals are simply better swimmers compared to other individuals regardless of environmental manipulations (e.g. Martinez et al., 2002). Performance traits, like swimming performance, that vary among individuals, are repeatable across time, and have the potential to affect overall fitness of the organism, can be selected on by natural selection, if the trait is heritable (Endler, 1986). This experiment has shown that HT is extremely variable among individuals of similar size (Table I.) and repeatable over time (Fig. 4). Hypoxia tolerance also has the potential to affect fitness since it is known to cause mortality, reduced growth, and predation efficiency (Breitburg, 1994). Thus it is important for future studies to determine whether HT is heritable to determine if HT has the potential to be under selection in nature (Nilsson & Ostlund-Nilsson, 2008).

Low social status can have negative impacts on an individual's physiological state, resulting in poor overall performance of that individual when physically challenged, as in a critical thermal maximum test (LeBlanc et al., 2011). Yet, the results of this study show that an individual's social status did not affect their performance when challenged with hypoxia. These results are contradictory to those of Thomas and Gilmour (2012) who found arterial blood oxygen levels of subordinate fish to be half that of dominant fish in severe hypoxia, leading them to conclude that low social status impaired hypoxia tolerance in rainbow trout. These authors exposed rainbow trout (*Oncorhynchus mykiss*) to hypoxia individually after confinement in pairs for 48-72 h, in contrast to the

group setting exposure of this study. In the literature it is common for experiments examining the effects of social status to be done in pairs of fish rather than small groups (Sloman and Armstrong, 2002). Having a larger group may alleviate some of the aggression received by subordinates, and increase the stress imposed on dominant fish to sustain rank, therefore increasing the dispersion of stress and the overall affects of social status (Griffiths and Armstrong, 1999). In fact, subordinates in the wild are rarely found to have chronically elevated stress hormone levels (Creel, 2001), and in some cases the dominant organism has higher stress hormone levels indicating a greater social stress load (Creel et al., 1996). Therefore, group size may explain why the present study found no effect of social status on hypoxia tolerance in juvenile striped bass.

Alternatively, the fish may have habituated to the stressors. Reid et al. (1994) observed desensitization of fish to stressors (i.e. chasing and fasting) when they were repeatedly exposed to those stressors. In this experiment, individuals were repeatedly exposed to varying social environments and hypoxia. If the striped bass became desensitized to the stressors, they would exhibit attenuated neuroendocrine responses (Reid et al., 1998). A reduction in response to social stress and/or hypoxic stress, may obscure any interaction of the two. Yet, without measurements of cortisol and catecholamine responses of these fish, desensitization can not be conclusively stated. However, HT did increase with repeated exposure to hypoxia, which may support that the fish had become desensitized to that particular stressor.

In the present study an individual's dominance rank did not correlate with growth rate. This result further supports the idea that the group environment alleviated or more evenly dispersed social stress, relative to experiments of pairs of fish. In studies that

utilized pairs of fish to study social stress, the dominant fish exhibited higher growth rate (e.g. Sloman et al., 2000), but in studies that utilized small groups this relationship was not always found (Sloman and Armstrong, 2002).

The present study found dominance rank to be repeatable across trials, regardless of social context. Many studies have found it very difficult to determine whether social rank is the cause for observed physiological differences, or if social rank is a result of those physiological differences (Overli et al., 2004). Some believe that fish exhibit distinct stress coping styles or personalities, and the characteristics of those coping styles determine social rank (Wilson et al., 1994; McCarthy, 2001). Henry (1977) described two distinct coping styles: proactive and reactive. Proactive individuals are more aggressive, territorial, and develop routines, while reactive individuals exhibit lower aggression, immobility in response to a stressor, and are more flexible in a variable environment (Koolhaas et al., 1999). These coping strategies have been observed in fish. For example, Sneddon (2003) found consistent personalities in rainbow trout, where bold individuals had higher activity levels and spent more time in open areas. The observation of repeatable dominance rank in the present study supports the hypothesis that dominance is determined by the fish's personality, rather than other factors such as relative size within the group, which was previously widely accepted as a determinate of social status (Archer, 1988). Consistent with this idea, no relationship between relative size and dominance rank was found among the striped bass in the present study, however the p-value was marginal ($p = 0.07$), so further experimentation is warranted.

In summary, a group setting likely causes a more even dispersion of social stress than pairs of fish, that results in dominance rank having no effect on an individual's

ability to cope with hypoxia. The increase in hypoxia tolerance with repeated exposure to hypoxia may be a result of persistently elevated [Hb] and Hct, and might suggest that environmental history has an effect on an organism's hypoxia tolerance. Lastly, hypoxia tolerance is highly variable, repeatable over time, and has the potential to affect the individual's fitness, which suggests it might be under selection in nature, if it is shown to be heritable.

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Table I. Results of selected characteristics for each individual across all experiments

Fish ID #	Mean Weight (g)	# of exposures to hypoxia in a group	Mean time between exposures (days)	Mean Dominance Rank	Mean Hypoxia Tolerance Rank (HT_{corr})[a]
1	71.7 ± 16.7	6	34.60 ± 25.60	2.50 ± 1.5	8.76 ± 4.44
3	31.5 ± 11.0	5	43.25 ± 33.75	3.00 ± 1.0	16.25 ± 3.48
4	39.7 ± 6.8	4	57.67 ± 34.70	6.00 ± 0.0	10.40 ± 5.75
5	31.2 ± 2.5	3	31.00 ± 22.63	5.00 ± 0.0	9.66 ± 3.37
8	45.4 ± 13.6	5	43.25 ± 33.26	1.67 ± 1.2	14.80 ± 3.92
9	27.5 ± 9.7	4	57.67 ± 26.50	4.00 ± 1.4	6.29 ± 2.62
10	59.6 ± 17.1	4	52.33 ± 27.80	3.25 ± 0.4	10.46 ± 5.97
11	32.2 ± 12.7	4	57.67 ± 26.50	2.50 ± 0.7	10.91 ± 2.38
12	51.0 ± 22.4	2	173.00 ± 0.0	-	14.74 ± 5.28
13	37.0 ± 11.3	4	57.67 ± 55.94	2.00 ± 0.7	9.11 ± 6.49
14	41.0 ± 16.4	5	43.25 ± 25.05	1.00 ± 0.0	8.95 ± 4.07
15	54.9 ± 23.4	4	57.67 ± 45.08	4.75 ± 0.4	5.17 ± 2.36
16	49.0 ± 16.1	4	57.67 ± 45.08	3.25 ± 1.8	4.72 ± 3.67
17	18.5 ± 2.24	3	22.00 ± 8.49	4.00 ± 2.8	19.32 ± 1.14
18	31.6 ± 12.2	5	43.25 ± 24.17	4.00 ± 0.0	16.53 ± 2.18
20	15.3 ± 0.9	4	35.67 ± 22.63	5.50 ± 0.6	12.33 ± 8.47

(a) HT_{corr} is a ranked value where the individual in the group with the greatest hypoxia tolerance is given a HT_{corr} of one, and the individual with the lowest a HT_{corr} of 20. Not all trials had equal sample size, so all trials are scaled up to 20. Values are mean ± S.D.

Table II. Dominance Rank correlations with relative growth rate or weight rank

		Relative Growth Rate (%)	Weight Rank
Dominance Rank	Spearman's rho	-0.24	0.49
	Sig. (2-tailed)	0.39	0.07
	N	15	15

Table III. Summary of Group Trials

Trial	Days After 1st Exposure	Mean Weight (g)	Mean TL[a] (mm)	Variation of length within groups (%)	Mean HT[b] (min)
Complete[c] I	0	25.46 ± 8.24	143.50 ± 14.61	32	52.11 ± 44.61
RGT[d] I	16	28.62 ± 12.55	148.83 ± 20.80	44	243.99 ± 133.49
SGT[e] I	29	20.13 ± 3.95	132.33 ± 6.12	20	83.80 ± 63.53
MGT[f] I	45	30.55 ± 6.44	149.17 ± 10.80	21	176.74 ±166.27
LGT[g] I	63	42.28 ± 12.43	167.83 ± 12.97	29	65.54 ± 40.50
SGT II	108	34.22 ± 12.01	150.40 ± 15.24	35	222.62 ±125.64
RGT II	137	43.98 ± 25.03	164.40 ± 28.43	57	231.36 ±119.62
LGT II	153	67.52 ± 13.91	188.00 ± 13.98	21	384.11 ± 36.15
Complete II	174	62.53 ± 17.86	183.23 ± 21.18	29	314.82 ± 96.84

(a)TL; Total length from snout to tip of caudal fin, (b) HT; Hypoxia tolerance measured as time to loss of equilibrium, (c) Complete; All healthy individuals (Complete I: N= 20, Complete II: N = 13), (d) RGT; Group of five or six randomly selected individuals, (e) SGT; Group of the five or six smallest individuals, (f) MGT; Group of two small, two large, and two medium sized fish, (g) LGT; Group of the six largest individuals. Values are mean ± S.D.

Table IV. Hematological analysis following the Complete II hypoxia challenge trial

	Mean	Equation	R^2	p-value
Hct[a] (%)	40.4 ± 13.1	HT[f] = 3.171 x + 206.00	0.67	0.002*
[Hb][b] (g dL^{-1})	9.5 ± 0.4	HT = 87.94 x - 505.68	0.42	0.031*
RBC[c] count (10^6*cells mm^{-3})	3.8 ± 0.7	HT = 29.68 x + 219.74	0.19	0.19
MCHC[d] (kg cell*10^{-14})	2.6 ± 0.5	HT = -30.687 x + 412.62	0.08	0.39
[Lac][e] (mM L^{-1})	11.2 ± 4.6	HT = 0.0298 x + 1.2219	0.11	0.311

(*) denotes significant linear regression, (a) Hct; hematocrit, (b) [Hb]; hemoglobin concentration, (c) RBC; red blood cell, (d) MCHC; mean hemoglobin concentration per erythrocyte, (e) [Lac]; plasma lactate concentration, (f) HT, hypoxia tolerance measured as time to loss of equilibrium (min). Hypoxic exposure consisted of 4 h at 10 % air saturation followed by subsequent hourly decrements of 2% air saturation. (N = 11). Values are mean ± S.D.

FIGURE CAPTIONS

FIG. 1. Protocol for subgroup trials consisted of being transferred to the experimental tank, followed by a day of fasting to ensure fish would feed for behavioral observations. On the three consecutive days following fasting, the fish were video recorded as one food item was introduced to the tank in the same location for 30 min, to assign dominance based on total number of food items taken by an individual. On the day following the final behavioral observation a hypoxia challenge trial was conducted.

FIG. 2. Individual hypoxia tolerance, measured as time to loss of equilibrium (min), increases with repeated exposures to hypoxia. Only individuals that participated in at least four trials are shown here. Hypoxia was established by decreasing oxygen saturation to 10% air saturation in approximately 30 min, where it was held for the first 4 h, followed by subsequent hourly decrements of 2% air saturation when necessary.

FIG. 3. Individual hypoxia tolerance, measured as time to loss of equilibrium (min), significantly increases with repeated exposures ($X^2 = 24.7$, df = 3, $p < 0.001$) to hypoxia after the third exposure ($p = 0.04$). Only individuals that participated in at least four trials were used in this analysis. Different letters indicate significant difference. Hypoxia was established by decreasing oxygen saturation to 10% air

saturation in approximately 30 min, where it was held for the first 4 h, followed by subsequent hourly decrements of 2% air saturation when necessary.

FIG. 4. An individual's relative hypoxia tolerance (HT_{corr}) was repeatable across trials (Kendall's coefficient of concordance; $W = 0.53$, $df = 12$, $p < 0.01$). HT_{corr} is a ranked value where the individual in the group with the greatest hypoxia tolerance is given a HT_{corr} of one, and the individual with the lowest a HT_{corr} of 20. Not all trials had equal sample size, so all trials are scaled up to 20. Only individuals that participated in at least four trials were used in this analysis.

FIG. 5. Proportion of the cumulative group time at hypoxia each rank contributed to the different subgroup trials: five to six randomly selected individuals (RGT I&II), the smallest five or six fish (SGT I&II), a gradient of all sized fish determined by listing the fish in size order, dividing the list into groups of three, and randomly selecting two individuals from each group (MGT I), and the six largest fish (LGT I&II).

FIG. 6. The relationship of (a) hematocrit ($r^2 = 0.11$, $N = 11$, $p = 0.32$) and (b) hemoglobin concentration ($r^2 = 0.42$, $N = 11$, $p = 0.03$) with hypoxia tolerance, measured as loss of equilibrium (min), in juvenile striped bass exposed to 10% air saturation for the first 4 h, followed by subsequent hourly decrements of 2% air saturation when necessary.

Fig. 1

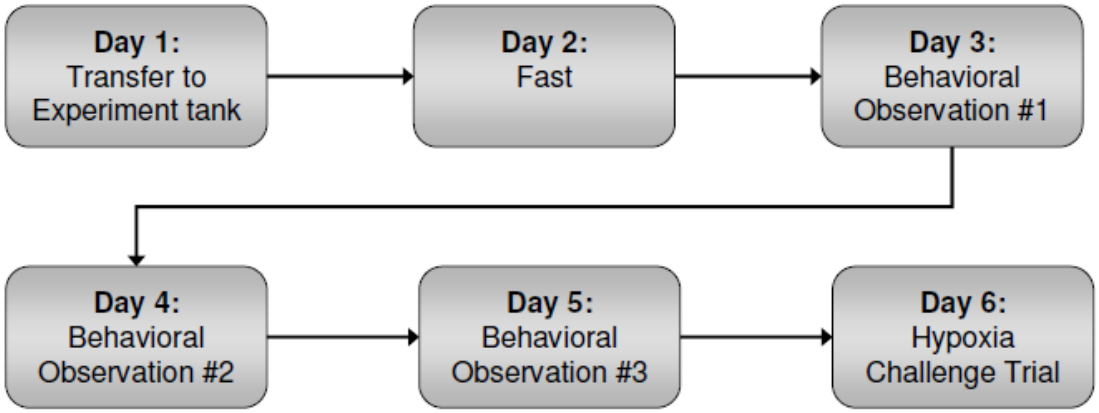

Day 1:
Transfer to
Experiment tank
Day 2:
Fast
Day 3:
Behavioral
Observation #1
Day 4:
Behavioral
Observation #2
Day 5:
Behavioral
Observation #3
Day 6:
Hypoxia
Challenge Trial

Fig. 2

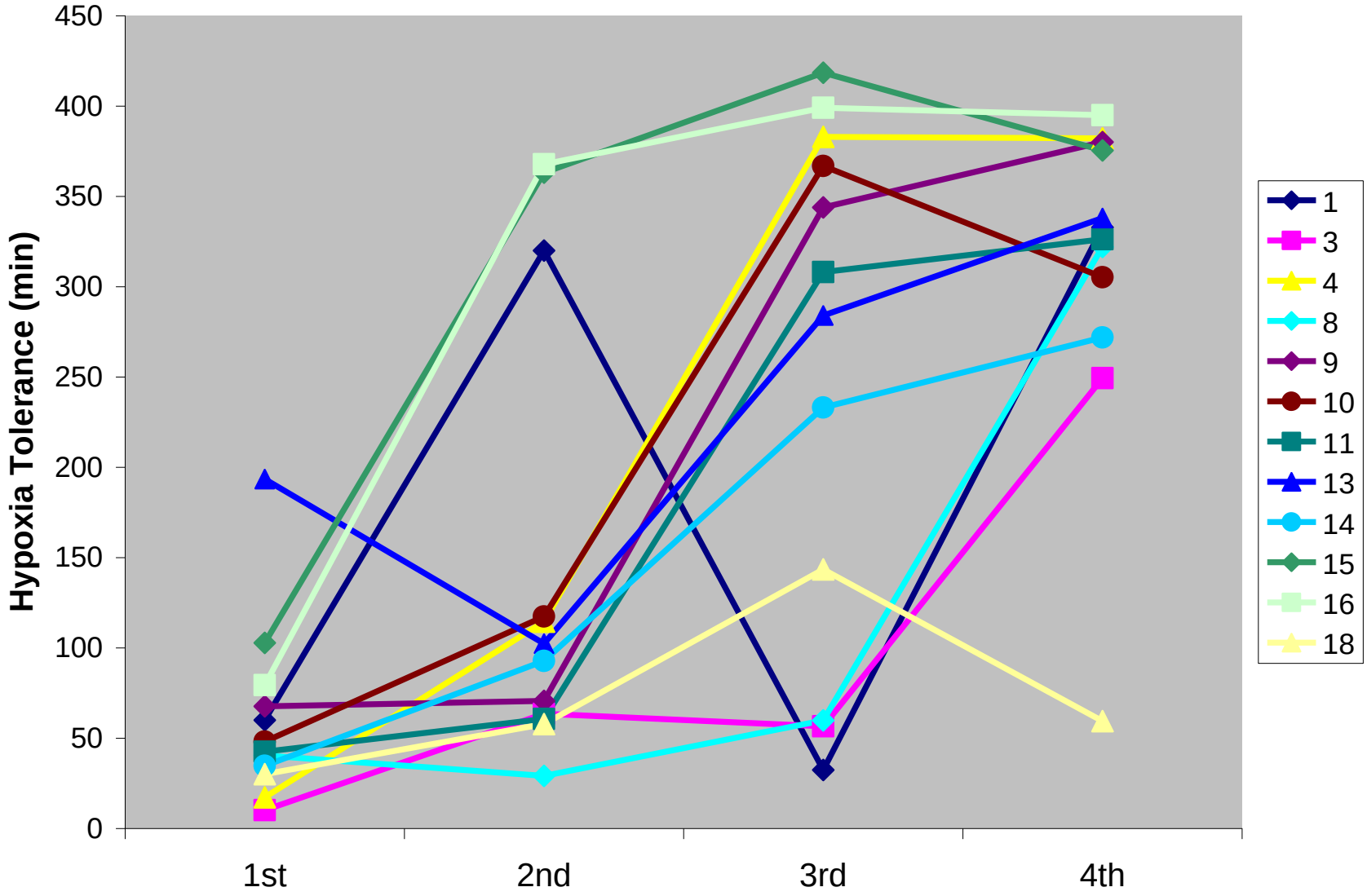

Hypoxia Tolerance (min)
450
400
350
300
250
200
150
100
50
0
1st
2nd
3rd
4th
1
3
4
8
9
10
11
13
14
15
16
18

Fig. 3

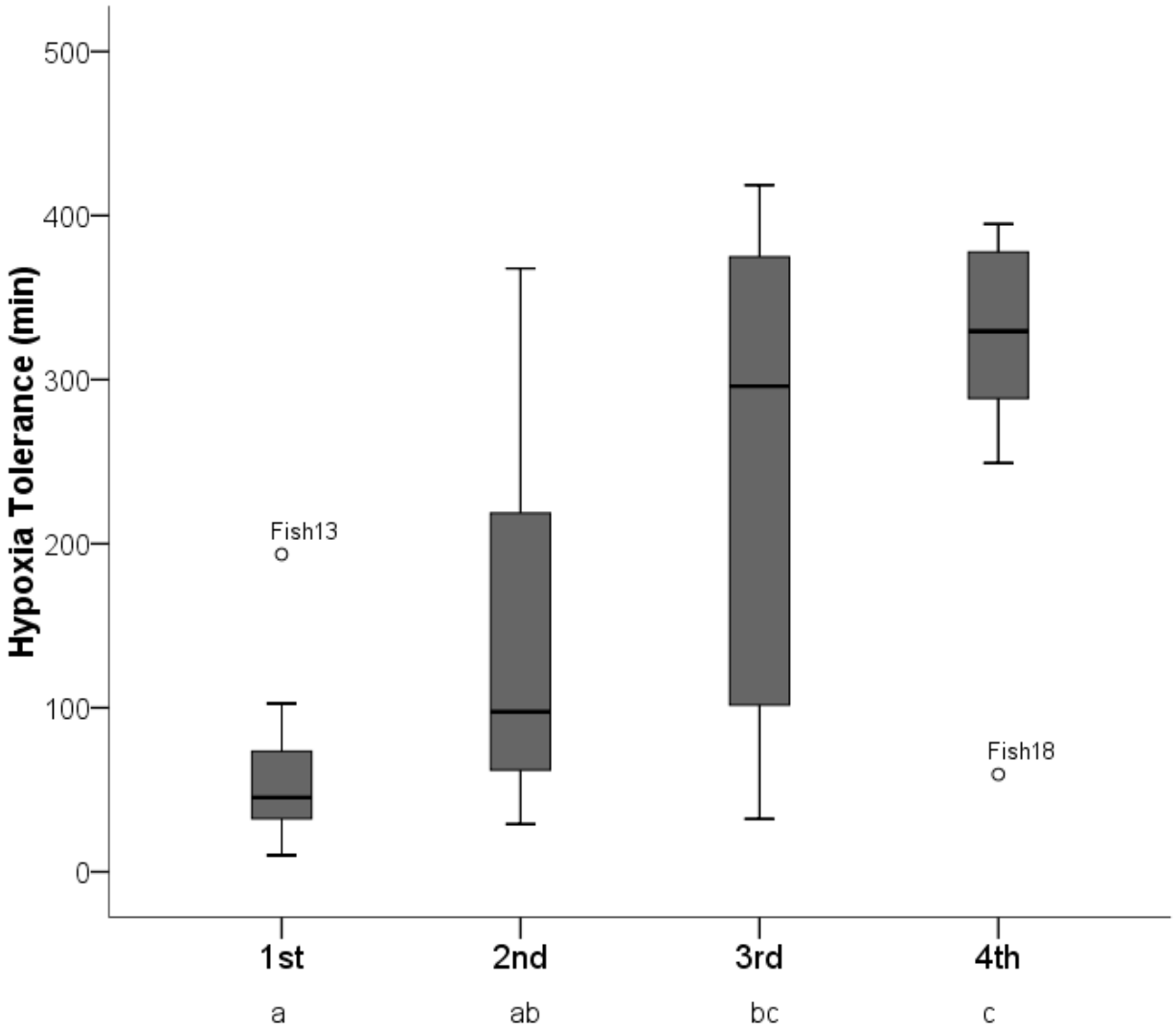

Hypoxia Tolerance (min)
500
400
300
200
100
0
Fish13
Fish18
1st
2nd
3rd
4th
a
ab
bc
c

Fig. 4

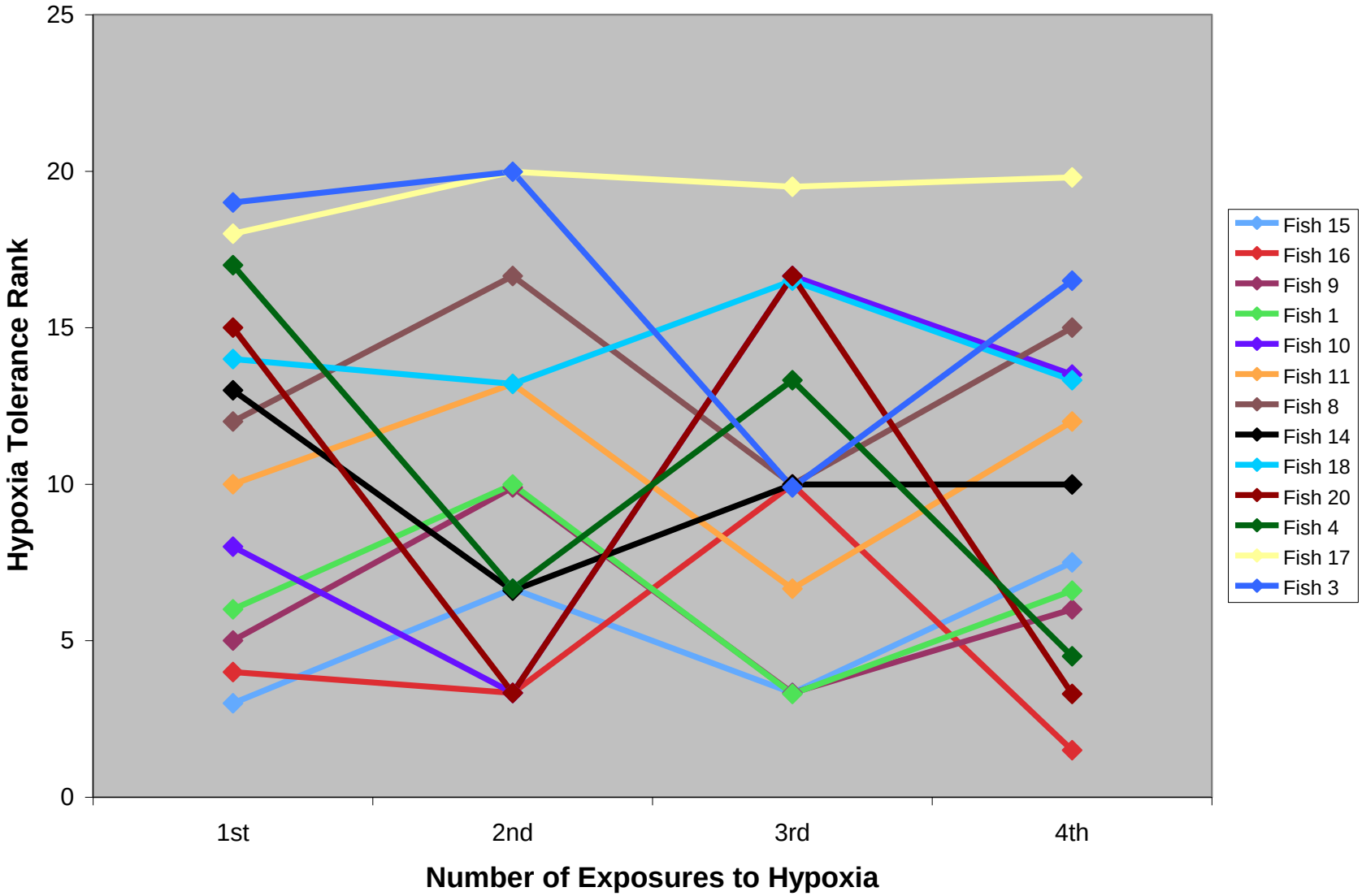

25
20
15
10
5
0
Hypoxia Tolerance Rank
1st
2nd
3rd
4th
Number of Exposures to Hypoxia
Fish 15
Fish 16
Fish 9
Fish 1
Fish 10
Fish 11
Fish 8
Fish 14
Fish 18
Fish 20
Fish 4
Fish 17
Fish 3

Fig. 5

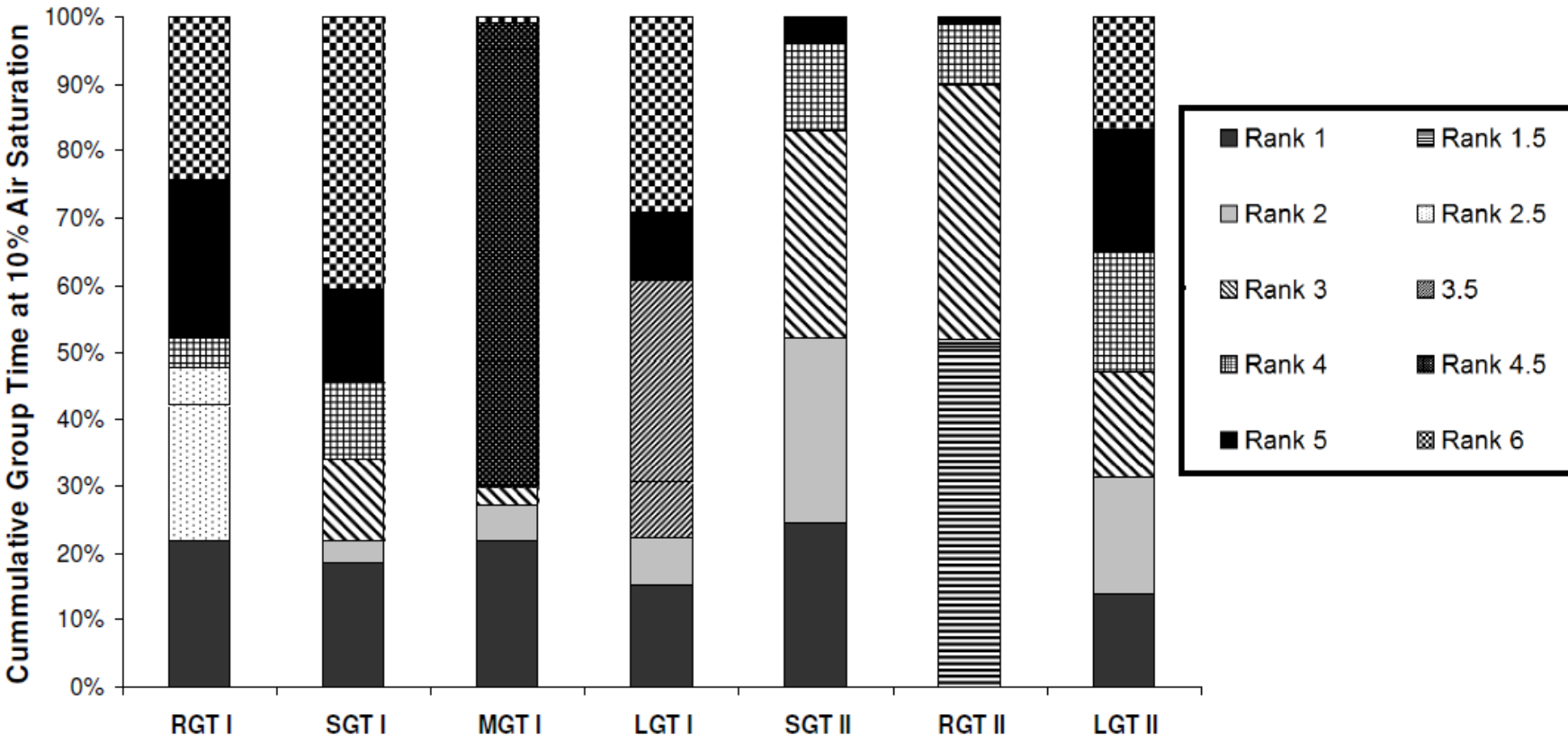
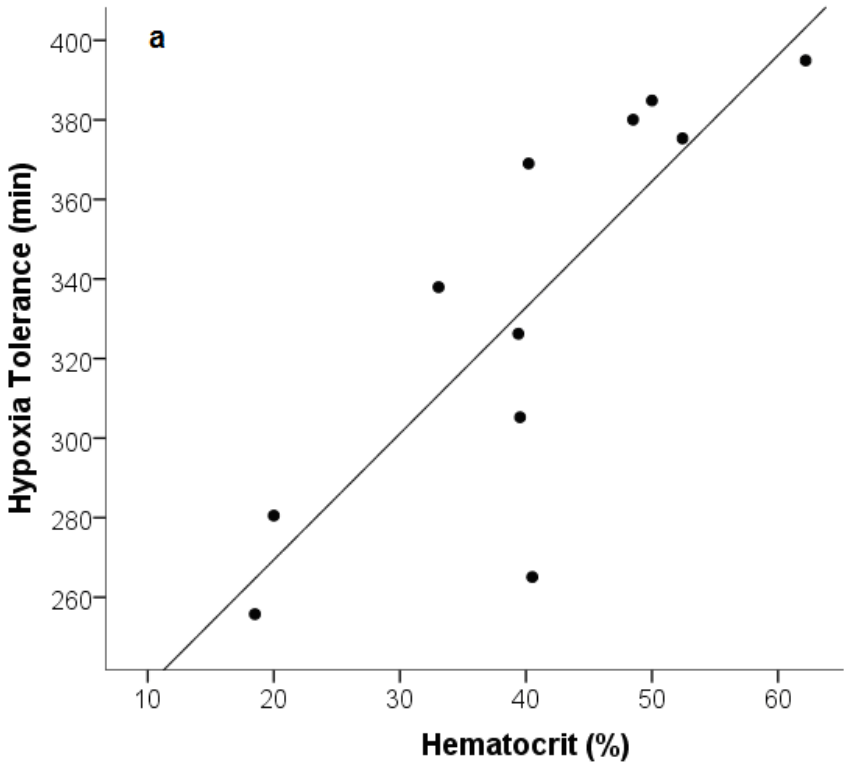
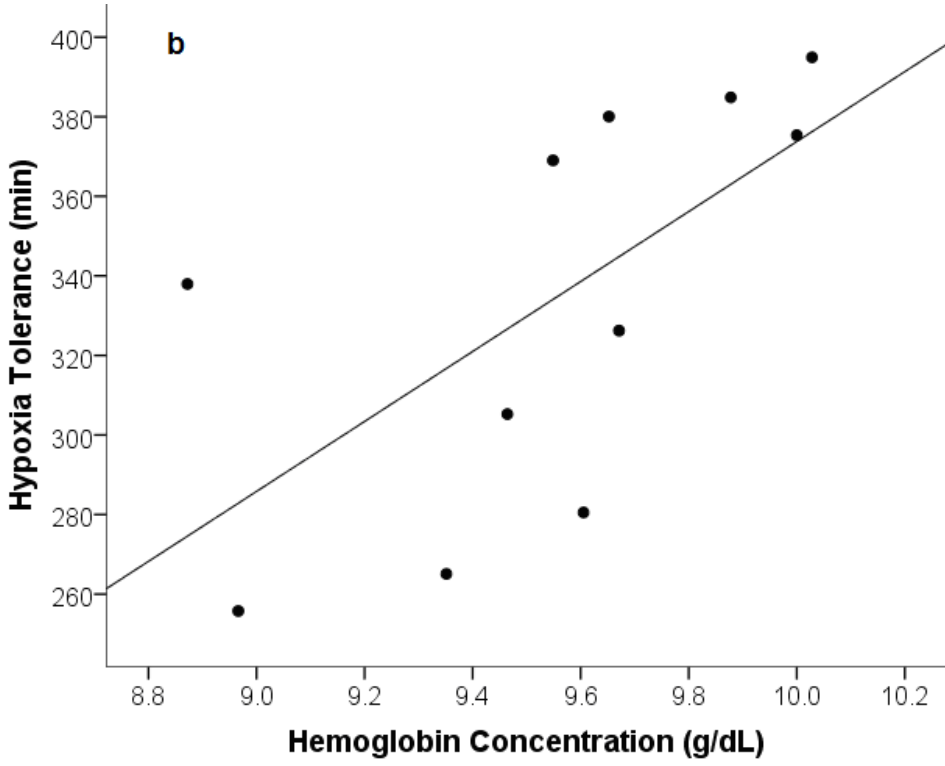

Cummulative Group Time at 10% Air Saturation
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
RGT I
SGT I
MGT I
LGT I
SGT II
RGT II
LGT II
Rank 1
Rank 1.5
Rank 2
Rank 2.5
Rank 3
3.5
Rank 4
Rank 4.5
Rank 5
Rank 6

Fig.6


a
Hypoxia Tolerance (min)
400
380
360
340
320
300
280
260
10
20
30
40
50
60
Hematocrit (%)


b
Hypoxia Tolerance (min)
400
380
360
340
320
300
280
260
8.8
9.0
9.2
9.4
9.6
9.8
10.0
10.2
Hemoglobin Concentration (g/dL)

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EDUCATION

2010 – Present M.S., Towson University, Biological Sciences (expected Dec 2012)

2005 – 2009 B.S., Virginia Tech, Biological Sciences (*Summa Cum Laude*, GPA: 3.92)

SCIENTIFIC RESEARCH

2010 – Present **Masters Research.** Towson University Fish Physiology Lab
Towson, MD
- Intraspecific variability of hypoxia tolerance in striped bass
- Laboratory hypoxia challenge experiments
- Dr. Jay Nelson, advisor

2009 (Aug-Dec) **Research Assistant.** Auburn University Marine Fish Lab
Fairhope, AL
- Artificial reefs as habitat for red snapper in the Gulf of Mexico
- SCUBA visual reef surveys
- Dr. Steve Szedlmeyer, supervisor

2007 (June-Aug) **Internship.** Mote Marine Laboratory
Sarasota, FL
- Effects of red tide on dolphin prey populations and habitat use
- Purse seining; Fish population sampling; Data entry
- Dr. Damon Gannon, supervisor

2006 - 2008 **Bachelors Research.** Virginia Tech, Biology Department
Blacksburg, VA
- Seasonal variation in stickiness of viscous capture threads
- Laboratory thread properties analysis
- Dr. Brent Opell, supervisor

TEACHING POSITIONS

2010 – Present **Teaching Assistant.** Towson University, Towson, MD
- Lab instructor for Introduction to Biology for the Health Professions

2010 – 2011 **Mathematics Tutor.** Hospitality High School, Washington, DC
- Subjects: algebra and geometry

RELATED WORK EXPERIENCE

2012	**Fish Museum Curator**. Towson University, Towson, MD
2008 - 2009	**Aquarist Intern.** National Aquarium, Baltimore, MD
2006	**Internship.** NOAA National Ocean Service, Silver Spring, MD

PUBLICATIONS & CONTRIBUTIONS TO SCIENCE

Lipkey, G.K., and J.A. Nelson. Hypoxia tolerance of swimming juvenile striped bass *Morone saxatilis* (Walbum 1792). Journal of Fish Biology. Submitted August 2012.

Opell, B.D., **Lipkey, G.K**., Hendricks, M.L. & Vito, S.T. 2009. Daily and seasonal changes in the stickiness of viscous capture threads in *Argiope aurantia* and *Argiope trifasciata* orb-webs. Journal of Experimental Zoology Part A: Ecological Genetics and Physiology. 311A: 217-225.

Lipkey, G.K., and J.A. Nelson. Swimming decreases hypoxia tolerance of juvenile striped bass (*Morone saxatilis*). Oral Presentation at 10th International Congress on the Biology of Fish. Madison, Wisconsin. July 2012.

Lipkey G.K., and J.A. Nelson. Swimming decreases hypoxia tolerance of juvenile striped bass (*Morone saxatilis*). Poster. Towson University Research Symposium. April 2012.

RELEVANT COURSE WORK

2012 Grad	Mechanisms of Animal Physiology (Towson)
2011 Grad	Evolutionary and Ecological Physiology (Towson)
2011-Grad	Data Analysis and Interpretation for Biologists (Towson)
2011-Grad	Biological Oceanography (UMD-Horn Point Laboratory)
2010-Grad	Animal Physiology (Towson)
2009-Grad	Reef Fish Ecology (Auburn)
2009-Undergrad	Fish Ecology (Virginia Tech)
2009-Undergrad	Marine Ecology (Virginia Tech)
2008-Undergrad	Ichthyology (Virginia Tech)
2007-U ndergrad	Biological Statistics (Virginia Tech)

SKILLS

Statistical Analysis	Experience with JMP, SAS, SPSS, and Excel

Web development	Experience with NVU
Otolith Analysis	Experience with whole otolith analysis (red snapper) Observed sectioned (red snapper) and crack and burn (arctic cod)
Grant preparation	Applied for EAPSI Fellowship 2012 Aided with SeaGrant application awarded to Dr. Jay Nelson 2010
Fish Sampling	Purse & beach seining, rod and reel fishing SCUBA visual fish surveys Identification, measurement, scale sampling, and tagging (PIT)
Boating	Maryland Boat Safety Certified Experience driving 22ft jet and outboard boats Minimal experience with 62ft inboard and blue ocean navigation
SCUBA Certified	Open water since 2007, logged 46 dives
Aquarist/Fish Husbandry	14 – 300 gallon tanks: fresh, brackish, and marine species Experience using anesthetics (MS-222) and antibiotics

AWARDS

2012	ICBF Student Travel Award
2010	Graduate Student Association Award for Thesis Research
2007	Deborah Ayers Koller Endowed Scholarship
2005 – 2009	Dean's List at Virginia Tech
2005	Martha S. Van Oss Scholarship