

**Quantifying the Interactive Effects of Hypoxia, Temperature, and Mycobacteriosis  
on Striped Bass (*Morone saxatilis*), and Their Impact on the Energetics and  
Ecology of These Fish**

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## Executive Summary

Despite the elevated abundance of Chesapeake Bay striped bass, concerns have developed recently with respect to the health of the Atlantic coastal migratory stock. The prevalence of mycobacteriosis, a bacterial disease characterized by granulomatous inflammation predominantly in the spleen and kidney, exceeds 90% in 4 to 5 yr-old Chesapeake Bay striped bass. Environmental stressors have been hypothesized to act as modulators of disease expression. The increasing eutrophication of Chesapeake Bay and the resulting expansion of hypoxic bottom waters, coupled with increases in water temperature due to global climate change, are forcing striped bass to inhabit waters outside their normal thermal range. Striped bass in Chesapeake Bay may experience a 'temperature–oxygen squeeze,' whereby hypoxic bottom water forces fish to inhabit more oxygenated but warmer and more stressful surface waters during the summer, predisposing them to this infectious disease. However, this 'temperature–oxygen squeeze' hypothesis has yet to be directly linked to the physiological requirements of wild striped bass. Moreover, the interactive effects of hypoxia, temperature, and infectious disease remain largely unexplored in finfishes. To address this knowledge gap, the present study focused on two objectives: first, to quantify the individual and combined influences of temperature and hypoxia on the metabolic performance of healthy and diseased wild adult striped bass from Chesapeake Bay; and second, to investigate the impact of seasonal hypoxia conditions in the Chesapeake Bay on healthy and *Mycobacterium*-infected adult striped bass. We used intermittent-flow respirometry to determine rates of oxygen consumption of healthy and *Mycobacterium*-infected fish under varying conditions of temperature and oxygen concentration. We determined physiological parameters such as standard metabolic rate, maximum metabolic rate under normoxia and hypoxia, critical oxygen saturation, aerobic scope, and excess post-hypoxic oxygen consumption. Our results indicate that acting synergistically, three simultaneous stressors (elevated water temperature, hypoxia, and mycobacteriosis) reduce aerobic scope more than any single stressor acting alone. Consequently, diseased fish under similar conditions of water temperature and hypoxia are likely to fatigue more rapidly, be restricted in their ability to elude predators or secure prey, have lower growth rates, and exhibit reduced reproductive performance and immune function. Our results also show that hypoxia does not impair the metabolic recovery of striped bass following exposure to oxygen levels below their critical oxygen saturation, suggesting that fish are able to recover

from short exposures to severe hypoxia in both normoxic and hypoxic environments. Overall, this project provides critical insights into how the interactions of an infectious disease and environmental stressors impact physiologic homeostasis in striped bass. We specifically argue that anthropogenic impacts on environmental quality, possible changes in forage abundance, the recent decline in striped bass condition, and the emergence of mycobacteriosis are interlinked processes. Therefore, because of the high prevalence and severity of mycobacteriosis in adult striped bass from Chesapeake Bay, and our demonstration of the disease's impact on their physiological capacities, models that attempt to quantify the impacts of environmental factors on striped bass should consider responses of healthy and diseased fish.

## BACKGROUND

The striped bass *Morone saxatilis* is an ecologically and economically important finfish inhabiting much of the US Atlantic coast. They are a dominant piscivore (Hartman and Brandt 1995) and constitute a key component of recreational and commercial fisheries in the Chesapeake Bay (Kirkley et al. 2000). In the mid-1980s, extremely low production prompted implementation of coast-wide restrictions on both commercial and recreational fisheries for this species (Richards & Rago 1999), but since the mid-1990s, striped bass abundance along the Atlantic coast has rebounded and fishing restrictions have been eased (Hartman & Margraf 2003). Although the abundance of Chesapeake Bay striped bass is currently considered high, concerns have developed with respect to the health of the Atlantic coastal migratory stock. Striped bass exhibiting poor body condition and clinical signs of mycobacteriosis (including severe skin ulceration in some fish) were first reported from Virginia waters in 1997 (Vogelbein et al. 1998). Concomitant with the first reports of this disease, significant increases in natural mortality for Chesapeake Bay striped bass were reported (Jiang et al. 2007). The disease is caused by acid-fast bacteria belonging to the genus *Mycobacterium*. In aquaculture situations, outbreaks of mycobacteriosis are associated with stressful environmental conditions and are invariably lethal (Nigrelli & Vogel 1963, Backman et al. 1990, Bruno et al. 1998, Chinabut 1999). *Mycobacterium spp.* have also been found in Chesapeake Bay fishes other than striped bass, including Atlantic menhaden *Brevoortia tyrannus* (Kane et al. 2007; Gauthier et al. 2010). In Chesapeake Bay striped bass, the disease is characterized by granulomatous inflammation with the presence of acid-fast bacteria, predominantly in the spleen and kidney. However, severe skin ulceration is also commonly observed (Vogelbein et al. 1999, Cardinal 2001). The prevalence of mycobacteriosis in Chesapeake Bay striped bass is high, exceeding 90% by the time fish are 4 to 5 yr of age (Overton et al. 2003, Gauthier et al. 2008). A recent study provided the first evidence of mortality associated with mycobacteriosis in Chesapeake Bay striped bass (Gauthier et al. 2008). However, the

magnitude of this disease-associated mortality, the role of environmental stressors in disease expression, and the specific physiological impacts of the disease remain largely unexplored.

Aside from the etiologic agents, the underlying causes of mycobacteriosis in striped bass are currently not known. Two predominant hypotheses proposing underlying environmental stressors as modulators of disease expression have recently been forwarded. The first hypothesis suggests that disease emergence may be linked to food limitation, as poor nutrition of laboratory-held fish resulted in severe systemic disease, high bacterial loads, and rapid progression to mortality in experimentally infected striped bass (Jacobs et al. 2009). A second hypothesis posits that increasing eutrophication of Chesapeake Bay and the resulting expansion of hypoxic ( $<4 \text{ mg O}_2 \text{ l}^{-1}$ ) bottom waters is forcing striped bass to inhabit waters outside their normal thermal range (Kemp et al. 2005, Martino & Secor 2009). Since the 1950s, eutrophication has adversely impacted dissolved oxygen conditions of coastal and estuarine ecosystems around the world (Diaz and Breitburg 2009). The recent increase in hypoxic events has had significant impacts on the productivity of Chesapeake Bay (Price et al. 1985, Adams et al. 2003, Breitburg et al. 2003, Kemp et al. 2005, Diaz & Breitburg 2009). In addition, decadal increases in water temperature due to global climate change have been recorded for the Chesapeake Bay and its tributaries (Austin 2002; Pyke et al. 2008; Kaushal et al. 2010). Adult striped bass prefer temperatures  $<25^\circ\text{C}$ , and in freshwater reservoirs, they have been demonstrated to use deeper, cooler waters as summer thermal refugia (Coutant 1985). It is possible that striped bass in Chesapeake Bay experience a 'temperature–oxygen squeeze,' whereby hypoxic bottom water forces them to inhabit more oxygenated but warmer, more stressful surface waters during the summer, predisposing them to this infectious disease. Nonetheless, this 'temperature–oxygen squeeze' hypothesis has yet to be linked to the physiological requirements of wild striped bass.

## **OBJECTIVES AND HYPOTHESES**

The interactive effects of hypoxia, temperature, and infectious disease remain largely unexplored in finfishes. To address this knowledge gap, this study focused on the following two objectives:

- 1) To quantify the individual and combined influences of temperature and hypoxia on the metabolic performance of healthy and diseased wild adult striped bass from Chesapeake Bay.
  - In teleosts, the spleen functions as a reservoir for red blood cells which can be ejected into the circulation to increase blood oxygen carrying capacity during periods of hypoxia and elevated metabolic oxygen demands (Yamamoto et al.

1980, 1983, Yamamoto 1987, Yamamoto & Itazawa 1989, Gallagher & Farrell 1998). Because the spleen is the primary target organ of mycobacteriosis in striped bass, we suggest that the disease compromises splenic function by interfering with the ability of fish to increase their hematocrit in the face of increasing oxygen demands. We thus hypothesized that mycobacteriosis exacerbates the negative consequences of hypoxia and elevated temperatures on metabolic performance, and that the deleterious effects of temperature, hypoxia, and mycobacteriosis are additive.

- We further contend that skin ulceration interferes with hydromineral balance, thereby increasing the osmoregulatory burden (Kieser et al. 1991, Briand et al. 2012). Hence, we hypothesized that severe dermal mycobacteriosis results in an increase in SMR, as well as an increase in the activity of sodium–potassium adenosine triphosphatase ( $\text{Na}^+\text{-K}^+\text{-ATPase}$ ).
- In order to test these two hypotheses, we determined standard metabolic rate (SMR), maximum metabolic rate under normoxia ( $\text{MMR}_N$ ), critical oxygen saturation ( $S_{\text{crit}}$ ), and MMR under a fixed level of hypoxia ( $\text{MMR}_H$ ) at normal ( $20^\circ\text{C}$ ) and elevated ( $28^\circ\text{C}$ ) temperatures, in both healthy and diseased fish. We also calculated aerobic scope ( $\text{AS}_N$ ,  $\text{AS}_H$ ) and factorial aerobic scope ( $\text{FS}_N$ ,  $\text{FS}_H$ ). In addition, to assess the ability of the spleen to serve as an erythrocyte reservoir, we determined hematocrit and hemoglobin concentration. Finally, we measured gill and intestinal  $\text{Na}^+\text{-K}^+\text{-ATPase}$  activity.

2) To investigate the impact of seasonal hypoxia conditions in the Chesapeake Bay on healthy and *Mycobacterium*-infected adult striped bass.

- We addressed this objective by quantifying the excess post-hypoxic oxygen consumption (EPHOC; Svendsen et al. 2012) of striped bass, in normoxia and mild hypoxia ( $3 \text{ mg O}_2 \text{ L}^{-1}$ ), and at an elevated temperature ( $28^\circ\text{C}$ ) believed to be stressful to these fish. Additionally, we determined peak oxygen consumption rate during the recovery period (peak  $M_{\text{O}_2}$ ), time to reach peak  $M_{\text{O}_2}$  ( $t_{\text{peak}}$ ), and time to recovery ( $t_{\text{recovery}}$ ). We hypothesized that it would require more time for a fish to recover under hypoxic conditions, and that a lower peak  $M_{\text{O}_2}$  would be reached, but that EPHOC would be greater compared with that of a fish recovering under normoxia. We also hypothesized that mycobacteriosis would exacerbate the effect of hypoxia on these parameters in adult striped bass.

## METHODS

### Fish collection and maintenance

Animal care and experimental protocols were approved by the College of William & Mary Institutional Animal Care and Use Committee (William & Mary IACUC-2010-11-02-6990-rwbril) and the College of William & Mary Institutional Biohazard Committee (IBC-2012-11-26-8307-wrvoge); all protocols complied with applicable US laws and guidelines.

Adult striped bass were captured by commercial pound net at the mouth of the Rappahannock River and in the Great Wicomico River; both rivers are tributaries to Chesapeake Bay. Fish of varying dermal disease severity were collected and transported to the Virginia Institute of Marine Science (Gloucester Point, VA). Fish were maintained in a filtered recirculating system supplied with a constant input of filtered and UV-sterilized York River water. Maintenance of the filtration system was performed daily, temperature and dissolved oxygen content were measured daily, and water quality conditions were monitored weekly. An artificial lighting regime matched the natural photoperiod cycle (revised monthly). Once acclimated to laboratory conditions, striped bass were fed *ad libitum* 3 times per week with commercially prepared food, blue crabs *Callinectes sapidus*, or goldfish *Carassius auratus*. Fish were acclimated for a minimum of 3 weeks prior to the beginning of the experiments, and food was withheld from fish for 24 hours prior to their use in an experiment.

### **Respirometry setup**

We determined oxygen consumption rates using an intermittent-flow respirometry system (Schurmann & Steffensen 1997, Horodysky et al. 2011, Roche et al. 2013). Two independent single cylindrical respirometry chambers were operated simultaneously. Oxygen levels in the respirometer were measured continuously with oxygen sensors mounted in a flow-through cell inserted in the water recirculation tubing. The digital outputs from the oxygen meters were recorded with computers running custom-designed software developed in DasyLab 9.02 (National Instruments, www.ni.com). The respirometers were submerged in separate temperature-controlled water basins bubbled with air (for normoxic conditions) or nitrogen (for hypoxic conditions). The flow of nitrogen was controlled by oxygen meters with galvanic oxygen sensors and solenoid valves to maintain fixed levels of oxygen saturation. The water surface of each basin was covered with a plastic sheet to minimize air–water exchange of gases, and an insulated cover was placed on top of the plastic sheet to minimize external stimuli.

The 10 min cycle required for a metabolic rate measurement consisted of a 5 min flush, followed by a 2 min equilibration interval, then 5 min of data recording for calculation of metabolic rate ( $MO_2$ ). At the conclusion of the data recording interval, the DasyLab software executed a call to an Excel macro routine which estimated the rate of change of  $O_2$  content with time ( $\Delta[O_2] t^{-1}$ ) from a linear regression of the recorded oxygen levels against elapsed time ( $t$ ). The Excel macro routine then estimated  $MO_2$  as:

$$MO_2 = [(\Delta[O_2] \text{ } \tau^{-1}) \times V] \times W^{-1} \quad (1)$$

where  $V$  = respirometer volume (l) corrected for fish volume and  $W$  = weight of the fish (kg). To account for variations in  $MO_2$  due to size differences among the fish,  $MO_2$  estimates were adjusted to a standard body weight of 1 kg using a weight exponent of 0.82 (Edwards et al. 1972):

$$X_s = \left(\frac{1}{W}\right)^{0.82} \times X_m \quad (2)$$

where  $X_s$  is the standardized  $MO_2$  value,  $W$  is the weight of the fish (kg), and  $X_m$  is the measured  $MO_2$  value. Background oxygen consumption ( $\Delta[O_2] \text{ } \tau^{-1}$ ) was measured and subtracted from  $\Delta[O_2] \text{ } \tau^{-1}$  measured when fish were in the respirometer.

## EXPERIMENTAL DESIGN: OBJECTIVE 1

### Maximum metabolic rate under normoxia

To measure  $MMR_N$ , individuals were transferred into a circular tank (chase tank) and then gently prodded to produce short periods of burst swimming until exhaustion (i.e. until fish showed no response to being handled and removed from the water; Black 1958, Lapointe et al. 2006). Fish were then immediately transferred to the respirometer where  $MO_2$  was measured for a minimum of 48 h, or until the  $MO_2$  displayed minimal to no variation. We defined  $MMR_N$  as the highest  $MO_2$  measured during the first 12 h based on results from preliminary experiments.

### Standard metabolic rate

SMR was estimated using the average of the 10 lowest routine  $MO_2$  values observed during the last 24 h of the 48 h period during which the fish were in the respirometer (Schurmann & Steffensen 1997, Claireaux et al. 2000, Lapointe et al. 2006). Under these conditions, the rate of oxygen consumption is influenced only by spontaneous activity because fish movements are restricted and external stimuli are minimal (Fry 1971).

### Critical oxygen saturation

Following the estimation of SMR,  $S_{crit}$  was determined by decreasing the oxygen content in the water basin and hence, respirometer, in a stepwise fashion (Schurmann & Steffensen 1997).  $MO_2$  was monitored at 9 levels of oxygen saturation (90, 80, 70, 60, 50, 40, 30, 20, and 10%), or until the fish was not able to maintain SMR as evidenced by a drastic decrease in  $MO_2$ . At each  $O_2$  level, 2 to 4  $MO_2$  measurements were performed, each of which lasted approximately 10 min. On average,  $S_{crit}$  trials were completed within 4 h.  $S_{crit}$  was determined using a 2-segment piecewise linear

regression of  $MO_2$  on the DO content of the water, with the first segment forced through the origin (Schurmann & Steffensen 1997, Capossela et al. 2012), using Sigmaplot 11 (Systat Software). After reaching  $S_{crit}$ , the oxygen content of the water basin and respirometer was restored to full saturation and the fish were left undisturbed until the next day, when they were transferred into the chase tank and allowed to recover for 24 h.

### **Maximum metabolic rate under hypoxic conditions**

Following the 24 h recovery period, striped bass were subjected to a second chase protocol. Once exhausted, fish were transferred into the respirometer, and the oxygen content was decreased to  $3 \text{ mg O}_2 \text{ l}^{-1}$ . The targeted  $O_2$  level was reached in  $36 \pm 9$  and  $18 \pm 5$  min (mean  $\pm 1$  SD) at  $20^\circ\text{C}$  and  $28^\circ\text{C}$ , respectively. The  $MO_2$  was recorded from the time of transfer into the respirometer until  $MO_2$  stabilized or for a maximum of 3 h. The highest  $MO_2$  recorded during that 3 h period corresponded to  $MMR_H$ . The oxygen content of the water was restored to full saturation and the fish were left undisturbed for at least 1 h before transfer to the chase tank. On the following day, they were fed, and the day after feeding, the temperature in the chase tank was increased to  $28^\circ\text{C}$  (over a 15 to 18 h period). Fish were then subjected to the protocols described above, at the increased temperature.

### **Calculated parameters**

$AS_N$  was calculated as the difference between  $MMR_N$  and SMR (Fry 1971), whereas  $FS_N$  was calculated by dividing  $MMR_N$  by SMR (Schurmann & Steffensen 1997). To determine AS and FS under a fixed level of hypoxia ( $AS_H$  and  $FS_H$ ),  $MMR_N$  was replaced by  $MMR_H$ .

## **EXPERIMENTAL DESIGN: OBJECTIVE 2**

Excess posthypoxic oxygen consumption (EPHOC; Svendsen et al. 2012) was determined at  $28^\circ\text{C}$  in 14 striped bass recovering in normoxic and hypoxic ( $3 \text{ mg O}_2 \text{ L}^{-1}$ ) conditions from exposure to severe hypoxia. Fish were transferred from the holding tank into a 900-L circular tank where they were given three hours to recover before the water temperature gradually increased from  $20^\circ\text{C}$  to  $28^\circ\text{C}$  over the course of 8 to 10 hours. Fish were held at  $28^\circ\text{C}$  for approximately 10 hours prior to being transferred into the respirometer.

Oxygen consumption rate before the exposure to hypoxia ( $M_{O_2pre-hyp}$ ) corresponded to the average of the  $M_{O_2}$  collected after it stabilized following transfer to the respirometer and before the exposure to severe hypoxia. Fish were subsequently exposed to severe hypoxia by decreasing the oxygen content in the water basin and hence, respirometer, in a stepwise fashion (Schurmann and Steffensen 1997) until their critical oxygen

saturation ( $S_{crit}$ ) was reached. Striped bass  $M_{O_2}$  was monitored at eight levels of oxygen saturation (80, 70, 60, 50, 40, 30, 20 and 10%) or until the fish was not able to maintain its  $M_{O_2}$ , as evidenced by a drastic decrease in oxygen consumption. After  $S_{crit}$  was reached,  $M_{O_2}$  during recovery from severe hypoxia ( $M_{O_2post-hyp}$ ) was recorded while fish were allowed to fully recover under normoxic conditions. Peak  $M_{O_2}$  was defined as the highest  $M_{O_2}$  measured at the beginning of the recovery period, and  $t_{peak}$  corresponded to the time at peak  $M_{O_2}$ . Fish were considered fully recovered when their  $M_{O_2}$  was within the  $M_{O_2pre-hyp}$  95% confidence interval. The following day, a second hypoxia challenge was performed on the same fish, followed by a recovery under hypoxic conditions (3 mg O<sub>2</sub> L<sup>-1</sup>).

## POST-EXPERIMENTATION PROCEDURES

Fish were euthanized after completion of the experimental protocols via immersion in a tricaine methanesulfonate (MS-222) solution (400 mg l<sup>-1</sup>), measured (fork length, cm), weighed (kg), and aseptically necropsied. Spleen, skin (if pigmented foci or ulcers were present), gill, gonad, and liver tissue samples were taken and placed in Z-fix fixative (Anatech) for histological processing. The spleen was divided into 6 pieces of equal size prior to fixation. Gill and intestinal tissue samples were collected for measurement of Na<sup>+</sup>K<sup>+</sup>-ATPase activity and immediately placed in a -80°C freezer (for Objective 1 only). Fulton's condition factor was calculated as  $K = \text{mass (g)} \times 100 \times \text{length}^{-3}$  (cm).

### Determination of disease status and severity

Fixed tissues were processed by routine methods for paraffin histology (Prophet et al. 1992). Six pieces of spleen were embedded in 2 paraffin blocks to facilitate simultaneous sectioning at 6 levels within the spleen. Tissues were sectioned at 5 µm on a rotary microtome and stained with hematoxylin and eosin. All sections were examined on a light microscope for the presence of granulomas. Quantification of mycobacterial disease severity was based on counting granulomas in the 6 sections of spleen obtained from each of the fish. Splenic lesions comprised mainly of epithelioid cells with or without a necrotic core were considered to be mycobacterial granulomas (Cotran et al. 1999) and were counted in all 6 splenic sections to obtain a total granuloma count. Granulomas containing helminth parasites were excluded from the counts. Digital image files of spleen sections examined for granulomas were then acquired using a scanner. Total area (mm<sup>2</sup>) of all 6 splenic sections was measured using MetaMorph software (Universal Imaging). A disease severity index (SI) was then calculated as  $\log_{10} ([\text{total count of granulomas in 6 splenic sections} \times \text{total splenic sectional area}^{-1}] + 1)$  (Latour et al. 2012).

Based on the number of granulomas  $\text{mm}^{-2}$  of spleen, fish were then assigned to mycobacterial visceral disease severity categories. Under Objective 1, the designations were: 'Healthy' if  $\text{SI} < 0.1$ ; 'Moderate' if  $0.1 < \text{SI} < 0.5$ ; and 'Heavy' if  $\text{SI} > 0.5$ . Under Objective 2, we grouped fish into 'Healthy' ( $\text{SI} \leq 0.1$ ) and 'Diseased' ( $\text{SI} > 0.1$ ) categories.

Dermal disease status was determined by visual examination of both sides of the fish. Lesions were classified as either 'ulcer,' for areas in which scales and epidermis were completely eroded, or 'pigmented focus,' a small ( $< 2 \text{ mm}$ ) pigmented (pale tan to brown) focal lesion. We have examined both of these types of lesions extensively using histological methods and have found that they are consistently associated with granulomatous inflammation and acid-fast bacteria (W. K. Vogelbein et al. unpublished). Fish with no skin lesions were classified as 'Healthy.' Fish with  $< 50$  pigmented foci and no ulcer  $> 2 \text{ cm}^2$  were considered 'Moderate,' and fish with  $> 50$  pigmented foci and/or ulcers  $> 2 \text{ cm}^2$  were classified as 'Heavy'. We have used this classification system extensively for tag–recapture studies of striped bass in the Rappahannock River, VA (Sadler et al. 2012). Dermal disease status was used to establish the groups for  $\text{Na}^+$ - $\text{K}^+$ -ATPase analyses.

### **$\text{Na}^+$ - $\text{K}^+$ -ATPase activity (Objective 1 only)**

$\text{Na}^+$ - $\text{K}^+$ -ATPase activity in gill and intestinal tissue was used to estimate osmoregulatory costs across the 3 dermal disease groups (Gibbs & Somero 1990, Kirschner 1995), as this enzyme plays an important role in active ion transport in fishes (Jampol & Epstein 1970). Frozen gill and intestinal tissue samples were thawed on ice and individually homogenized. The homogenate was centrifuged and the supernatant was removed and stored on ice.

The assay procedure used to determine  $\text{Na}^+$ - $\text{K}^+$ -ATPase activity was modified from that described by Kültz & Somero (1995). The assay is based on the coupling of ATPase activity to the conversion of NADH to NAD measured spectrophotometrically at 340 nm. All assays were run in duplicate and performed at  $24^\circ\text{C}$  using a temperature-controlled spectrophotometer.  $\text{Na}^+$ - $\text{K}^+$ -ATPase activity was determined as the difference between total ATPase activity and that measured in controls. Total protein content was measured using a bicinchoninic acid protein assay kit. Activities were expressed in IU (mmol of substrate converted to product  $\text{min}^{-1}$ )  $\text{mg}^{-1}$  of protein and  $\text{IU g}^{-1}$  of wet tissue weight.

### **Hematocrit and blood hemoglobin concentration**

Following the first bout of exhaustive exercise at, blood samples were taken via direct puncture of the caudal vein to determine hematocrit and hemoglobin concentration. Microhematocrit tubes were filled with whole blood and centrifuged. Whole blood hemoglobin concentration was measured using Drabkin's reagent.

## STATISTICAL ANALYSES – OBJECTIVE 1

Mean morphological parameters, hematocrit, blood hemoglobin levels, and Na<sup>+</sup>-K<sup>+</sup>-ATPase activities among groups were compared using simple ANOVAs with *a posteriori* multiple comparison (Tukey-Kramer) tests in SigmaPlot 11.0 (Systat Software). All tests used the 5% significance level, and the normality and homogeneity of variance assumptions were verified using the Shapiro-Wilk and Levene Median tests, respectively.

To evaluate the effect of temperature and disease status on SMR and S<sub>crit</sub>, and the effect of temperature, disease status, and oxygen level on MMR, AS, and FS, we used a multivariate repeated-measures model implemented with the MIXED procedure in SAS 9.3 (SAS Institute) (Seco et al. 2007, Withers & Cooper 2011). Responses (i.e. SMR, S<sub>crit</sub>, MMR, AS, or FS) were not independent and were thus modeled using a repeated measures approach (Seco et al. 2007). SMR and S<sub>crit</sub> were modeled as a function of the disease status (Healthy, Moderate, or Heavy), temperature (20 or 28°C), and the interaction of status and temperature; MMR, AS, and FS were similarly modeled, but this model included the effect of oxygen condition (normoxia or hypoxia), and all 2- and 3-way interactions between the main effects. Individual fish were treated as the subject of these analyses; furthermore, because disease state could not be assigned to each fish in a randomly sequential order, the proper treatment of such data required nesting individual fish within disease state (see e.g. Littell et al. 2006).

We modeled the heterogeneity in responses among fish assigned to the 3 disease states using the group option in the MIXED procedure in SAS and specified the Kenward-Roger method for calculating the degrees of freedom (Kenward & Roger 1997). Several covariance structures were fit to the data using restricted maximum likelihood estimation, and Akaike's Information Criterion adjusted for small sample sizes (AICc) was used to identify the model with the appropriate random structure (Littell et al. 2006). The model for SMR and S<sub>crit</sub> was best fit using a double-banded unstructured covariance structure, whereas the model for MMR, AS, and FS was best fit using a banded main diagonal unstructured covariance structure; these are highly flexible but complex structures that account for the unequal correlations and variances among observed measures, in contrast to the simple (but unrealistic) compound symmetric structure typically assumed by repeated measures models (Littell et al. 2006). Finally, *a priori* contrasts of least-squares means were obtained using the LSM estimate statement in the MIXED procedure and used to make inferences about the effects of temperature, hypoxia, and disease state on the 5 measured responses.

## STATISTICAL ANALYSES – OBJECTIVE 2

Mean morphological parameters, hematocrit, and blood hemoglobin levels between groups were compared using either a t-test or a Mann-Whitney Rank Sum Test in SigmaPlot 11.0 (Systat Software, Inc., Chicago, IL, USA). All tests used the 5% significance level, and normality and homogeneity of variance assumptions were verified using the Shapiro-Wilk and Levene Median tests, respectively.

For each fish, recovery data were included until two measurements of  $M_{O_2\text{post-hyp}}$  were below the upper 95% confidence limit of the mean  $M_{O_2\text{pre-hyp}}$ . At the individual fish level, the relationship between time (h) and the  $M_{O_2\text{post-hyp}}$  was quantified using a double exponential model implemented in SAS 9.3 (Proc NLIN, SAS Institute, Inc. Cary, NC, USA):

$$M_{O_2\text{post-hyp}} = \alpha \times \exp(\beta \times t) + \delta$$

If the model did not converge, a simpler model was employed:

$$M_{O_2\text{post-hyp}} = \alpha \times \exp(\beta \times t)$$

All adjustable parameters ( $\alpha$ ,  $\beta$ , and  $\delta$ ) were estimated using nonlinear regression. Individual recovery periods were terminated when the modeled curve intercepted the upper 95% confidence limit of the mean  $M_{O_2\text{pre-hyp}}$  ( $t_{\text{recovery}}$ ). EPHOC was determined as the area delimited by the upper 95% CI of  $M_{O_2\text{pre-hyp}}$  ( $t_{\text{recovery}}$ ), and the double exponential equation.

To evaluate the effect of hypoxia and disease status on EPHOC, peak  $M_{O_2}$ ,  $t_{\text{peak}}$ , and  $t_{\text{recovery}}$ , we used a multivariate repeated-measures model implemented with the MIXED procedure in SAS 9.3 (SAS Institute, Inc. Cary, NC, USA) (Seco et al. 2007; Withers and Cooper 2011). Responses (i.e., EPHOC, peak  $M_{O_2}$ ,  $t_{\text{peak}}$ , and  $t_{\text{recovery}}$ ) were not independent and were thus modeled using a repeated measures approach (Seco et al. 2007). Responses were modeled as a function of the disease status (“Healthy”, or “Diseased”), oxygen condition (“Normoxia”, or “Hypoxia”), and the interaction of disease status and oxygen condition. Individual fish were treated as the subject of these analyses; furthermore, because disease state could not be assigned to each fish in a randomly sequential order, the proper treatment of such data required nesting individual fish within disease state (see e.g., Littell et al. 2006).

We modeled the heterogeneity in responses among fish assigned to the two disease states using the group option in the MIXED procedure in SAS and specified the Kenward-Roger method for calculating the degrees of freedom (Kenward and Roger 1997). Several covariance structures were fit to the data using restricted maximum likelihood estimation, and Akaike’s Information Criterion adjusted for small sample sizes

(AICc) was used to identify the model with the appropriate random structure (Littell et al. 2006). The model was best fit using a Variance Components (VC) covariance structure. Finally, *a priori* contrasts of least-squares means were obtained using the LSM estimate statement in the MIXED procedure and used to make inferences about the effects of hypoxia and disease state on the four measured responses.

Data were log-e transformed prior to being used in the aforementioned analysis. Therefore, the model estimated means were back-transformed and bias-adjusted to obtain estimates of the mean in the original units.

## RESULTS AND INTERPRETATION – OBJECTIVE 1

Mean fish weight did not differ among groups, whereas mean length and condition factor did (Table 1). Striped bass in the Moderate and Heavy groups had a significantly lower condition factor compared with Healthy fish (Table 1). Mean values of post-exercise hematocrit and blood hemoglobin levels did not significantly differ among groups, and gill and intestinal Na<sup>+</sup>-K<sup>+</sup>-ATPase activities were not significantly higher in diseased fish (Table 1). Mycobacteriosis damages and in some instances completely destroys significant portions of the splenic parenchyma, which is largely replaced by granulomatous inflammatory tissues (Fig. 1), likely compromising splenic function of striped bass. This may effectively reduce or completely eliminate the spleen's ability to serve as an erythrocyte reservoir. We therefore expected hematocrit and blood hemoglobin concentration in diseased animals to be significantly below those of non-diseased fish during periods of high oxygen demand (i.e. after exercise). Although post-exercise hematocrit and blood hemoglobin concentration tended to be lower in heavily-diseased animals compared with the healthy group (Table 1), we found no significant differences in these parameters among disease states. Thus, in adult striped bass, compromised splenic function associated with mycobacteriosis may not translate into a significantly reduced blood oxygen carrying capacity, although this was not directly tested in the present study. Our results suggest the presence of compensatory mechanisms that enable diseased fish to maintain near optimal circulating erythrocyte concentrations (hematocrit) and hemoglobin values, thereby maintaining blood oxygen carrying capacity in the face of compromised splenic function. Given the chronic nature of mycobacteriosis, with severe disease developing slowly over extended time periods (months to years), it is probable that these blood parameters are maintained by elevated levels of hematopoiesis within the anterior kidney.

Table 1. Morphometric characteristics, blood parameters, and Na<sup>+</sup>-K<sup>+</sup>-ATPase activities of striped bass from 3 groups determined based on the visceral disease severity index: Healthy, Moderate, and Heavy. For Na<sup>+</sup>-K<sup>+</sup>-ATPase, groups were determined based on the dermal disease. Data are shown as means ± 95% CI. Different letters indicate a significant difference ( $p < 0.05$ ) between groups based on a simple ANOVA. Absence of letters indicates that all groups were statistically similar. n: number of individuals; M: male; F: female; U: unknown; K: Fulton's condition factor; FL: fork length; Ht: hematocrit; Hb: hemoglobin concentration

Parameter	Healthy	Moderate	Heavy
<b>n</b>	16	6	10
<b>Sex ratio (M / F / U)</b>	9 / 4 / 3	5 / 0 / 1	9 / 1 / 0
<b>FL (mm)</b>	464 ± 17 <sup>a</sup>	489 ± 27 <sup>ab</sup>	510 ± 27 <sup>b</sup>
<b>Mass (kg)</b>	1.3 ± 0.1	1.3 ± 0.3	1.4 ± 0.2
<b>K</b>	1.24 ± 0.04 <sup>a</sup>	1.1 ± 0.1 <sup>b</sup>	1.00 ± 0.06 <sup>b</sup>
<b>Ht (%)</b>	33 ± 3 (n = 4)	32 ± 6 (n = 5)	26 ± 5 (n = 5)
<b>Hb (mg/ml)</b>	152 ± 10 (n = 3)	144 ± 13 (n = 5)	125 ± 25 (n = 6)
<b>Na<sup>+</sup>-K<sup>+</sup>-ATP<sub>ase</sub></b>	(n = 4)	(n = 8)	(n = 13)
<b>Gill</b>	5.2 ± 0.9	5.3 ± 0.8	4.3 ± 0.8
<b>Intestine</b>	3.6 ± 0.3	3.8 ± 1.0	2.8 ± 0.8

$S_{crit}$  was significantly greater under elevated water temperature compared with values observed at 20°C, across all groups (Fig. 2a). Although  $S_{crit}$  was not different among the 3 disease groups at 20°C, it was significantly higher in fish classified as heavily diseased at 28°C (Fig. 2a). In addition, the relative difference between the Heavy group and the other 2 groups was the same (1.2 fold) regardless of water temperature, suggesting that severe mycobacteriosis alone reduces hypoxia tolerance in striped bass. Furthermore, elevated water temperature in this study increased the  $S_{crit}$  of striped bass, impacting their ability to cope with oxygen deprivation. Critical oxygen saturation was 1.4-fold higher at 28°C compared to 20°C, indicating that a fish's capacity to sustain its oxygen consumption rate under hypoxic conditions is significantly reduced at elevated water temperatures. This was expected, as similar results have been observed in other fishes (e.g. Fry & Hart 1948, Fernandes & Rantin 1989, Schurmann & Steffensen 1997).

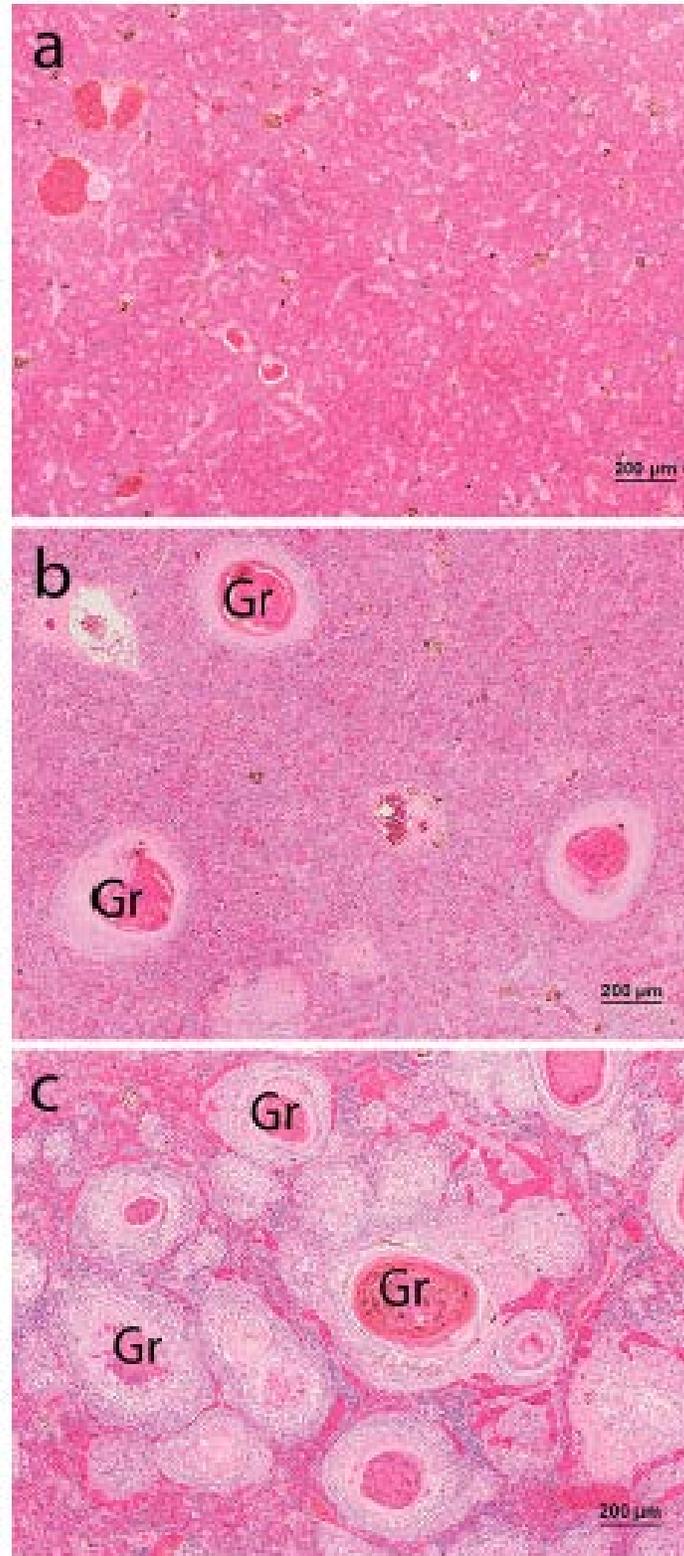


Figure 1. Splenic mycobacteriosis in Chesapeake Bay striped bass. (a) Healthy spleen. (b) Moderately affected spleen (Gr: mycobacterial granulomas). (c) Severe splenic mycobacteriosis (Gr: mycobacterial granulomas)

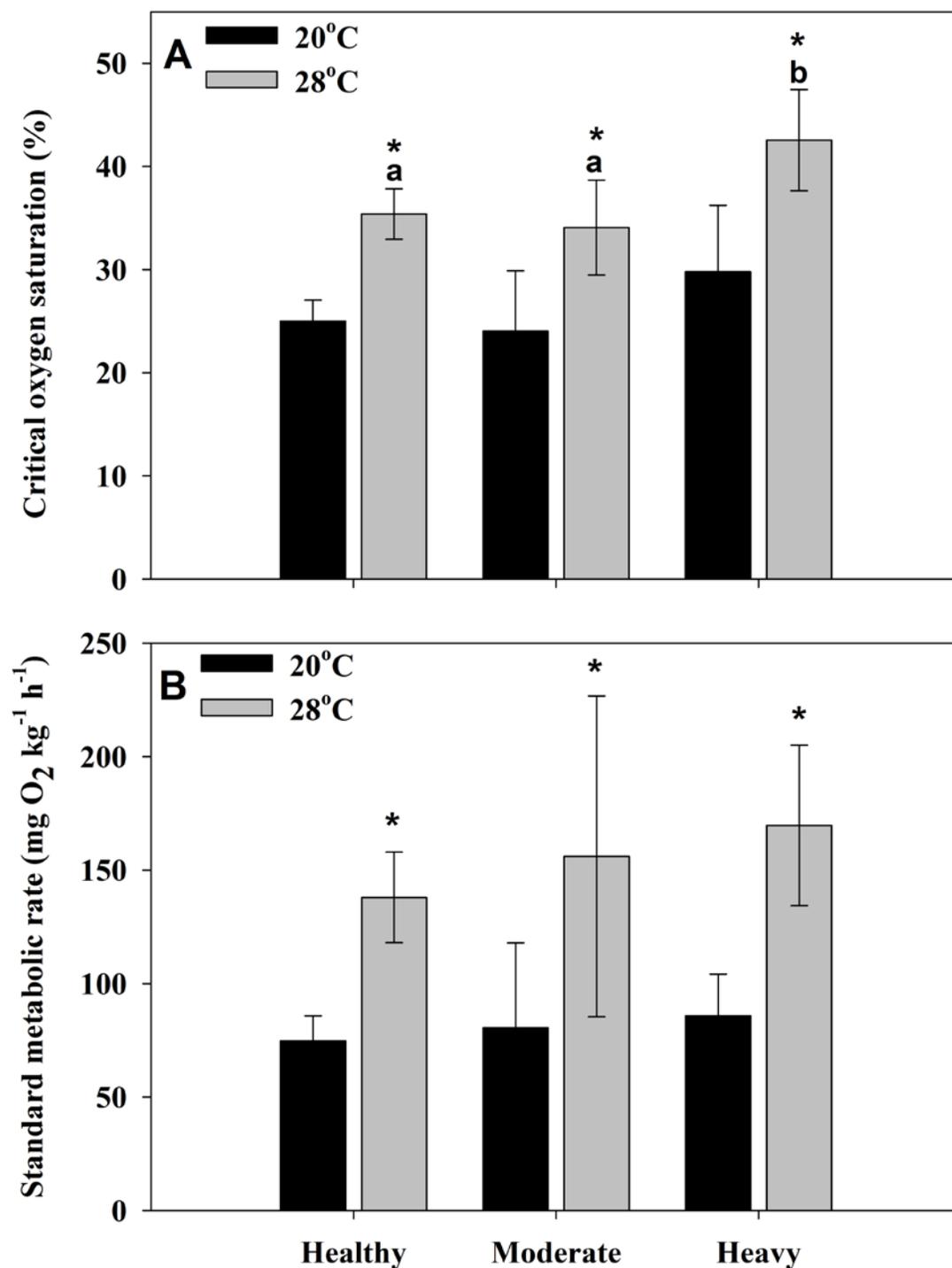


Figure 2. (a) Critical oxygen saturation and (b) standard metabolic rate measured at 20 and 28°C for 3 groups of striped bass classified according to their disease severity. Data are shown as model estimates of the mean  $\pm$  95% CI. Stars indicate a significant effect of temperature on the measured responses for a given group. Different letters indicate a significant difference between groups at a given temperature. Absence of letters indicates no significant differences.

As expected, SMR significantly increased with increasing temperature (Fig. 2b). This has been shown to be true for other species (e.g. Fry 1947, Beamish 1964, Kruger & Brocksen 1978, Schurmann & Steffensen 1997, Claireaux & Lagardère 1999, Claireaux et al. 2000, Sylvestre et al. 2007). However, we measured no significant effect of mycobacteriosis on SMR at either 20 or 28°C (Fig. 2b). The skin of fishes presents an effective protective barrier to the aqueous environment. Given that mycobacteriosis causes severe skin ulceration in striped bass, we hypothesized that osmoregulatory burden would increase with increasing dermal disease severity. This should manifest as an increase in SMR and  $\text{Na}^+\text{-K}^+\text{-ATPase}$  activity in the gill and intestine of heavily diseased fish (in this case based on dermal rather than splenic disease severity). Surprisingly, dermal disease severity had no effect on any of these parameters (Fig. 2b, Table 1). Histological examination of the dermal lesions provides a possible explanation. Among tissue samples displaying the range of dermal disease, most but not all lesions had become re-epithelialized (Fig. 3). This was true for both small incipient pigmented foci (Fig. 3a) and many expansive ulcers (Fig. 3b). Most of these skin lesions exhibited a prominent re-growth of the epidermis, although in some large ulcers this was not the case and the entire epidermal layer and scales had been lost (Fig. 3c), exposing the underlying dermis and the prominent granulomatous inflammatory lesions to the environment. It is plausible that in many instances, regrowth of epidermis minimizes the increase in osmoregulatory burden associated with ulceration and may explain why we did not see evidence of increased SMR and  $\text{Na}^+\text{-K}^+\text{-ATPase}$  activity in gill and intestine of these fish.

Mean  $\text{MMR}_N$  and  $\text{MMR}_H$  were higher after transfer to 28°C, but this increase was significant only for the Healthy group (Fig. 4a). The absence of a significant increase in  $\text{MMR}_N$  and  $\text{MMR}_H$  with increasing temperature in moderately and heavily diseased striped bass suggests that mycobacteriosis shifted the relationship between maximum metabolic rate and temperature compared to Healthy fish. This was particularly evident for  $\text{MMR}_H$  measured in the heavily diseased group. Therefore, mycobacteriosis appears to reduce maximum rates of oxygen delivery by the cardio-respiratory system at elevated water temperatures under hypoxic conditions.

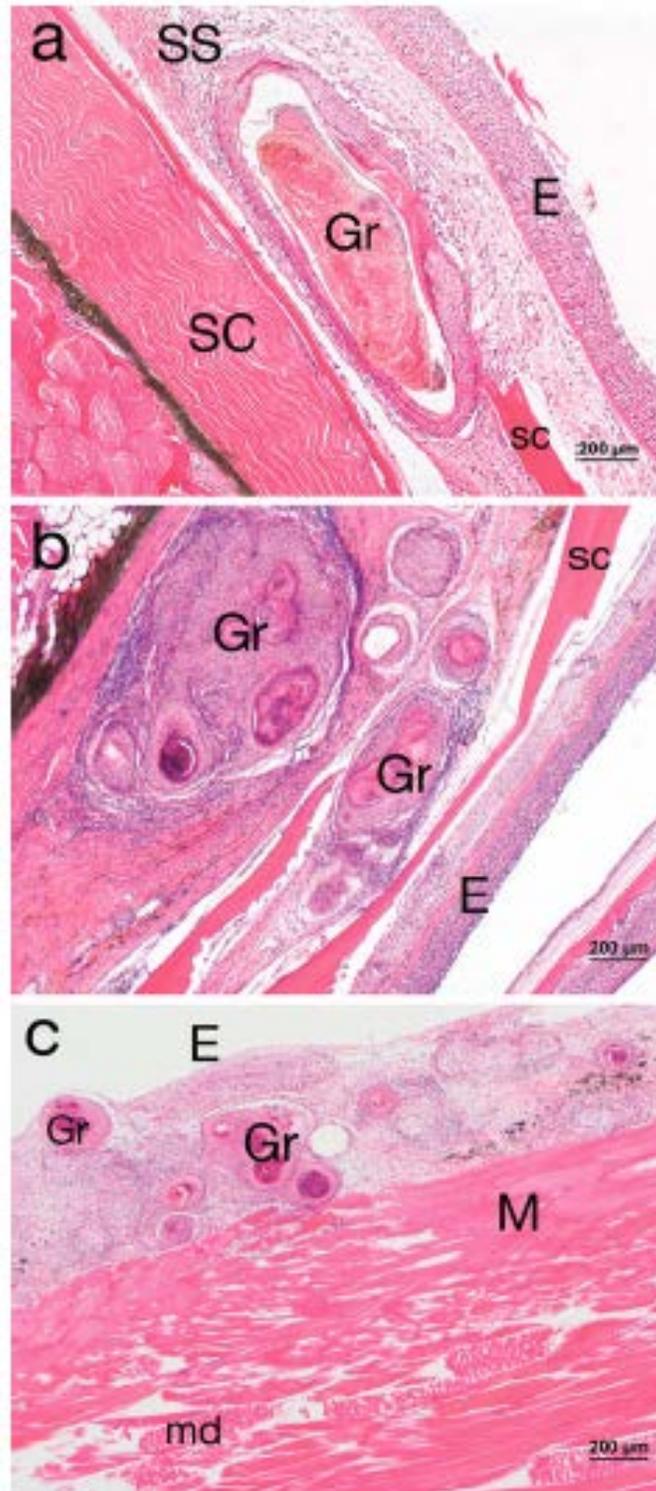


Figure 3. Dermal mycobacteriosis in Chesapeake Bay striped bass. (a) Re-epithelialization (E) of small pigmented focus. Gr: granuloma; SS: stratum spongiosum of dermis; SC: stratum compactum of dermis; sc: scale. (b) Re-epithelialization (E) of large ulcer. Gr: granuloma. (c) Large ulcer exhibiting widespread loss of epidermis and scales (E). sc: scale; Gr: granuloma; M: muscle, md: myodegeneration.

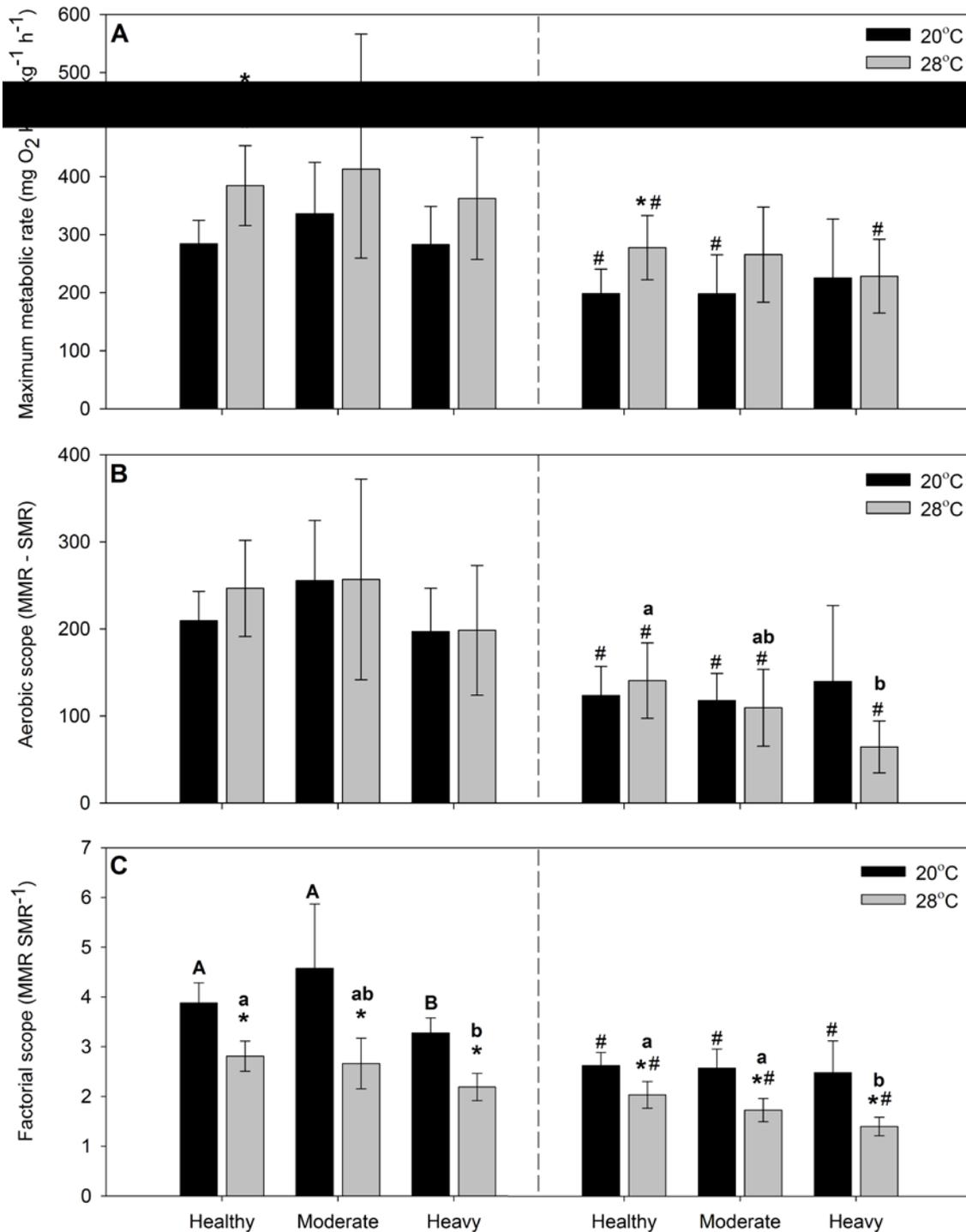


Figure 4. (a) Maximum metabolic rate (MMR), (b) aerobic scope, and (c) factorial scope measured at 20 and 28°C, under normoxic and hypoxic conditions, for 3 groups of striped bass classified according to their disease severity. Data are shown as model estimates of the mean  $\pm$  95% CI. Stars indicate a significant effect of temperature on the measured responses for a given group and oxygen level. Different letters indicate a significant difference between groups at a given temperature. Absence of letters indicates no significant differences. The dash symbols indicate a significant effect of oxygen level on the measured responses for a given group and at a given temperature. SMR: standard metabolic rate.

As for the effect of hypoxia,  $MMR_H$  was generally significantly lower than  $MMR_N$  regardless of temperature or disease state (except for the heavily diseased group at 20°C and the moderately diseased group at 28°C; Fig. 4a). This is consistent with other studies where oxygen consumption during activity, or following exhaustive exercise, was found to decrease in concert with reductions in ambient oxygen (Fry 1947, Basu 1959, Claireaux & Lagardère 1999, Claireaux et al. 2000, Dutil et al. 2007). Surprisingly,  $MMR_N$  and  $MMR_H$  did not differ between disease groups, under any set of conditions. However, this result is consistent with the absence of the effect of disease state on blood parameters.

To the best of our knowledge, this is the first study to report the interactive effects of temperature, oxygen levels, and infectious disease in finfishes. Our results indicate that act synergistically, three simultaneous stressors (elevated water temperature, hypoxia, and mycobacteriosis) reduce aerobic scope (AS) more than any single stressor acting alone (Fig. 4b). We measured no significant influence of temperature on either  $AS_N$  or  $AS_H$ . AS in fishes has long been hypothesized to increase with temperature until an optimal temperature is reached, and then decline with further increases in temperature, taking the form of a bell-shaped curve (Claireaux & Lagardère 1999, Claireaux et al. 2000, Pörtner & Farrell 2008, Farrell 2009, Pörtner 2010). Alternatively, it has been recently hypothesized that AS continues to increase beyond the optimal temperature, until close to the upper critical temperature (Clark et al. 2013). In both instances, the temperature dependence of AS is driven by the failure of maximum metabolic rate to continue increasing with temperature, while SMR exponentially increases until temperature approaches a lethal level (Fry 1947, Fry & Hart 1948, Farrell 2009). Thus, following the first hypothesis, the absence of a significant difference between AS measured at 20 and 28°C in adult striped bass may indicate that these 2 temperatures are respectively below and above the preferred or optimal temperature for adult striped bass. However, following the second hypothesis, our results for AS may suggest that 28°C is close to their upper critical temperature, and that mycobacteriosis narrows their thermal window. Alternatively, the absence of a significant influence of temperature on AS might be caused by the high inter-individual variability observed in this study.

In contrast to temperature, hypoxia significantly reduced AS.  $AS_H$  was significantly lower than  $AS_N$  at both temperatures, except for the heavily diseased group at 20°C (Fig. 4b). Interestingly,  $AS_H$  of heavily diseased fish under elevated temperature was significantly lower than  $AS_H$  of the healthy group under the same conditions (Fig. 4b). For striped bass in the healthy group,  $AS_H$  was 1.70- and 1.75-fold lower than  $AS_N$  measured at 20 and 28°C, respectively. This reduction in AS under hypoxia was similar to that reported for European sea bass (Claireaux & Lagardère 1999), common sole (Lefrançois & Claireaux 2003), and Atlantic cod (Claireaux et al. 2000, Dutil et al. 2007).

Disease alone did not influence  $AS_N$ . However,  $AS_H$  at 28°C was significantly lower in heavily diseased fish compared to the healthy group. Under the influence of all 3 stressors (28°C, hypoxia, severe disease), the AS was reduced to approximately one-third of that of healthy fish held at 20°C under normoxia.

Temperature, hypoxia, and mycobacteriosis had a synergistic effect on FS, similar to the observed effects of these factors on AS of striped bass (Fig. 4c). Because of the large differences in SMR and MMR among fish species (e.g. Brill & Bushnell 2001, Korsmeyer & Dewar 2001), we argue that FS is a more informative measure for inter-species comparisons, especially when considering the effects of temperature. In fact, FS appeared to be a more sensitive indicator of physiological impairment because temperature alone elicited a significant effect. Both  $FS_N$  and  $FS_H$  were reduced at elevated temperature (Fig. 4c), regardless of disease severity. In line with our results, a reduction in FS with increasing water temperature was previously reported for Atlantic cod (*Gadus morhua*) measured at an acclimation temperature of 7°C and following an acute transfer to 11°C (Sylvestre et al. 2007). However, in Atlantic cod acclimated to different water temperatures (acclimation period ranged from 3 weeks to several months), FS did not vary with temperature (Schurmann & Steffensen 1997, Claireaux et al. 2000). In contrast, in European sea bass acclimated for 10 to 15 d to different temperatures, FS increased with increasing acclimation temperature (Claireaux & Lagardère 1999).

Additionally,  $FS_H$  was significantly lower than  $FS_N$ , regardless of water temperature and/or disease severity (Fig. 4c). A similar reduction in FS under hypoxia has been reported for European sea bass (Claireaux & Lagardère 1999) and Atlantic cod (Claireaux et al. 2000).

Disease state also significantly influenced FS. Striped bass in the heavily diseased group had a significantly lower  $FS_N$  than fish in the healthy group, regardless of temperature. To our knowledge, this is the first report of a significant reduction in FS associated with an infectious disease. Likewise,  $FS_H$  was significantly lower in fish from the heavily diseased group compared with the healthy and moderately diseased groups, but in this case only at 28°C. Assuming that the value obtained for  $FS_N$  within the healthy group in normoxia at 20°C represents the value for healthy adult striped bass under preferred temperature and oxygen conditions, hypoxia, temperature, and severe mycobacteriosis occurring alone reduced the FS by 1.5-, 1.4-, and 1.2-fold, respectively. When the stressors of hypoxia and elevated temperature were combined, however, FS was reduced by 2.8-fold (Fig. 4c). Under the influence of all 3 stressors (28°C, hypoxia, severe disease), like AS, FS was reduced to approximately one-third of that of healthy fish held at 20°C under normoxia.

## RESULTS AND INTERPRETATION – OBJECTIVE 2

Mean fish weight, fork length, condition factor, hematocrit, and blood hemoglobin levels were not significantly different between diseased and healthy fish (Table 2).

EPHOC, peak  $M_{O_2}$ , time to peak  $M_{O_2}$  ( $t_{peak}$ ), and time to recovery ( $t_{recovery}$ ) were determined in healthy and *mycobacterium*-infected striped bass recovering from exposure to severe hypoxia under normoxic and hypoxic conditions. To the best of our knowledge, this is the first study to report on EPHOC and its associated parameters in wild-caught adult striped bass. Following the synergistic effect of elevated water temperature, hypoxia, and mycobacteriosis on the aerobic scope we observed in adult striped bass while addressing Objective 1 of this study, we hypothesized that EPHOC would be greater when a fish recovers under hypoxia compared to normoxia, and that mycobacteriosis would exacerbate any effect of hypoxia on EPHOC characteristics.

Table 2. Morphometric characteristics of striped bass from two groups determined based on the visceral disease: Healthy, Diseased. Data are shown as mean  $\pm$  95% C.I. No statistically significant differences ( $P < 0.05$ ) between groups were observed. Abbreviations are as follows: n: number of individuals. M: male. F: female. K: Fulton's condition factor; FL: fork length; Ht: hematocrit; Hb: hemoglobin concentration.

Parameter	Healthy	Diseased
n	11	3
Sex ratio (M / F)	9 / 2	3 / 0
FL (mm)	530 $\pm$ 15	549 $\pm$ 33
Mass (kg)	1.9 $\pm$ 0.1	2.1 $\pm$ 0.4
K	1.27 $\pm$ 0.03	1.24 $\pm$ 0.09
Ht (%)	39 $\pm$ 3 (n=10)	31 $\pm$ 5 (n=2)
Hb (mg/ml)	148 $\pm$ 10 (n=10)	149 $\pm$ 7

In healthy striped bass, EPHOC was significantly reduced during recovery under hypoxic conditions compared with normoxia ( $P=0.01$ ; Fig. 5). This result contrasts with our contention that EPHOC would be greater under hypoxia compared to normoxia.

Peak  $M_{O_2}$  was significantly reduced during recovery under hypoxia conditions compared with normoxia, for both healthy and diseased fish ( $P=0.003$  and  $0.05$ , respectively; Fig. 6). This result follows our hypothesis and is consistent with those reported for domesticated rainbow trout (*Oncorhynchus mykiss*) (Svendsen et al. 2012), as well as with previous demonstrations that reductions in ambient oxygen induced a decrease in

oxygen consumption during activity, or following exhaustive exercise (Fry 1947, Basu 1959, Claireaux & Lagardère 1999, Claireaux et al. 2000, Dutil et al. 2007, Lapointe et al. *In press*).

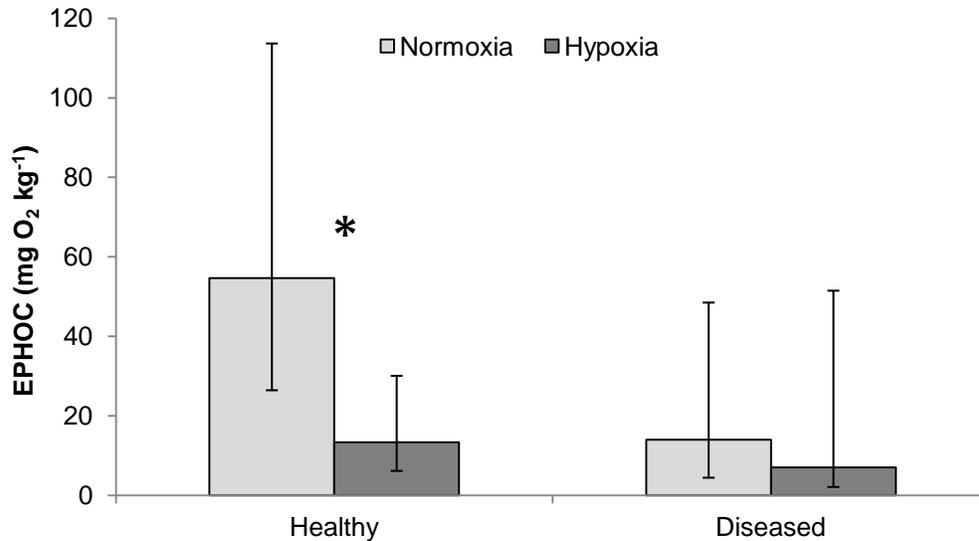


Figure 5. Excess post hypoxic oxygen consumption (EPHOC) measured at 28°C in healthy and *mycobacterium*-infected (Diseased) adult striped bass under two oxygen conditions (normoxia, hypoxia). Data are shown as bias-adjusted model estimates of the mean  $\pm$  95% C.I. Stars indicate a significant effect of oxygen level for a given group ( $P \leq 0.05$ ).

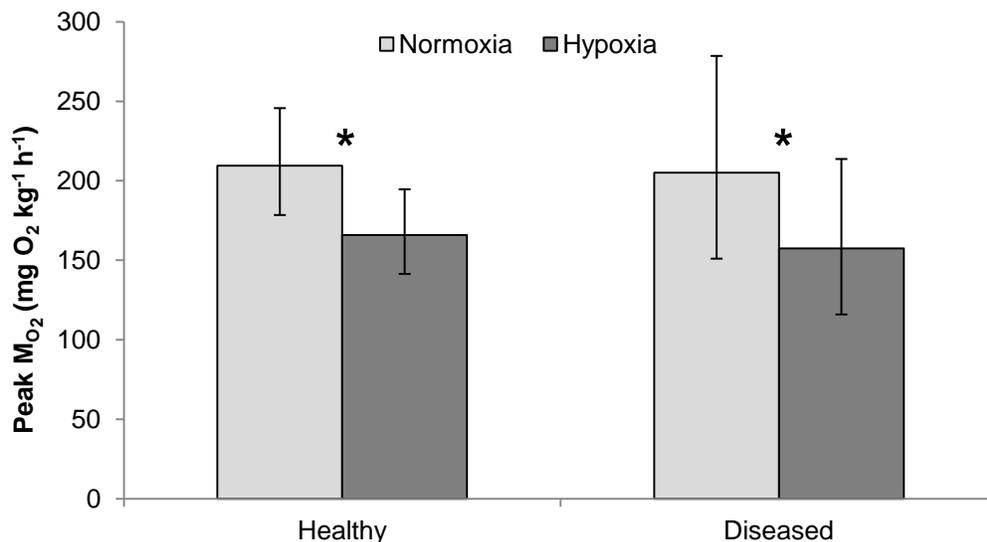


Figure 6. Peak oxygen consumption rate (peak  $M_{O_2}$ ) measured at 28°C in healthy and *mycobacterium*-infected (Diseased) adult striped bass. Data are shown as bias-adjusted model estimates of the mean  $\pm$  95% C.I. Stars indicate a significant effect of oxygen level for a given group ( $P \leq 0.05$ ).

In contrast, oxygen concentrations during the recovery period did not significantly influence time to peak  $M_{O_2}$  and time to recovery (Figs. 7 and 8, respectively). These results contrast with our contention that complete metabolic recovery following exposure to severe hypoxia would be delayed under hypoxic conditions. In rainbow trout, peak  $M_{O_2}$  was delayed and time to recovery was almost twice as long, when recovery occurred under hypoxic conditions (Svendsen et al. 2012).

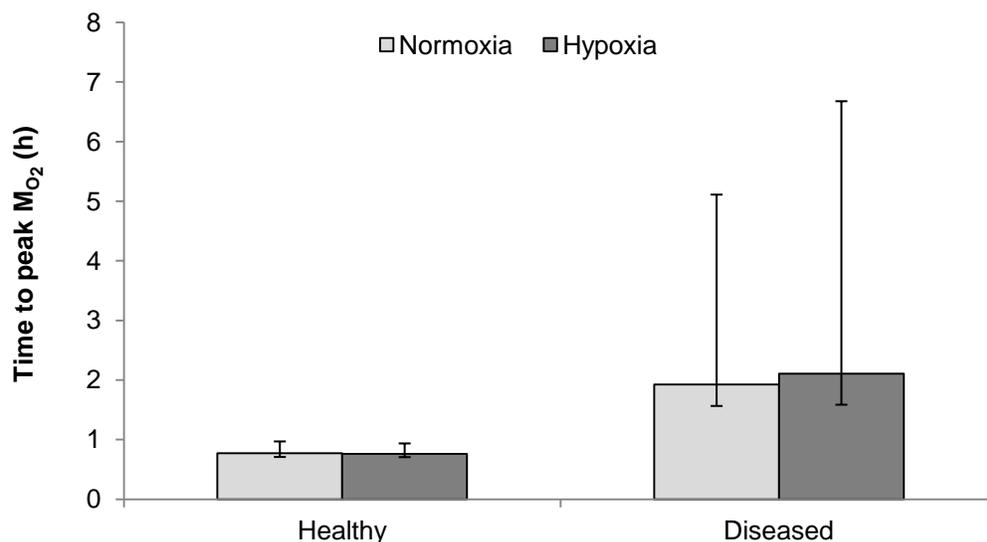


Figure 7. Time to peak  $M_{O_2}$  ( $t_{peak}$ ) measured at 28°C in healthy and *mycobacterium*-infected (Diseased) adult striped bass. Data are shown as bias-adjusted model estimates of the mean  $\pm$  95% C.I.

Finally, we hypothesized that mycobacteriosis would exacerbate the effect of hypoxia on EPHOC characteristics in adult striped bass. Surprisingly, we found no significant interaction between oxygen level and disease status. Moreover, we found no significant differences in the responses of healthy and diseased striped bass for the four EPHOC characteristics we measured. However, EPHOC under normoxia tended to be lower in diseased fish compared to healthy striped bass ( $P=0.06$ ; Fig. 5), while peak  $M_{O_2}$  seemed delayed (higher  $t_{peak}$ ) in diseased fish compared with healthy fish, regardless of oxygen concentration (Fig. 7). We believe that our small sample sizes may have hindered our ability to detect significant differences, but this warrants additional investigation.

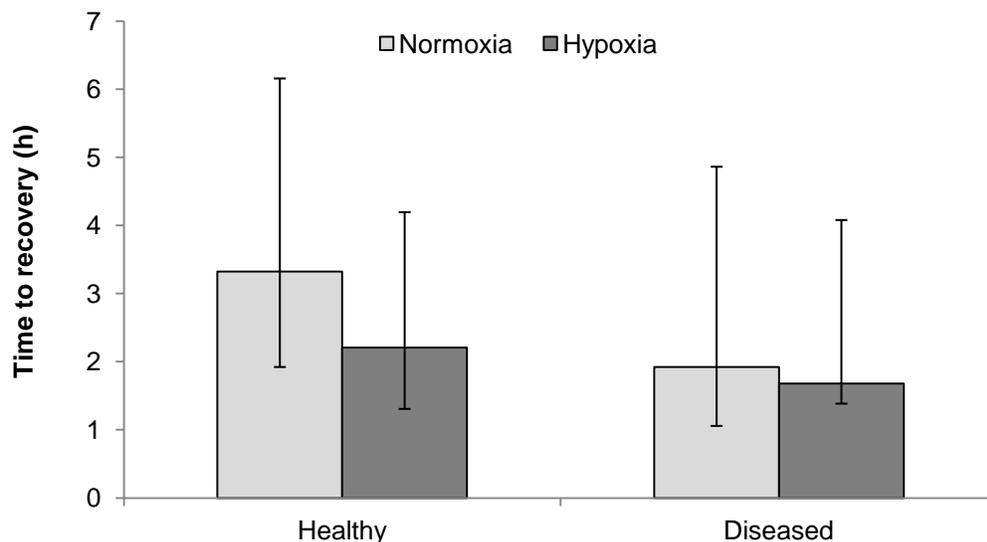


Figure 8. Time to recovery  $M_{O_2}$  ( $t_{\text{recovery}}$ ) measured at 28°C in healthy and *mycobacterium*-infected (Diseased) adult striped bass. Data are shown as bias-adjusted model estimates of the mean  $\pm$  95% C.I.

## CONCLUDING REMARKS

- Given that the scope for activity (both AS and FS) of diseased striped bass in warm hypoxic waters is greatly compromised, diseased fish under similar conditions are likely to fatigue more rapidly, be restricted in their ability to elude predators or secure prey, have lower growth rates, and exhibit reduced reproductive performance and immune function.
- Because of the high prevalence and severity of mycobacteriosis in adult striped bass from Chesapeake Bay, and our demonstration of the disease's impact on their physiological capacities (especially scope for activity under elevated temperature and hypoxia), models that attempt to quantify the impacts of environmental factors on striped bass must consider responses of healthy and diseased fish.
- The increasing occurrence and severity of summer hypoxic events in Chesapeake Bay may have negative impacts on striped bass populations. Indeed, there are documented increases in natural mortality rates associated with mycobacteriosis (Jiang et al. 2007, Gauthier et al. 2008), and a recent modeling analysis of adult striped bass from Chesapeake Bay showed that growth in disease-positive fish was compromised (Latour et al. 2012).

- We specifically argue that anthropogenic impacts on environmental quality, possible changes in forage abundance, the recent decline in striped bass condition, and the emergence of mycobacteriosis are completely interlinked processes. Therefore, we contend that summer temperature–oxygen conditions increase the prevalence and severity of mycobacteriosis, and that in turn, the disease reduces the tolerance of striped bass to elevated water temperature and hypoxia. This situation creates multiple positive feedback loops, possibly resulting in the observed high prevalence of disease, reduced growth rates, and elevated rates of natural mortality.
- Hypoxia did not impair the metabolic recovery of striped bass following exposure to oxygen level below  $S_{crit}$ , suggesting that fish are able to recover from short exposures to hypoxia in both normoxic and hypoxic environments. Whereas peak  $M_{O_2}$  was lower under hypoxia compared with normoxia, oxygen conditions had no effect on the duration of the recovery period.
- Healthy and diseased striped bass had similar responses after exposure to hypoxia, or at least our sample size did not allow the detection of significant differences in EPHOC characteristics between the two groups.

**Impacts and accomplishments** - This project provides critical insights into how the interactions of an infectious disease and environmental stressors (e.g., elevated temperature and hypoxia) impact physiologic homeostasis in a critically important finfish species. Currently, the direct effects of aquatic habitat alteration on fish populations in the Bay are difficult to predict. However, the sustainability of commercial and recreational fisheries will depend on this knowledge. We argue that this information is fundamental to the development of an ecosystem-based fisheries management plan for Chesapeake Bay that takes into account the adverse impacts of anthropogenic environmental change on disease expression and disease impacts at the highest levels of biological organization. Our results also contribute to an understanding of habitat use and availability during the summer months when environmental stressors may negatively affect behavior, health and survival of striped bass, and may serve as a model for predicting the effects of hypoxia on other fish species from Chesapeake Bay and its tributaries. Results from this study may also assist in defining effective management alternatives that necessarily must account for slower growth rates and lower survival to address changing habitat conditions within Chesapeake Bay. Mycobacteriosis is a serious fish health issue within Chesapeake Bay. Therefore, this project has direct value to both Maryland and Virginia users of the striped bass resource. Furthermore, striped bass are a highly migratory species, with significant value to stakeholders in coastal Atlantic states from Maine to Florida.

**Future research** - Projects complementing our laboratory studies would include the application of acoustic telemetry to examine the effects of hypoxia on striped bass movements in Chesapeake Bay. Using such an approach, we could determine if healthy and diseased fish respond to hypoxic conditions in dissimilar fashion, and ultimately test hypotheses and models developed using laboratory data with direct field observations of movements and distributions, and vice versa.

## Metrics

### *Publications*

Lapointe, D., Vogelbein, W. K., Fabrizio, M. C., Gauthier, D. T. and Brill, R. W. (In press) Temperature, hypoxia, and mycobacteriosis: effects on adult striped bass *Morone saxatilis* metabolic performance. *Diseases of Aquatic Organisms*. DOI: 10.3354/dao02693.

Lapointe, D., Vogelbein, W. K., Fabrizio, M. C., Gauthier, D. T. and Brill, R. W. Oxygen consumption in striped bass (*Morone saxatilis*) following exposure to severe hypoxia. Manuscript in preparation for submission to *Estuaries and Coasts*.

### *Conferences / Presentations*

Lapointe, D., Vogelbein, W.K., Fabrizio, M.C., Gauthier, D.T., and Brill, R.W. Température, hypoxie et mycobacteriose : Effets sur le métabolisme du bar d'Amérique (*Morone saxatilis*). Platform presentation at the Annual meeting of Chapitre Saint-Laurent (SETAC-SRA). Montréal (QC) June 6-7, 2013.

Fabrizio, M. C. Effects of Hypoxia on Chesapeake Bay Fishes. Presentation at the Smithsonian Environmental Research Center, Edgewater, MD. May 2013.

Fabrizio, M. C. Effects of Hypoxia on Chesapeake Bay Fishes. Keynote address at the Mid-Atlantic Meeting of the Ecological Society of America. Dover, DE. April 2013.

Lapointe, D., Vogelbein, W.K., Fabrizio, M.C., Gauthier, D.T., and Brill, R.W. Individual and interactive influence of temperature, hypoxia and mycobacteriosis on the metabolism of striped bass.

- a) Seminar presented in the Fisheries Science Department Seminar Series. VIMS. December 12, 2012.
- b) Seminar presented in the Environmental and Aquatic Animal Health Department Seminar Series. VIMS. November 13, 2012

Lapointe, D., Vogelbein, W.K., Fabrizio, M.C., Gauthier, D.T., and Brill, R.W. Effects of temperature, hypoxia and mycobacteriosis on striped bass (*Morone saxatilis*) metabolism. Platform presentation at the 10<sup>th</sup> International Congress on the Biology of Fish. Madison (WI) July 15-19, 2012.

Lapointe, D., Fabrizio, M.C., Vogelbein, W.K., Gauthier, D.T., and Brill, R.W. Quantifying the interactive effects of hypoxia, temperature, and mycobacteriosis on striped bass, and their impact on the energetics and ecology of these fish. Poster presented at the annual VA Sea Grant Workshop. Richmond, VA, February 1<sup>st</sup>, 2012.

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