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Individual- and Population-Level Effects of Temperature and Hypoxia on Two Demersal Fishes in Chesapeake Bay

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APPROVAL PAGE

This dissertation is submitted in partial fulfillment of

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DEDICATION

This dissertation is dedicated to my son,
Gregory John Marcek, born on 11/29/2017.
I hope that in the coming years I can pass on my love of science
and fascination with the natural world to you.

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ABSTRACT

Anthropogenically-induced climate change has resulted in increases in water temperature and the frequency and severity of hypoxic events in coastal areas worldwide. Temperature and hypoxia affect fishes' energetics which can, in turn, be reflected in changes in reproductive success and shifts in spatial distributions. In an effort to quantify these changes in Atlantic croaker (*Micropogonias undulatus*) and spot (*Leiostomus xanthurus*) in Chesapeake Bay. I:

(1) estimated standard and maximum metabolic rates and hypoxia tolerances at five temperatures (10, 15, 20, 25 and 30°C) using intermittent-flow respirometry,
(2) examined the effects of hypoxia exposure on metrics of reproductive potential and,
(3) developed an individual-based, dynamic-seascape model of Atlantic croaker and spot based on data from the respirometry trials,

The first set of experiments showed that metabolic scope (i.e., the difference between standard and maximum metabolic rates, and within which all aerobic metabolic processes must operate) increased with increasing temperature in both species between 10 and 20°C, but plateaued above 25°C in Atlantic croaker and above 20°C in spot. Except at 10°C, the metabolic scope of Atlantic croaker was less than that of spot at all temperatures. In contrast to previous studies with Atlantic croaker from the Gulf of Mexico, the relative expression of hypoxia-inducible factors and metrics of reproduction (gonadosomatic index, most-advanced oocyte stage, and proportion of atretic oocytes) did not differ between Atlantic croaker captured under normoxic and hypoxic conditions in Chesapeake Bay. Simulations of the movements and distribution of Atlantic croaker and spot using individual-based models suggested that these species would occupy areas with warmer and better-oxygenated water than indicated by trawl survey observations from 1988-2014. Additionally, simulations indicated that a greater proportion of Atlantic croaker and spot in the Virginia waters of Chesapeake Bay would occupy the lower portion of Chesapeake Bay than indicated by capture rates from the trawl survey. My research suggests Atlantic croaker and spot are well-adapted to the environmental conditions of Chesapeake Bay during summer and are likely not affected by the frequent hypoxic episodes occurring in the subestuaries of the lower Chesapeake Bay. The apparent larger effect of elevated temperature on the metabolic scope of spot may provide them a greater capacity for movement, growth, and reproduction in warmer conditions and thus, a competitive advantage over Atlantic croaker as water temperatures continue to rise due to anthropogenically-induced climate change. My results indicate that intermittent exposure to hypoxic conditions is unlikely to negatively affect the reproductive potential of Atlantic croaker. Additional research, however, is necessary to better understand how this intermittent hypoxia exposure affects the endocrine pathways controlling reproduction. Finally, although climate-change science frequently focuses on the effects of rising coastal water temperature, and fisheries science and management on the effects on fish distributions, the results of my individual-based models suggest that predicting the effects of anthropogenically-induced climate change should not focus on temperature alone, as this may not be the most important driver of changes in fish distribution. More specifically, other factors such as time-area specific hypoxic events,

prey availability, and predator avoidance likely contribute to the spatial distributions of these species in Chesapeake Bay.

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AUTHOR'S NOTE

The chapters that comprise this dissertation were written in manuscript format for a scientific publication. Thus, the formatting for each chapter follows the guidelines of the publication to which the manuscript was, or will be, submitted. At the time of writing, citations for individual chapters are as follows:

Chapter 2

Marcek, B.J., Brill, R.W., Fabrizio, M.C. In Review. Metabolic scope and hypoxia tolerance of Atlantic croaker (*Micropogonias undulatus* Linnaeus, 1766) and spot (*Leiostomus xanthurus* Lacepède, 1802), with insights into the effects of acute temperature change. Journal of Experimental Marine Biology and Ecology.

Chapter 3

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(intended for submission to PLOS One)

Chapter 4

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Individual- and Population-Level Effects of Temperature and Hypoxia on Two Demersal Fishes in Chesapeake Bay

STRUCTURE OF THE DISSERTATION

This dissertation is comprised of five chapters – an introduction, three chapters reporting my research results, and a fifth chapter of concluding remarks. The three chapters on my research results describe:

1. the results of my laboratory experiments investigating the effect of temperature on the metabolic scope and hypoxia tolerance of Atlantic croaker and spot using intermittent-flow respirometry (Chapter 2).
2. the effects of hypoxia exposure on metrics of reproductive potential (gonadosomatic index, most-advanced oocyte stage, and proportion of atretic oocytes) for Atlantic croaker captured in the Virginia subestuaries of Chesapeake Bay (Chapter 3).
3. development an individual-based, dynamic-seascape model of fish distribution in the lower Chesapeake Bay and its subestuaries, the James, York, and Rappahannock rivers for 1988-2014 (Chapter 4).

In Chapter 3 I describe the use of quantitative polymerase chain reaction (qPCR) to investigate the hypoxia exposure history of Atlantic croaker through the expression of hypoxia-inducible factors and the results of modeling efforts to determine the impact of hypoxia exposure on the reproductive potential of Atlantic croaker in Chesapeake Bay. Chapter 4 includes data from field-based sampling, and simulation modeling to investigate the individual- and population-level effects of temperature and hypoxia on the movements and distribution of Atlantic croaker and spot in Chesapeake Bay. These include movement submodels within the individual-based model such that simulated individuals move to areas where environmental conditions optimize their metabolic

scope. I compare the results of these simulations to the observed distributions of Atlantic croaker and spot from the VIMS Juvenile Fish Trawl Survey over the same period (1988-2014). I then discuss the results of these comparisons and suggest improvements to the individual-based models. Chapter 5 summarizes of the results of my dissertation and suggests future research.

CHAPTER 1

Introduction

Stock assessments, which monitor the status and trends of harvested fish populations and play a vital role in the management of marine species (NMFS 2001), are founded on the principals of population dynamics. In recent years, the identification and delineation of essential fish habitat were recognized as critical components of stock assessments and ultimately fisheries management plans (Rosenberg et al. 2000; Levin and Stunz 2005; Valavanis et al. 2008). Stock assessment methods, however, typically ignore spatial variability in vital rates of fish populations or stocks. The ability to incorporate spatially-explicit and dynamic information into stock assessments is becoming more critical as scientists and managers recognize the need to understand the effect of environmental conditions (and especially the effects of anthropogenically-induced climate change) on the observed distribution and abundance of fishes (NRC 1999; NMFS 2001).

Distributions of a particular fish species change in space and time and reflect habitat selection decisions (e.g., substrate, temperature, salinity, dissolved oxygen) of individuals within the constraints imposed by their physiological abilities and tolerances. Habitat selection is thus shaped by the ability of an individual to detect, tolerate, and respond to changes in environmental conditions (Kramer et al. 1997; Cardona 2000; Fulford et al. 2011; Horodysky et al. 2015; Cooke et al. 2016). Fluctuations in environmental conditions may lead to short-term variability in the productivity of a population. However, long-term directional changes in estuarine habitat quality, such as those predicted under climate-change scenarios, may lead to increases in the extent and duration of suboptimal environmental conditions (Hayhoe et al. 2007; Najjar et al. 2010; Harding et al. 2015). I therefore contend (as have others; Secor et al. 2009; Tian et al.

2009; Kerr et al. 2010) that such changes can affect the productivity, stability, and resilience of fish populations.

In temperate estuaries, environmental conditions are dynamic across a broad range of temporal frequencies (e.g., tidal, daily, seasonal, annual) and are known to influence the movements and distribution of fishes. In contrast, anthropogenically-induced, directional changes in water temperature and dissolved oxygen concentrations will influence the movements and distribution of fishes in a sustained directional manner (Murawski 1993; Eaton and Scheller 1996; Roessig et al. 2004; Craig and Crowder 2005; Perry et al., 2005; Sabatés et al., 2006; Brady and Targett, 2013; Buchheister et al., 2013). Such directional increases in water temperature are already apparent in many rivers in the United States, including tributaries of Chesapeake Bay (Kaushal et al. 2010). Hypoxic areas in Chesapeake Bay and its tributaries, which were historically restricted to the deep channels in summer months (Officer et al. 1984; Hagy et al. 2004), have likewise increased in magnitude, spatial extent, and duration due to anthropogenically-induced, directional climate change (Cooper and Brush 1991; Hagy et al. 2004; Murphy et al. 2011). Increases in the severity and extent of hypoxic episodes are likely to have detrimental effects on commercially and recreationally important fisheries targeting Atlantic croaker (*Micropogonias undulatus*), a species that commonly inhabits the deeper parts of Chesapeake Bay during summer. Other sympatric (and likewise important to both commercial and recreational fisheries) species such as spot (*Leiostomus xanthurus*) appear, however, to be better able to cope with hypoxia than Atlantic croaker (Bell and Eggleston 2005, Eby et al. 2005), although this conclusion is based solely on field-based observations.

Increased temperature and hypoxic events are known to negatively impact both the distribution and abundance of fishes (Breitburg 2002; Craig and Crowder 2005; Buchheister et al. 2013). Many studies have investigated the relationship between environmental factors and the distribution and abundance of inshore fishes and several have proposed mechanistic explanations for these relationships (see Humston et al. 2000; 2004; Fulford et al. 2011; 2014; 2016; Rose et al. 2013a; 2013b; 2018a; 2018b). Studies proposing a mechanism for habitat selection often suggest that selection is based on a species' preference for environmental conditions (e.g., a specific temperature or salinity). Another possible explanation for the link between environmental characteristics and fish movements and distribution is based on the concept of metabolic scope (i.e., the difference between the minimum and maximum aerobic metabolic rates, and within which life processes (e.g., movement, gonadal and somatic growth, etc.) must operate (Fry 1947; 1971; Claireaux and Lefrançois 2007; Horodysky et al. 2015)). I contend, therefore, that it is plausible that fish alter their distribution to maintain an optimal metabolic scope, or at least a metabolic scope within set limits. Furthermore, a reduction in metabolic scope under sub-optimal environmental conditions is likely to result in decreased somatic growth (Pihl et al. 1992; Eby et al. 2005; Powers et al. 2005; Long and Seitz 2008; Brandt et al. 2009; Stierhoff et al. 2009) and impaired reproduction (Wu et al. 2003; Thomas et al. 2006; 2007; Wang et al. 2008; Thomas and Rahman 2009a; 2009b; 2012; Wu 2009; Tuckey and Fabrizio 2016). The latter effect is particularly significant because impaired reproduction reduces the ability of a fish population to sustain a given level of fishing mortality or a given population size. For example, simulations in which annual mild, intermediate, and severe hypoxic conditions were randomly selected with

probabilities of 0.18, 0.52, and 0.30, respectively (see Rose et al. 2018a for details), predicted a 25% reduction in the Atlantic croaker population in the northern Gulf of Mexico during a 100-year period (Rose et al. 2018b).

To understand the effects of environmental conditions on fish populations, we must first understand effects on individuals within the population. In this study, I integrated individual- and population-level effects of temperature and hypoxia on Chesapeake Bay fishes by:

(1) investigating the effect of temperature on the metabolic scope and hypoxia tolerance of Chesapeake Bay Atlantic croaker and spot,

(2) incorporating the relationship between temperature and metabolic scope and hypoxia tolerance into an individual-based, dynamic-seascape model of fish distribution in the Virginia waters of Chesapeake Bay,

and

(3) examining the relationship between hypoxia exposure and reproductive potential of Atlantic croaker in the Virginia subestuaries of Chesapeake Bay.

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CHAPTER 2

Metabolic Scope and Hypoxia Tolerance of Atlantic Croaker (*Micropogonias undulatus* Linnaeus, 1766) and Spot (*Leiostomus xanthurus* Lacepède, 1802), with Insights into the Effects of Directional Climate Change

Abstract

The magnitude, extent, and frequency of hypoxic waters have increased in coastal and estuarine environments as a result of anthropogenic nutrient inputs and greater stratification of the water column due to rising temperatures. Under current climate change forecasts, it is likely that temperatures will continue to rise, thus exacerbating hypoxic conditions and affecting fishes that reside in these habitats. Increases in temperature will lead to an increase in the cost of maintaining homeostasis and may result in a decrease in metabolic scope and, therefore, an individual's ability to undergo aerobic processes such as growth, reproduction, and movement. Increasing temperatures can also decrease hypoxia tolerance in fishes, which may result in a decrease in the amount of available habitat. I used intermittent-flow respirometry to determine the effects of temperature on the metabolic scope and hypoxia tolerance of two economically and ecologically important species, Atlantic croaker and spot. Metabolic scope increased from 10 to 25°C but did not change from 25 to 30°C for Atlantic croaker. Similarly, metabolic scope increased from 10 to 20°C but did not change between 20 and 30°C for spot. The metabolic scope of Atlantic croaker was lower than that of spot at all temperatures examined, except at 10°C. Hypoxia tolerance did not differ by species or temperature. Our results indicate that Atlantic croaker and spot are well-adapted to the conditions currently experienced in Chesapeake Bay. As directional climate change results in warmer waters, however, the greater metabolic scope of spot may result in a competitive advantage over Atlantic croaker because it provides spot a greater capacity for movement, growth, and reproduction under warmer conditions.

1. Introduction

Throughout the world, seasonal hypoxic episodes (herein defined as waters with oxygen concentration < 2 mg L^{-1}) occur in coastal and estuarine environments, frequently during warmer months (Diaz and Rosenberg, 2008). Hypoxia is often driven by a combination of oxygen consumption through the decomposition of organic matter and density-driven stratification of the water column which isolates bottom waters from exchange with oxygen-rich surface waters (Taft et al., 1980; Bishop et al., 2006; Tyler et al., 2009). The extent and severity of these hypoxic events are, however, exacerbated by increasing temperatures as well as expanded urbanization and increasing agricultural runoff (Diaz and Rosenberg, 2008; Lyman et al., 2010; Rabalais et al., 2010). Such degradation in environmental conditions impacts fishes at the individual level through changes in both routine and maximum metabolic rates (e.g., Pörtner, 2010; 2012; Claireaux and Chabot, 2016).

Increases in the metabolic rates of fishes with increasing water temperature are well-documented across taxa and have been used as a means of determining the temperature at which a species performs optimally through the concept of aerobic metabolic scope (hereafter "metabolic scope") (e.g., Fry, 1947; 1971; Bozinovic and Pörtner, 2015). Metabolic scope is the difference between standard metabolic rate (SMR), the minimum metabolic rate necessary for the maintenance of homeostasis, and maximum metabolic rate (MMR). Metabolic scope in fishes generally increases to a thermal optimum, then decreases rapidly with further increases in temperature (Pörtner and Peck, 2010; Clark et al., 2013; McBryan et al., 2013). Rapidly changing environmental conditions, and the resultant changes in metabolic scope, therefore have

major implications for fishes because metabolic scope represents the state-space within which all aerobic activities must occur (Brander, 2015; Marras et al., 2015; McKenzie et al., 2016). Like metabolic rates, metabolic scope responds to changes in temperature but the relationship is species-specific (e.g., Guderly and Pörtner, 2010; Marras et al., 2015; Gunderson et al., 2016). Because metabolic scope governs processes such as feeding, movement, and gonadal and somatic growth rates, changes in metabolic scope resulting from variations in temperature and oxygen conditions have population-level impacts (Pankhurst and Munday, 2011; Peck et al., 2016). Understanding the relationship between environmental conditions and metabolic scope is, therefore, critical to understanding and predicting population-level processes (such as the ability of a species to withstand various levels of fishing mortality) and the development of effective fishery management and resource conservation plans and policies (Horodysky et al., 2015; Cooke et al., 2016; Townhill et al., 2017).

Effective fisheries management is especially important in temperate estuaries because these areas provide nursery and foraging grounds for many commercially and recreationally important species. For instance, Chesapeake Bay is inhabited by more than 350 species of fish throughout the year (Murdy and Musick, 2013), many of which are subject to heavy fishing pressure. The effects of environmental conditions on fish metabolism have only been investigated in a handful of these species (Horodysky et al. 2011; Capossela et al., 2012; Lapointe et al., 2014). This is concerning because Chesapeake Bay, like other temperate estuaries, is experiencing increasing water temperatures which intensifies stratification, resulting in a reduction in turnover between the warm, oxygen-rich surface waters and the cooler bottom waters. This process

increases the magnitude, duration, and spatial extent of seasonal hypoxic events (Cooper and Brush, 1991; Hagy et al., 2004; Kemp et al., 2005; Kaushal et al., 2010). Such conditions have been hypothesized to result in a temperature-oxygen squeeze whereby fish that would normally use deeper, cooler waters as a thermal refuge during summer are forced into suboptimal hypoxic habitats (Coutant, 1985).

To determine the potential impacts of increases in temperature on the metabolism and hypoxia tolerance of estuarine fishes, we used intermittent-flow respirometry to investigate differences in the metabolic scope and hypoxia tolerance of Atlantic croaker (*Micropogonias undulatus* Linnaeus, 1766) and spot (*Leiostomus xanthurus* Lacepède, 1802) at temperatures commonly experienced in Chesapeake Bay. These two sciaenid species are common along the U.S. Atlantic seaboard and use estuaries as nursery and foraging grounds from spring to fall, migrating offshore to spawn during fall (Moser and Gerry, 1989; Barbieri et al., 1994). In Chesapeake Bay, Atlantic croaker and spot support important commercial fisheries accounting for combined annual landings ranging from 3000 to 8500 metric tons (from 2000 to 2015; https://www.st.nmfs.noaa.gov/commercial-fisheries/commercial-landings/).

2. Methods

All animal capture, handling, and experimental procedures followed approved Institutional Animal Care and Use Committee protocols (IACUC-2014-06-13-9557-mcfabr and IACUC-2015-04-29-10380-mcfabr) and all applicable U.S. regulations.

2.1. Animal Subjects

Atlantic croaker and spot were captured using either a commercial pound net (September 2, 2014 and August 26, 2015) or a 9.14 m otter trawl (August – September, 2014 and April – September, 2015). All fish were transported to the VIMS Seawater Research Laboratory where they were measured for length (total length (TL) for Atlantic croaker, fork length (FL) for spot) to the nearest mm (range: 235 – 317 mm TL for Atlantic croaker, 194 – 234 mm FL for spot) (Table 1). Fish masses (to the nearest gram) were obtained prior to the respirometry trials. Atlantic croaker masses ranged from 137 to 388 g whereas spot masses ranged from 112 to 195 g (Table 1). Masses were converted to kg for the calculation of metabolic rates (see below). Kruskal-Wallis tests were used to investigate potential differences in the length and mass of fish subjected to trials at different temperatures.

Prior to transferring fish to holding tanks, elastomer tags, color coded by location of capture (the main stem of Chesapeake Bay, or the James, York, or Rappahannock rivers) were injected subdermally, posterior to the right eye (FitzGerald et al., 2004). Fish were held in two 1800 L recirculating holding tanks for a minimum of two weeks before being subjected to respirometry trials. Holding tanks were maintained at 20°C and a salinity of 22.2 ± 0.2‰ (i.e., ambient salinity at the mouth of the York River). Fish were

fed to satiation three times per week using commercial pellets. Water quality was checked twice per week and water changes performed as necessary.

2.2 Respirometry

Intermittent-flow respirometry (Steffensen, 1989) was used to determine the maximum metabolic rate (MMR), standard metabolic rate (SMR), and critical oxygen saturation (S_{crit}) for Atlantic croaker and spot at five temperatures at which Atlantic croaker and spot commonly occur in Chesapeake Bay (10, 15, 20, 25, and 30°C). Respirometry trials were conducted in either a 7.5-L or 4.1-L static respirometry chamber to maintain chamber volumes at ~20-50 times the mass of the fish as recommended by Forstner (1983) and Svendsen et al. (2016). Temperature, species, and individuals were pseudo-randomly selected prior to each trial.

The metabolic rates of either two Atlantic croaker or two spot at one of the five trial temperatures were simultaneously measured (mg O_2 kg^{-1} hr^{-1}) in independent respirometry chambers. Chambers were submerged in separate, temperature-controlled water baths bubbled with air to maintain normoxic conditions and covered to reduce visual stimuli. Oxygen levels in the respirometers were measured using fluorescence oxygen sensors (Presens, Regensburg, Germany). Sensors were mounted either in a flow-through cell inserted in the water circulation tubing or directly in the respirometer. Computers recorded oxygen saturation (%) every second using custom-designed software in Dasylab 13.0 (National Instruments, www.ni.com) and converted oxygen saturation to concentration (mg O_2 L^{-1}) using standard equations (Richards, 1965) within the software routines.

Metabolic rate (MO_2) was measured using a 10-15 min cycle consisting of a 5 min flush, a 1-1.5 min equilibration period, and a 4-9.5 min data recording interval. Measurement periods varied based on the mass of the fish relative to respirometer volume. Following Lapointe et al. (2014), the rate of change of O_2 concentration over time ($\Delta[O_2]\ t^{-1}$) was calculated using a linear regression of recorded oxygen concentrations against elapsed time (t) after the conclusion of the data recording interval. MO_2 was then calculated as:

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

where: V is the respirometer volume (L) corrected for fish volume, and W is the weight of the fish (kg). MO_2 was adjusted to a body weight of 1 kg to account for variations in MO_2 due to differences in size among fish using a weight exponent of 0.82 (Edwards et al., 1972) using the equation:

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

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. To account for bacterial respiration during respirometry trials, background oxygen consumption was measured at the completion of each experiment, and subtracted from respiration values measured when fish were in the respirometer.

Prior to respirometry trials, fish were transferred to a 260 L holding tank at 20ºC. The temperature in the holding tank was adjusted to the trial temperature (10, 15, 20, 25, or 30°C) during a 3-6 hr period, and fish were allowed to acclimate to the trial temperature for ~36 hrs during which they were not fed. Fish were then exercised to exhaustion (determined as the point when they no longer avoided handling) and subjected to a brief (~1 min) air exposure (Ferguson and Tufts, 1992; Donaldson et al., 2010; Clark

et al., 2012; Roche et al., 2013) during which they were weighed (g). Following air exposure, fish were introduced to the respirometer and the MMR of each individual was determined as the single highest MO_2 obtained under normoxic conditions. Fish remained in the respirometer overnight to allow recovery from exercise and handling. Because the MO_2 data were highly variable and included the acclimation period, the SMR was calculated using the 15% quantile method recommended by Chabot et al. (2016). Metabolic scope was calculated as the difference between MMR and SMR.

To determine the critical oxygen saturation (S_{crit}), the oxygen content of the water in the outer bath was reduced in a stepwise fashion by bubbling nitrogen into the system. Reductions in ambient oxygen continued to levels at which metabolic rate decreased simultaneously with decreases in ambient oxygen. S_{crit} was determined using a piecewise regression fitted to the metabolic rate-oxygen saturation data where S_{crit} was the oxygen saturation at which metabolic rate began to decrease below the estimated SMR (Schurmann and Steffensen, 1997; Lapointe et al., 2014; Brill et al., 2015). To account for differences in the solubility of oxygen at different temperatures and salinities (albeit, over a narrow range of salinities), S_{crit} values were converted to critical oxygen concentration (C_{crit}, mg O_2 L^{-1}) using an online algorithm (*baliga.systemsbiology.net/drupal/sites/default/.../DO-percent-to-mg-per-L-Calculator....*).

At the conclusion of each respirometry trial, fish were euthanized via immersion in an ice-water slurry (Blessing et al., 2010). Following euthanasia, the sex of each fish was determined and condition was measured using the Distell Fish Fatmeter, which uses

microwaves to measure subdermal lipid content (Distell.com), and by calculating Fulton's K (Schloesser and Fabrizio, 2017) using the equation:

$$K = 100 \times (W \times L^{-3}) \quad (3)$$

where W is the fish's weight in grams and L is its length in centimeters. Finally, the age of individual fish was determined from the otoliths following Barbieri et al. (1994). Ages were assigned by three readers and agreement between at least two readers was necessary to assign an age to an individual. Chang's coefficient of variation was calculated to determine the degree of reader agreement (Chang, 1982).

2.3 Analysis

Because multiple, correlated responses were measured during the respirometry trials (Table 2), a multivariate approach was used to analyze the data (Tabachnick and Fidell, 2007). Additionally, because a combination of fixed effects and a random effect of individual fish were included in the model, we used a generalized linear mixed model (Littell et al., 2006; Bolker et al., 2008). To identify the most appropriate model for these data, we followed the model fitting procedures outlined in Henderson et al. (2014) and Marcek et al. (2016). First, potential predictors were investigated graphically prior to incorporation in the model and included species, temperature, age, sex, condition, chamber (two chambers were used), and location of capture (main stem of Chesapeake Bay or the James, York, or Rappahannock rivers). Graphical analysis indicated that the metrics of condition (fatmeter readings and Fulton's K) were correlated and therefore only one condition metric could be included in the final models. The graphical analysis suggested that temperature and condition were likely the only important predictors of

metabolic parameters for both spot and Atlantic croaker, although age appeared to be an important predictor of metabolic parameters for Atlantic croaker.

Due to small sample sizes of Atlantic croaker greater than age 4, all individuals of age 5 and older were pooled into a 5+ group and the potential effects of age were investigated using an Analysis of Variance which supported the inclusion of age in the model describing the metabolic rates and critical oxygen saturations of Atlantic croaker. Because age appeared to be an important predictor for Atlantic croaker but not for spot, separate statistical models were used to describe the metabolic responses of each species to temperature, condition, and age.

Following the identification of appropriate fixed effects, the covariance structure of the models was determined using the Akaike's Information Criterion corrected for small sample sizes (AIC_c). Four covariance structures were investigated prior to fitting the models: variance components (vc), compound symmetry (cs), Toeplitz (toep), and unstructured (un). For both Atlantic croaker and spot, AIC_c was minimized using a Toeplitz covariance matrix (Atlantic croaker: $AIC_{c,\ vc}$ = 1988.8, $AIC_{c,\ cs}$ = 1965.9, $AIC_{c,\ toep}$ = 1779.2, $AIC_{c,\ un}$ = dnc; spot: $AIC_{c,\ vc}$ = 1354.0, $AIC_{c,\ cs}$ = 1341.0, $AIC_{c,\ toep}$ = 1224.9, $AIC_{c,\ un}$ = dnc; dnc = did not converge), indicating that a Toeplitz covariance matrix best described the random variation in the metabolic rate observations of both species. Following these preliminary model-building investigations, the multivariate model describing the effect of temperature (Temp), condition (Cond), and age on the metabolism and hypoxia tolerance of Atlantic croaker followed the form:

$$SMR_{ijk}, MMR_{ijk}, MS_{ijk}, S_{crit,\ ijk} \quad (4)$$
$$= Intercept + Temp_j + Cond + Age_k + \varepsilon_{ijk},$$

where the metabolic parameters of individual *i* were modeled as a function of temperature *j*, condition, and age *k*. *Intercept* represents the overall mean response, *Temp* represents the effect of trial temperature, *Cond* represents the effect of fish condition as measured by Fulton's k, *Age* represents the effect of fish age, and ε_{ijk} represents the random, unexplained variation in the model. The model describing how temperature and condition affected the metabolism and hypoxia tolerance of spot followed the form:

$$SMR_{ij}, MMR_{ij}, MS_{ij}, S_{crit,\ ij} = Intercept\ +\ Temp_j\ + Cond\ + \varepsilon_{ij}. \qquad (5)$$

Similar to the model for Atlantic croaker, the metabolic parameters of individual *i* were modeled as a function of temperature *j* and condition. *Intercept* represents the overall mean response, *Temp* represents the effect of trial temperature, *Cond* represents the effect of fish condition as measured by Fulton's k, and ε_{ijk} represents the random, unexplained variation in the model. Analyses were performed using the MIXED procedure in SAS version 9.3 (SAS Institute, Cary, NC).

3. Results

3.1 Animal Subjects

Mean fish lengths (± standard error) for Atlantic croaker and spot were 274 ± 3 mm and 209 ± 1 mm, respectively. All individuals were considered adults. Mean fish masses (± standard error) were 248 ± 10 g for Atlantic croaker and 147 ± 2 g for spot. Length and mass did not differ significantly among individuals exposed to different temperature treatments for either Atlantic croaker (length: $\chi^2 = 4.65$, $P = 0.33$; mass: $\chi^2 = 7.50$, $P = 0.11$) or spot (length: $\chi^2 = 6.03$, $P = 0.20$; mass: $\chi^2 = 8.52$, $P = 0.07$). Atlantic croaker ranged in age from 3 to 10 years, whereas spot ages ranged from 1 to 2 years. Chang's coefficient of variation was 4.4%, indicating a high degree of reader agreement and is below the 5% threshold recommended by Campana et al. (2001).

3.2 Respirometry

Multivariate models that included the effects of temperature, condition, and age on metabolic rate and hypoxia tolerance explained approximately 53% of the total variance in responses (i.e., SMR, MMR, metabolic scope, and S_{crit}) of Atlantic croaker and 52% of the total variance in responses of spot. These models indicate that temperature was the only significant predictor of responses measured for Atlantic croaker and spot ($F = 7.46$, $P < 0.01$ and $F = 15.39$, $P < 0.01$, respectively).

As expected, mean SMRs increased with increasing temperature in both species (Figure 1), but were similar between species at all temperatures; except 30°C where the mean SMR of spot (mean: 263.4, 95% CI: 223.2-303.6 mg O_2 kg^{-1} hr^{-1}) was greater than that of Atlantic croaker (mean: 171.6, 95% CI: 136.1-207.1 mg O_2 kg^{-1} hr^{-1}). Mean

MMRs also increased with increasing temperature in both species (Figure 2). In contrast to SMR, the mean MMR of spot was greater than that of Atlantic croaker at all temperatures except 10°C (spot – mean: 256.9, 95% CI: 216.8-297.0 mg O_2 kg^{-1} hr^{-1}; Atlantic croaker – mean: 231.5, 95% CI: 178.7-284.2 mg O_2 kg^{-1} hr^{-1}). Similar to SMR and MMR, the mean metabolic scope of Atlantic croaker and spot increased with increasing temperature (Figure 3). The mean metabolic scope of spot exceeded that of Atlantic croaker at all temperatures except 10°C (spot – mean: 194.2, 95% CI: 154.0-234.3 mg O_2 kg^{-1} hr^{-1}; Atlantic croaker – mean: 170.8, 95% CI: 118.0-223.6 mg O_2 kg^{-1} hr^{-1}). Unlike SMR and MMR, however, the mean metabolic scope of both species appeared to plateau at higher temperatures (Figure 3). Mean metabolic scope was not significantly different between 25 and 30°C for Atlantic croaker, nor between 20, 25, and 30°C for spot.

Although mean S_{crit} appeared to increase with increasing temperature, there were no significant changes in mean S_{crit} with temperature for either species due to the large amount of variation (Figure 4). Mean estimates of S_{crit} ranged from 16.3% at 10°C to 34.8% at 30°C for Atlantic croaker, and from 19.1% at 10°C to 29.0% at 30°C for spot. Estimates of mean S_{crit} were also similar between species (Figure 4). Mean estimates of C_{crit} ranged from 1.6 mg O_2 L^{-1} to 2.3 mg O_2 L^{-1} for Atlantic croaker and from 1.5 mg O_2 L^{-1} to 2.0 mg O_2 L^{-1} for spot. There were no significant differences in mean C_{crit} with temperature or species (Figure 5).

4. Discussion

Mean SMR, MMR, and metabolic scope increased with increasing temperature for both Atlantic croaker and spot, which is consistent with other marine ectotherms and is indicative of the pervasive effect of temperature on the physiology of these organisms (e.g., Schulte, 2015; Whitney et al., 2016). The mean SMRs of Atlantic croaker and spot were similar at all temperatures tested, except 30°C. At this temperature, the mean SMR of spot exceeded that of Atlantic croaker indicating that the minimum metabolic requirements of spot to maintain homeostasis are greater than those of Atlantic croaker. In contrast, the mean MMR and mean metabolic scope of spot exceeded that of Atlantic croaker at all temperatures except 10°C. These findings are consistent with those of Horodysky et al. (2011), where at 25°C, the SMR, MMR, and metabolic scope of spot were greater than those of Atlantic croaker. The differences in metabolic scope of these species, found in both studies, indicate that spot have a greater aerobic state-space within which movement, growth, and reproduction can be undertaken. This is demonstrated by faster growth in spot, where 84% of cumulative growth occurs in the first year and 99% occurs by age 2 (Piner and Jones, 2004), than in Atlantic croaker, where 64% of cumulative growth occurs in the first year and 84% occurs by age 2 (Barbieri et al., 1994). Additionally, spot attain a greater weight-at-length than Atlantic croaker (Barbieri et al., 1994; Piner and Jones, 2004). Faster growth rates are likely to confer a competitive advantage to spot as suitable habitat space is compressed under changing environmental conditions.

The expectation under the Oxygen and Capacity-Limited Thermal Tolerance (OCLTT) hypothesis is that metabolic scope will decrease as temperature increases

beyond a species' thermal optimum (Pörtner, 2010). Interestingly, the metabolic scope of Atlantic croaker and spot plateau at warm temperatures, which may indicate a broad range of temperature optima for both species (25-30°C for Atlantic croaker; 20-30°C for spot) and suggests that they are well-adapted to the temperature conditions they currently experience in Chesapeake Bay. Although observations from the Chesapeake Bay Program (1986 – 2016; http://www.chesapeakebay.net/data/) and the VIMS Juvenile Fish Trawl Survey (1988 – 2014) indicate that bottom temperatures rarely exceed 30°C in Chesapeake Bay, Atlantic croaker have been documented in waters up to 31.4°C in South Carolina estuaries (Miglarese et al., 1982) and 31.9°C in the Gulf of Mexico (Craig and Bosman, 2013). Observations of Atlantic croaker inhabiting waters > 30°C suggest that the upper critical temperature for Atlantic croaker is likely greater than 30°C, the maximum temperature tested in this study, and may explain why a decrease in metabolic scope was not observed. Because spot occupy habitats similar to Atlantic croaker throughout the Atlantic Ocean and Gulf of Mexico, it is likely that the upper critical temperature of spot is also greater than 30°C. Understanding the thermal limitations of Atlantic croaker and spot is important when considering climate change scenarios, as it is likely that water temperatures in Chesapeake Bay will continue to increase (Hayhoe et al., 2007; Najjar et al., 2010) and, therefore, may approach or exceed the thermal tolerance of Atlantic croaker and spot in the future. Increasing water temperatures will decrease the amount of habitat available to fishes and may result in decreased abundances of Atlantic croaker and spot as temperatures that exceed thermal optima result in increased mortality (Clark et al., 2003; Pörtner and Knust, 2007; Eliason et al., 2011) and shifts in distribution (Perry et al., 2005).

Temperature is a major factor driving metabolic rates in fishes and has also been associated with increases in S_{crit} (i.e., decreased hypoxia tolerance) (Schurmann and Steffensen, 1997; Capossela et al., 2012; Lapointe et al., 2014; Borowiec et al., 2016). The mean S_{crit} of both Atlantic croaker and spot showed a general increase with increasing temperature; there were, however, no detectable differences in mean S_{crit} for either species at any temperature tested due to the large amount of variation of individual S_{crit} values. Our results suggest that neither species is more tolerant of hypoxic conditions than the other and support the findings of Eby and Crowder (2002). In contrast, based on correlations of distributional and abundance data, Bell and Eggleston (2005) and Pihl et al. (1991) concluded that spot are more tolerant of hypoxia than Atlantic croaker. An important difference is that our experiments explicitly tested the hypoxia tolerance of Atlantic croaker and spot using established respirometry techniques, whereas the previous studies inferred hypoxia tolerance of fishes from survey data. Employing survey data to make inferences on species-specific hypoxia tolerances may lead to biased estimates of hypoxia tolerance because: (1) other abiotic and biotic factors (e.g., salinity, substrate, tidal stage, prey availability, presence of predators, conspecific density) can influence fish distribution and (2) fish may aggregate in the most suitable habitat conditions.

Mean C_{crit} did not differ with temperature or species and suggests that Atlantic croaker and spot are unable to maintain aerobic metabolism at dissolved oxygen concentrations below ~2 mg L^{-1} and therefore would require anaerobic processes to survive in hypoxic conditions (as herein defined). It should be noted, however, that while Atlantic croaker and spot may be able to survive brief exposures to dissolved oxygen

concentrations as low as 2 mg L^{-1}, sublethal effects such as decreases in gonadosomatic index, fecundity, and ovarian lipid content (Thomas and Rahman, 2009; Tuckey and Fabrizio, 2016) have been associated with exposure to hypoxic conditions and may occur at oxygen concentrations higher than the mean C_{crit}. Indeed, decreases in consumption and growth across many species have been associated with dissolved oxygen concentrations as high as 4.5 mg L^{-1} (Hrycik et al., 2017). Given that sublethal effects of hypoxia (e.g., decreases is metabolic scope) occur at dissolved oxygen concentrations higher than a species' critical limit, it is likely that fish will avoid waters that exceed their C_{crit} value. This avoidance behavior has been inferred for a broad range of fishes in Chesapeake Bay (Buchheister et al., 2013) and is likely to intensify as temperatures increase.

4.1 Conclusions

Our results demonstrate that temperature has pervasive effects on the metabolic rates of Atlantic croaker and spot but, at levels currently observed in Chesapeake Bay, does not affect hypoxia tolerance. Additionally, in contrast to the findings of studies correlating ambient oxygen and fish distribution, our results suggest that Atlantic croaker and spot are equally tolerant of hypoxic conditions regardless of temperature. This indicates that neither species has a competitive advantage in regards to exploiting hypoxic areas. At temperatures common to Chesapeake Bay, however, spot generally have a greater metabolic scope and therefore a greater state-space for aerobic activities such as growth, reproduction, and movement. If nutrient input to Chesapeake Bay continues at the current levels and temperatures continue to increase, it is likely that

hypoxic zones will increase in size and severity, limiting the habitat available to Atlantic croaker and spot. As habitat availability decreases, the distribution of both spot and Atlantic croaker may shift outside of Chesapeake Bay to areas where these conditions are more favorable; however, prey availability and predator abundance in these alternate habitats may further shape habitat use. Additionally, because they occupy similar niches, the decreased availability of suitable habitats in Chesapeake Bay may result in increased competition for resources between these species. In this scenario, the greater metabolic scope of spot may lead to a competitive advantage and result in a decrease in abundance of the Atlantic croaker population in Chesapeake Bay, and eventually coast-wide. To better assess the ecological impacts of climate change on Atlantic croaker and spot, additional research regarding the effect of other environmental conditions (e.g., salinity, acidification, prey availability) and their interactions on the metabolism of these fishes will be necessary.

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Tables

Table 1. Length, weight, and number (n) of Atlantic croaker and spot used at each temperature in the respirometry trials. Results are reported as means (standard errors).

	Atlantic Croaker			Spot		
Temperature (°C)	Total Length (mm)	Weight (g)	n	Fork Length (mm)	Weight (g)	n
10	281.0 (10.4)	289.9 (25.3)	5	207.7 (2.2)	146.1 (5.7)	9
15	276.0 (4.9)	250.5 (15.3)	10	207.9 (0.9)	145.3 (2.9)	11
20	267.4 (5.3)	218.8 (13.4)	5	214.1 (3.1)	156.2 (6.1)	11
25	264.7 (8.4)	221.2 (28.6)	9	208.6 (2.7)	149.6 (6.5)	10
30	280.6 (7.2)	268.9 (21.1)	8	205.3 (1.6)	137.1 (2.8)	9
Overall	273.8 (3.3)	248.4 (10.4)	37	208.9 (1.1)	147.2 (2.4)	50

Table 2. Correlations between standard metabolic rate (SMR), maximum metabolic rate (MMR), metabolic scope (MS), and critical oxygen saturation (S_{crit}) data used in the multivariate analysis.

Atlantic croaker

Response	SMR	MMR	MS	S_{crit}
SMR		0.81	0.53	0.79
MMR			0.93	0.66
MS				0.44
S_{crit}				

Spot

Response	SMR	MMR	MS	S_{crit}
SMR		0.85	0.58	0.50
MMR			0.92	0.39
MS				0.23
S_{crit}				

Figures

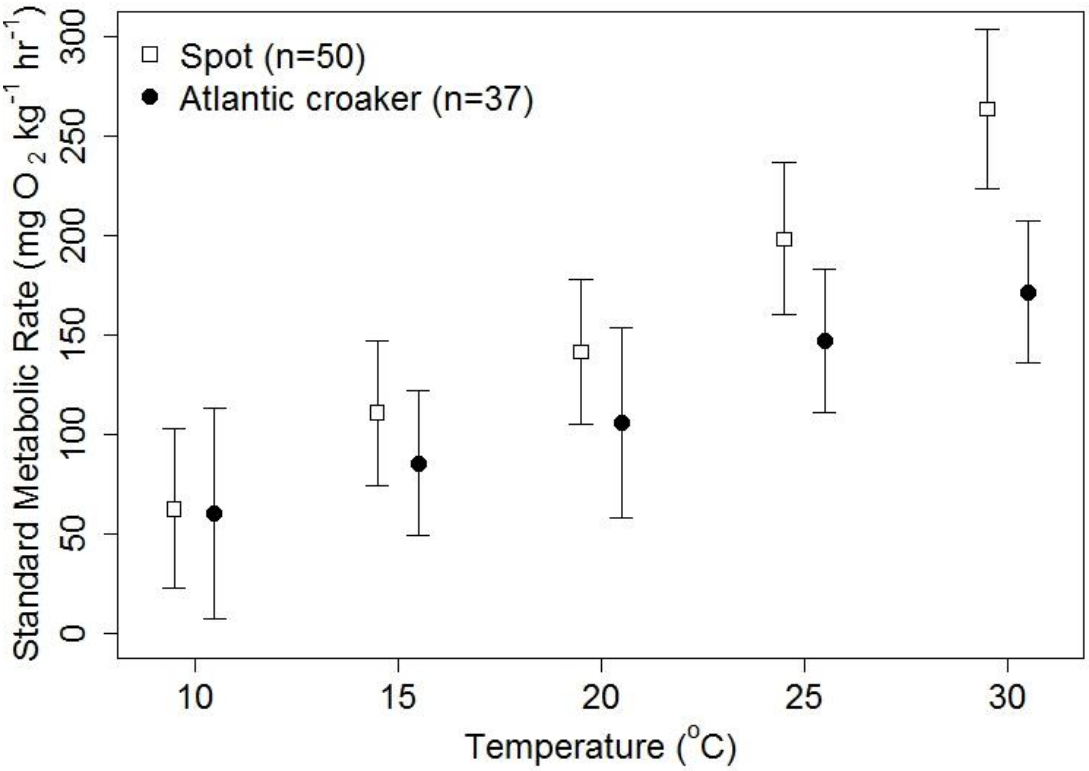

Figure 1. The standard metabolic rate of Atlantic croaker and spot at five temperatures. Mean model estimates (±95% confidence intervals) are shown for spot (open squares) and Atlantic croaker (filled circles).

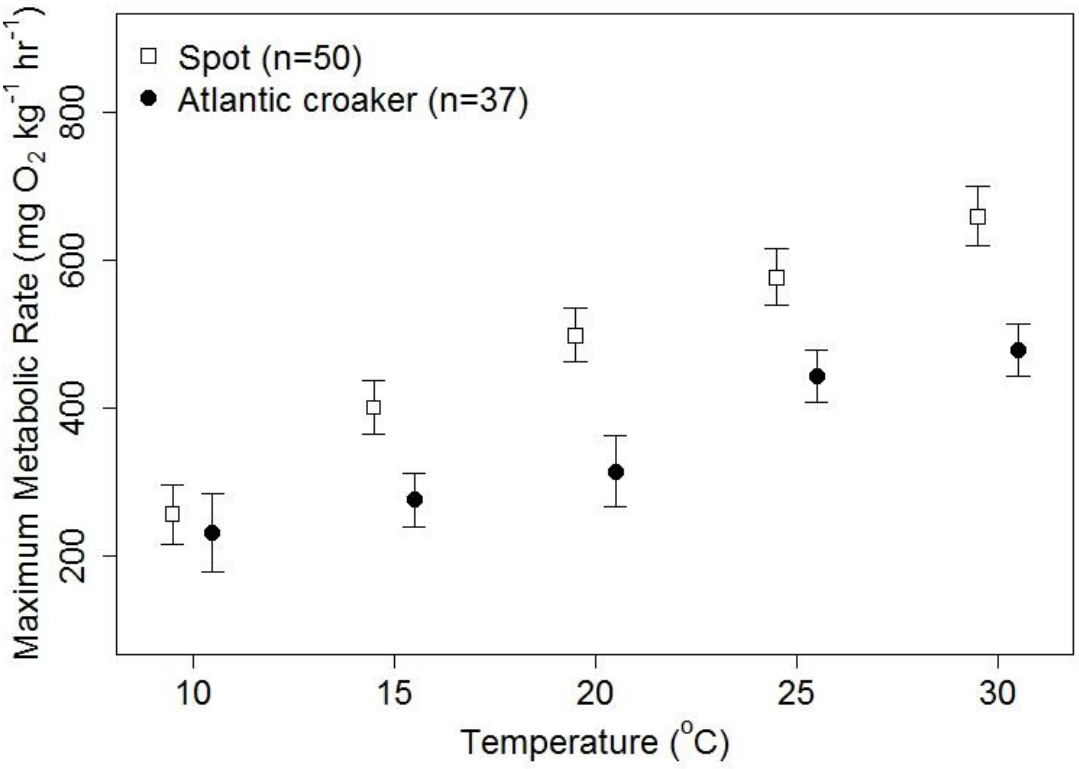

Figure 2. The maximum metabolic rate of Atlantic croaker and spot at five temperatures. Mean model estimates (±95% confidence intervals) are shown for spot (open squares) and Atlantic croaker (filled circles).

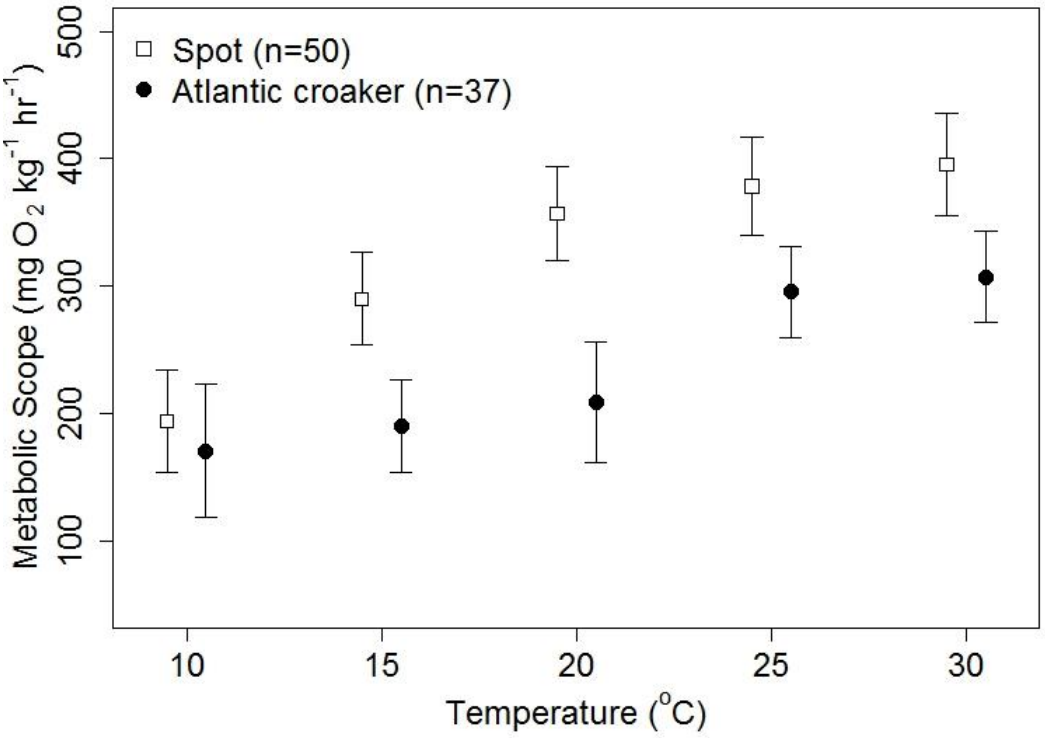

Figure 3. The metabolic scope of Atlantic croaker and spot at five temperatures. Mean model estimates (±95% confidence intervals) are shown for spot (open squares) and Atlantic croaker (filled circles).

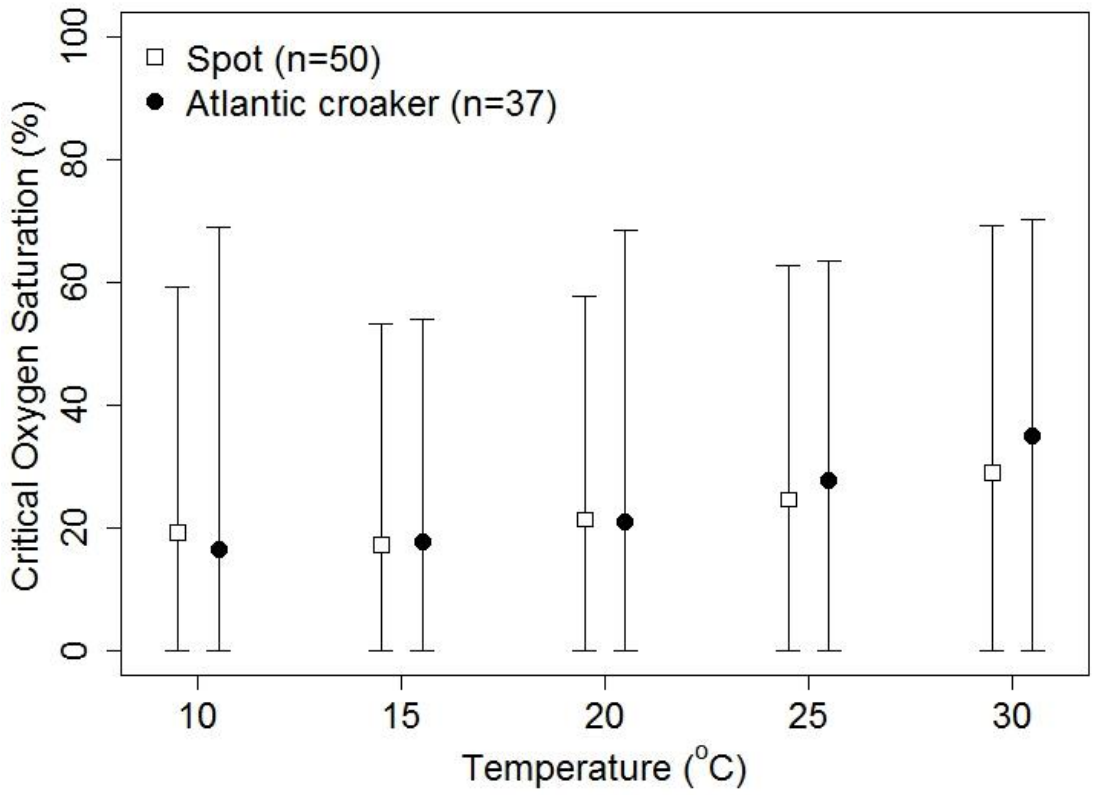

Figure 4. The critical oxygen saturation of Atlantic croaker and spot at five temperatures. Mean model estimates (±95% confidence intervals) are shown for spot (open squares) and Atlantic croaker (filled circles). Lower confidence intervals are truncated at 0%.

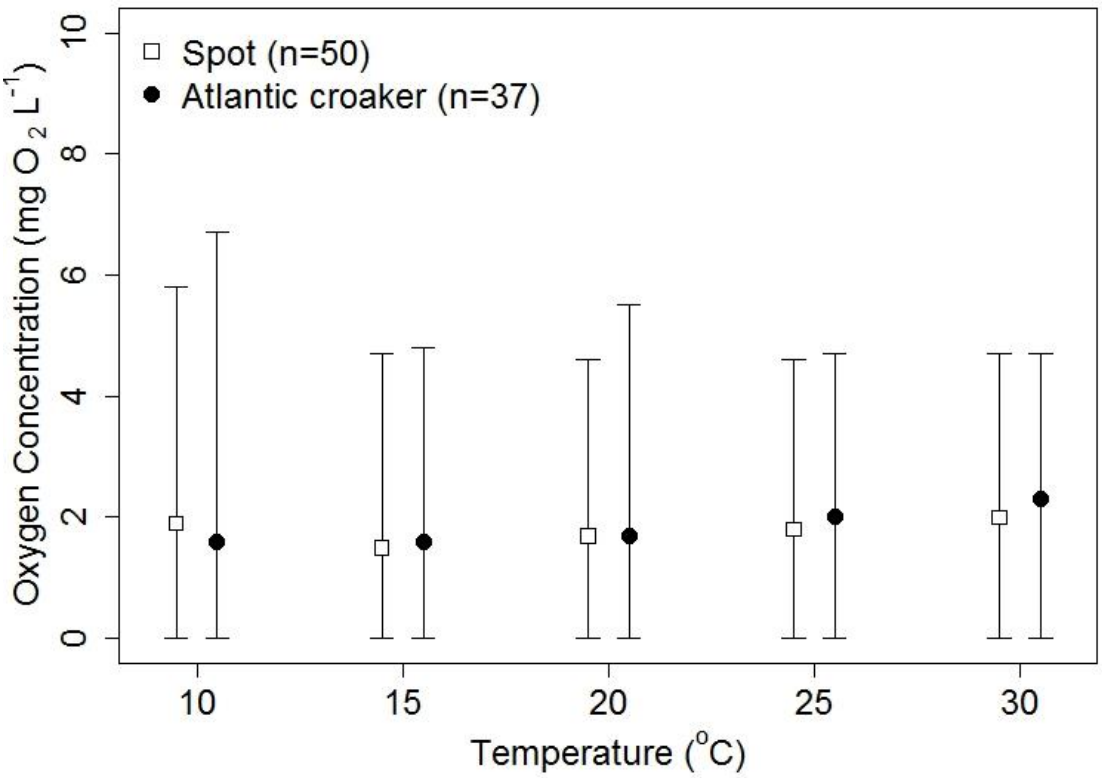

Figure 5. The critical oxygen concentration of Atlantic croaker and spot at five temperatures. Mean model estimates (±95% confidence intervals) are shown for spot (open squares) and Atlantic croaker (filled circles). The mean and confidence limits were converted to concentration from model estimates of critical oxygen saturation. Lower confidence intervals are truncated at 0 mg O_2 L^{-1}.

CHAPTER 3
Effects of Hypoxia on the Reproductive Potential of Atlantic Croaker in Chesapeake Bay

Abstract

Eutrophication, caused by anthropogenic nutrient inputs, has increased the spatial extent, frequency, and severity of hypoxic events in coastal and estuarine waters throughout the world. In Chesapeake Bay, these specific trends in hypoxia have led to concerns about their effects on economically and ecologically important species. One such effect at the individual level is the disruption of reproductive development, which if widespread, could lead to reductions in population size and alter community dynamics. To address a gap in knowledge about the direct effects of hypoxia exposure on the reproduction of Chesapeake Bay fishes, this study focused on female Atlantic croaker (*Micropogonias undulatus*) captured in Virginia waters of the Bay during 2016. I investigated the utility of using hypoxia-inducible factors (HIFs) to detect hypoxia exposure, while examining the effect of hypoxia exposure on these same individuals, as measured by three metrics of reproductive potential: the gonadosomatic index, the most-advanced oocyte stage, and the proportion of atretic oocytes. The relative expression of HIFs and the three metrics of reproductive potential did not differ between fish captured in normoxic versus hypoxic waters (< 3.5 mg O_2 L^{-1}). My results suggest that, in 2016, female Atlantic croaker captured in Virginia waters of Chesapeake Bay were not subjected to chronic hypoxia exposure, but were likely exposed to hypoxia only intermittently. This is in contrast to the evidence for effects of hypoxia on Atlantic croaker reproductive potential in Chesapeake Bay and in the northern Gulf of Mexico during years of more widespread hypoxia. I conclude, that the reproductive potential of Atlantic croaker residing in Virginia subestuaries of Chesapeake Bay was not affected by exposure to hypoxic conditions in 2016. To understand the potential effect of hypoxia on

the reproduction of the Atlantic croaker population in Chesapeake Bay however, additional sampling in years of chronic, severe hypoxia and in Maryland waters of this estuary is required.

1. Introduction

Hypoxia is among the most widespread, deleterious processes occurring in aquatic environments [1]. Hypoxia is a naturally-occurring phenomenon driven by high nutrient loads and water column stratification, but the duration, frequency, and spatial extent of hypoxic events has increased as a result of eutrophication caused by anthropogenic activities such as intense agriculture and urbanization [1-4]. Commonly, 2.0 mg O_2 L^{-1} is used as the threshold for hypoxia [1] because organisms, especially those inhabiting the benthos, experience high mortality rates at dissolved oxygen (DO) concentrations below this level [5]. The use of a strict mortality-driven threshold for defining hypoxia may not, however, be appropriate because sublethal impacts of exposure to low DO conditions may occur and because oxygen requirements and tolerances are species- and ontogeny-specific [6, 7].

Sublethal effects on marine fishes associated with exposure to low DO conditions include alterations in spatial distribution [8-16], changes in metabolic rates [17-21], and reductions in growth [7, 22-24], consumption [7, 23 ,24], and reproductive potential [25-31]. Reproduction in fishes is particularly sensitive to disruption by environmental stressors including hypoxia [28, 32, 33]. Impairment of reproductive function resulting from exposure to hypoxia has been described for common carp (*Cyprinus carpio*) [34], Gulf killifish (*Fundulus grandis*) [35, 36], and Atlantic croaker (*Micropogonias undulatus*) [26, 30], where DO concentrations leading to impaired function ranged from 1.3 mg L^{-1} [35] to 3.5 mg L^{-1} [30]. Exposure to hypoxic conditions can impair reproductive function through delays in oocyte development, decreases in gonadosomatic index (GSI), impaired gametogenesis, decreased oocyte size, decreased sperm motility,

reduced fertilization success, reduced hatching rate, decreased fecundity, ovarian masculinization, and alterations to processes controlling sex differentiation [26, 29, 30, 34, 35, 37, 38]. Stressful environmental conditions, including hypoxia, have additionally been linked to increased degeneration of oocytes (commonly referred to as atresia) in fishes [39-46]. Hypoxic conditions in Chesapeake Bay have worsened in recent decades [47-49], although the direct impacts of hypoxia exposure on the reproductive function of fish inhabiting this estuary are still largely unknown.

Disruption or impairment of the reproductive function of individuals can lead to population declines and may have long-term impacts on affected populations [32, 33, 50, 51]. If the effects of hypoxia are ignored, stock assessment models may yield overly optimistic estimates of population growth, especially if fecundity is assumed to be stable regardless of environmental conditions. Tuckey and Fabrizio [31] demonstrated that indirect exposure to hypoxia affects the reproductive potential of Atlantic croaker in Chesapeake Bay; verification of the hypoxia-exposure history of individual Atlantic croaker is, however, necessary to assess the direct effects of hypoxia exposure on their reproductive potential. Biomarkers of exposure to hypoxia, specifically the expression of hypoxia-inducible factors (HIFs) in the tissues of fish and other organisms captured in hypoxic areas, can be used to assess hypoxia exposure [26, 28, 52-54].

HIFs are transcription factors involved in the maintenance of oxygen homeostasis [55] through the regulation of gene expression and metabolic processes [30, 38, 52, 53, 56-62]. Increased expression of HIFs has been linked with the disruption of reproductive function in field and laboratory studies [26, 29, 30, 34]. Reproductive impairment resulted from the chronic exposure of fish to hypoxic conditions [26, 29, 30, 34]. The

effect of acute or intermittent exposure to hypoxia on the expression of HIFs and reproductive impairment is, however, poorly understood. Because the frequency, extent, and severity of hypoxic conditions are system-specific, it is difficult to generalize the impacts of exposure to hypoxic conditions on fish reproduction from one system to another. In contrast with previously studied systems, hypoxia in Chesapeake Bay is typically restricted to deep channels [48, 63] and the adjacent shallower waters are well-oxygenated. A variety of hypoxic conditions can be observed during summer in the subestuaries in the lower portions of Chesapeake Bay. For example, hypoxia does not occur in the James River, whereas the York River has mild, periodic hypoxic episodes, and the Rappahannock River has severe, seasonal hypoxia [31]. Hypoxia in the Rappahannock River has been shown to result in reductions in the reproductive potential of Atlantic croaker [31]. Due to the restricted or episodic extent of hypoxia in subestuaries of the lower Chesapeake Bay described above, fishes and other mobile organisms can avoid exposure to hypoxic conditions simply by moving away from hypoxic areas.

I compared the expression of two hypoxia-inducible factor subunits (HIF-1α and HIF-2α) in three tissues (brain, gill, and heart) of fish captured in hypoxic conditions with those of fish captured in normoxic conditions specifically to investigate the utility of HIFs as biomarkers of hypoxia exposure in Atlantic croaker captured in Chesapeake Bay. Additionally, I determined the effects of hypoxia exposure on the reproductive potential of Atlantic croaker by comparing metrics of reproductive potential (GSI, most-advanced oocyte stage, and proportion of atretic oocytes) of fish captured in hypoxia to those of fish captured in normoxia. Atlantic croaker are a common, seasonal resident of

Chesapeake Bay [64], and do not display a strong avoidance response to hypoxia [9, 65]. The utility of HIFs as biomarkers of hypoxia and reproductive impairment as a result of hypoxia exposure has also been demonstrated for this species in the northern Gulf of Mexico and Pensacola Bay Estuary [25, 26, 28-30, 52, 60]. Based on the results of these previous studies, I hypothesize that:

(1) the relative expression of HIFs will be greater in the tissues of fish captured in hypoxic conditions relative to those captured in normoxic conditions, and

(2) fish captured in hypoxia will display a reduced GSI, oocytes will be in an earlier stage of development, and atresia will occur in a larger proportion of oocytes relative to fish captured in normoxia.

Sublethal effects of dissolved oxygen concentrations are observed at levels greater than 2 mg L^{-1} [7, 16, 21, 26, 30], and disruptions to the reproductive processes of Atlantic croaker have been linked to exposure to dissolved oxygen concentrations as high as 3.7 mg L^{-1} [26, 30]. I, therefore, defined hypoxic conditions as oxygen concentrations $\leq$ 3.5 mg L^{-1}.

2. Methods

All capture and handling techniques described in this manuscript were approved by the Institutional Animal Care and Use Committee (IACUC-2015-06-30-10455-mcfabr) at the College of William & Mary and complied with all applicable U.S. guidelines.

2.1 Tissue collection

Adult, female Atlantic croaker (≥ 240 mm total length, TL; n = 87) were captured at 41 stations in the James (n = 12), York (n = 31), and Rappahannock (n = 44) rivers in July and August 2016 (Fig 1) using a 9.14 m otter trawl. Forty-one fish were captured in hypoxic conditions (DO < 3.5 mg L^{-1}) with 20 of these fish captured at stations where DO ≤ 2 mg L^{-1} and 46 were captured in normoxic conditions. Fish were captured at hypoxic and normoxic stations in the York and Rappahannock rivers. Because hypoxia does not occur in the James River, the relative expression of HIFs in the tissues of fish captured there was, therefore, used as a control against which the relative expression of HIFs in the tissues of fish captured in the York and Rappahannock rivers could be compared. Following capture, fish were euthanized in an ice-water slurry [66] prior to being weighed (g) and measured (mm; total length, TL). The sex of each individual was determined by examination of the gonads macroscopically. The brain, gill, and heart tissues were extracted and frozen in liquid nitrogen until processing. Total weight, somatic weight, and gonad weight were measured to the nearest 0.1 g where total weight refers to the mass of whole animal, somatic weight refers to that of the eviscerated

animal, and gonad weight was measured using both lobes of the ovary. The gonadosomatic index (GSI) was calculated as:

$$GSI = \frac{Gonad\ Weight}{Somatic\ Weight}. \quad (1)$$

The right ovary was preserved on ice and transported to the laboratory, fixed in 10% buffered formalin for a minimum of two weeks, then preserved in 70% ethanol until histological analysis. Otoliths were removed for aging.

2.2 Tissue-specific expression of hypoxia-inducible factors

The expression of HIF-1α and HIF-2α relative to 18S ribosomal RNA (18S rRNA), hereafter referred to as "relative expression", was examined in the brain, gill, and heart tissues of fish captured in the Virginia subestuaries of Chesapeake Bay as described above. To calculate the relative expression of HIFs, mRNA was extracted from approximately 30 mg of homogenized tissue using a RNeasy minikit (Qiagen, Hilden, Germany). Total mRNA was quantified for each tissue with a Qubit 2.0 Fluorometer (Life Technologies, Carlsbad, CA). Purity of the RNA was assessed using a Nanodrop (ND-2000, Thermo Fisher Scientific, Waltham, MA). First-strand cDNA was reverse-transcribed from mRNA using a Quantitect RT kit, which integrates removal of genomic DNA, following the manufacturer's instructions (Qiagen, Hilden, Germany).

Expressions of HIF-1α, HIF-2α, and an internal reference gene (18S rRNA) were quantified using a 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA). Gene-specific primers for HIF-1α, HIF-2α, and 18S rRNA, designed and optimized

by Rahman and Thomas [52], were used for this analysis. Reactions were performed using the Fast SYBR Green Master Mix (Applied Biosystems, Foster City, CA) in a 10-µl reaction mixture which contained 5 µl of master mix, 1 µM of each primer, and 0.84 ng cDNA. Thermocycling conditions were 95°C for 20 s followed by 40 repetitions of 95°C for 3 s, 60°C for 30 s, and 75°C for 30 s. Dissociation curve analyses were conducted immediately following the amplification cycles at 95°C for 15 s, 60°C for 1 min, 95°C for 15 s, and 60°C for 15 s. Due to primer-dimer formation in the HIF-2α samples, thermocycling conditions were modified for these trials by replacing the 75°C step with a step at 80.6°C for 30 s. All other steps were identical. Each reaction was performed in triplicate and the mean threshold cycle (C_t) used for subsequent analyses. The expression of HIF-1α and HIF-2α mRNA relative to 18S rRNA in each tissue was calculated using the comparative C_t method which allows for presentation of the data as "fold change" whereby the relative expression of HIFs in "treatment" groups (fish from the Rappahannock or York rivers) are assessed relative to the control group (fish from the James River) [67]. To determine the validity of using the comparative C_t method [67], a dilution series was created and the amplification efficiencies of each tissue-primer combination calculated (Table 1).

2.3 Oocyte development

Ovaries were sectioned and a portion from the middle of each ovary was used for subsequent analysis. Ovary sections were dehydrated, cleared, and infiltrated with embedding medium using a Thermo Excelsior ES tissue processor (Thermo Fisher Scientific, Waltham, MA). Following infiltration, tissue samples were embedded in

molten paraffin and allowed to cool. Tissues were then cut into 5-μm sections and mounted on slides for staining. To visualize the oocytes, the sectioned ovaries were stained with hematoxylin and eosin (H&E) using a Shandon Varistain Gemini automatic stainer (Thermo Fisher Scientific, Waltham, MA).

Ovary sections were examined for the most-advanced oocyte stage (Table 2; Fig 2) and the proportion of atretic oocytes. Oocyte staging and terminology followed Grier [68] and stages were coded numerically for analysis (Table 2). The percentage of oocytes undergoing atresia was estimated as none, 0-5%, or > 5% for each sample. For subsequent analysis, these categories were coded numerically as 0, 1, and 2, respectively.

2.4 Analysis

Ages of individual fish were estimated by three readers using polished sections of the sagittal otolith following Barbieri et al. [69]. Agreement between at least two readers was necessary to assign an age to an individual. If agreement did not occur between at least two readers, all readers reexamined the otolith and assigned a new age. The degree of reader agreement was determined using Chang's coefficient of variation [70, 71].

2.4.1 Relative expression of hypoxia-inducible factors

Significant linear correlations were noted between the relative expressions of HIF-1α and HIF-2α in several tissue-gene combinations using the comparative C_t method (Table 3). Therefore, to address the potential correlations among the relative expressions of HIF subunits from individual fish (n = 87), a multivariate approach, implemented in the MIXED procedure in SAS 9.3 (SAS Institute, Cary, NC), was used to analyze the

relative expressions of HIFs. I examined the utility of HIFs as biomarkers of hypoxia exposure in the subestuaries of the lower Chesapeake Bay by modeling the effect of the oxygen condition at the location of capture (hypoxic or normoxic) and the subestuary in which the fish was captured (James, York, or Rappahannock rivers) on HIF expression. Because all levels of oxygen condition did not occur in all subestuaries (hypoxia does not occur in the James River), I used a nested design to examine the potential effect of hypoxia on the expression of HIFs. The full model therefore sought to explain the variation in the relative expressions of HIF-1α (HIF1) and HIF-2α (HIF2) in each tissue (brain, b; gill, g; heart, h) for individual *i* as a function of subestuary *j* and dissolved oxygen condition *k* nested within subestuary; where *subestuary* indicates the effect of the James, York, or Rappahannock rivers, *DO* indicates the effect of hypoxic or normoxic conditions within each subestuary, and ε_{ijkl} represents the random unexplained error. A random effect of the station *l* at which fish were captured was also considered in the model to account for spatial variation among observations. I investigated compound symmetric, variance components, and unstructured variance-covariance matrices to describe the correlations and variances among capture locations. Model selection was performed using Akaike's Information Criterion corrected for small sample size (AIC_c), where the model that best describes the data had the lowest AIC_c score. The full model was:

$$HIF1_{b,ijkl}, HIF2_{b,ijkl}, HIF1_{g,ijkl}, HIF2_{g,ijkl}, HIF1_{h,ijkl}, HIF2_{h,ijkl} \quad (2)$$
$$= subestuary_j + DO_{k(j)} + station_l + \varepsilon_{ijkl}.$$

For all statistical analyses, I used an alpha level of 0.05 to determine significant effects.

2.4.2 Metrics of reproductive potential

I examined graphically the potential effects of fish length, weight, and age on metrics of reproductive potential. No patterns or associations were apparent. Length, weight, and age were therefore excluded from subsequent analyses.

Multinomial logistic ANOVAs were used to analyze the most-advanced oocyte stage and the proportion of atretic oocytes (GLIMMIX procedure, SAS 9.3, SAS Institute, Cary, NC) because these data were treated as categorical, multilevel responses. GSI is a proportion bounded in the interval [0, 1]; GSI data are skewed and violate the assumptions of normality and homogeneity of variance necessary for general linear models. I therefore used an ANOVA with a beta distribution [72] to analyze GSI (GLIMMIX procedure). Similar to the analysis of relative HIF expression, I treated oxygen condition as a nested factor within subestuary. Fish were not captured in both normoxic and hypoxic conditions during each month of sampling within each subestuary. As a result, month of capture was nested within oxygen condition, resulting in a doubly nested design. To account for variation in observations due to the location of capture, the random effect of station *l* was considered in models of metrics of reproductive potential. The full model considered for each metric of reproductive potential was:

$$Y_{ijklm} = subestuary_j + DO_{k(j)} + month_{m(k(j))} + station_l + \varepsilon_{ijklm}, \qquad (3)$$

where *Y* represents the most-advanced oocyte stage, proportion of atretic oocytes, or GSI for individual *i* from subestuary *j* and month *m* is nested within dissolved oxygen condition *k* which is nested within subestuary *j*; *subestuary* represents the effect of the James, York, or Rappahannock rivers, *DO* represents the effect of oxygen condition (normoxic or hypoxic), *month* represents the effect of the month of capture (July or

August), and ε_{ijklm} represents the random, unexplained error in the model. As a result, the ε_{ijklm}s are distributed as a multinomial or beta distribution, depending on the distribution of the response. AIC_c was used to assess the contribution of the random effect of station. Results of these analyses are presented as odds ratios which compare the probability of the occurrence of an outcome for individuals in one group with the probability of the occurrence of that outcome for individuals in a different group (i.e., the likelihood that the most-advance oocyte stage was a later stage of development for fish captured in August compared with those captured in July).

2.4.3 Intranuclear inclusions

Histological analysis of sectioned ovaries indicated a high prevalence of intranuclear inclusions (Fig 2 B, C, H) in the oocytes of Atlantic croaker. The presence of intranuclear inclusions in fish oocytes has been linked to contaminants and can lead to oocyte atresia and reductions in reproductive potential [73-75]. I therefore investigated the relationships between environmental conditions and the proportion of oocytes containing intranuclear inclusions, as well as the relationships between the proportion of oocytes containing intranuclear inclusions and metrics of reproductive potential. Intranuclear inclusions occurred in all samples examined in this study, but they occurred only in the primary growth stages of oocyte development. I therefore excluded samples for which the most-advanced oocyte stage was a secondary growth oocyte or a more mature stage (i.e., an ovary with a high proportion of secondary growth oocytes has a low proportion of intranuclear inclusions) to avoid potential bias. Fifty-nine ovary samples were used for this analysis. The proportion of oocytes containing intranuclear inclusions

ranged from <10% to >50%. For analysis, the proportion of oocytes with intranuclear inclusions were discretized into two categories (0-25% and >25) and coded as 1 (n = 34) or 2 (n = 25), respectively.

I investigated the effects of subestuary, dissolved oxygen condition, and month on intranuclear inclusions using a logistic regression to determine if environmental conditions impacted the proportion of oocytes that contained intranuclear inclusions in the ovaries of Atlantic croaker. The model considered for the proportion of oocytes that contained intranuclear inclusions was:

$$Intranuclear\ Inclusion_{ijklm} = subestuary_j + DO_{k(j)} + month_{m(k(j))} + station_l + \varepsilon_{ijklm}. \quad (4)$$

The proportion of oocytes that contained intranuclear inclusions in the ovary of individual *i* was modeled as a function of subestuary *j* and month *m* nested within dissolved oxygen condition *k* which is nested within subestuary *j*. *Subestuary* represents the effect of the James, York, or Rappahannock rivers, *DO* represents the effect of dissolved oxygen condition (normoxic or hypoxic), *month* represents the effect of the month of capture (July or August), and ε_{ijklm} represents the random, unexplained error in the model. AIC_c was used to assess the importance of the random effect of station.

I conducted a multinomial logistic ANOVA for the most-advanced oocyte stage and the proportion of atretic oocytes, and an ANOVA with a beta distribution for GSI as described above to examine the effect of the proportion of oocytes containing intranuclear inclusions on metrics of reproductive potential. Because I used only data for which the most-advanced oocyte stage was in primary growth stages (PGmn-PGca or categories 2-4; n = 59), these models were developed separately from models of metrics

of reproductive potential and included intranuclear inclusions as the only predictor. Separate models were used for each metric of reproductive potential following:

$$Y_{im} = intranuclear\ inclusions_m + \varepsilon_{im}. \quad (5)$$

In this case, *Y* is the most-advanced oocyte stage, proportion of atretic oocytes, or GSI for individual *i* as a function of the proportion of oocytes containing intranuclear inclusions *m*. *Intranuclear inclusions* is the effect of an individual having 0-25% or >25% of oocytes containing intranuclear inclusions and ε_{im} is the random, unexplained error. Results from this analysis are presented as odds ratios.

3. Results

The mean ± standard error (se) size of adult Atlantic croaker was 258 ± 1 mm TL and 208 ± 4 g total weight. Fish ages ranged from 3 to 7 years. Chang's CV was 4%, indicating a high degree of agreement on fish age among readers [76].

3.1 Relative expression of hypoxia-inducible factors

The mean relative expressions of HIF-1α and HIF-2α in the brain, gill, and heart tissues were not significantly affected by subestuary (i.e., Rappahannock, York, or James rivers, $F = 1.18$, $P = 0.31$; Fig 3) or dissolved oxygen condition nested in subestuary ($F = 1.21$, $P = 0.29$; Fig 4). Inclusion of the random effect of station resulted in a lower AIC_c score indicating that differences among stations explained a significant amount of the variability in the relative expression of HIFs among fish.

3.2 Metrics of reproductive potential

3.2.1 Most-advanced oocyte stage

The most-advanced oocyte stage was significantly affected by month and dissolved oxygen condition nested within subestuary ($F = 3.33$, $P = 0.01$). This parameter was, however, not different between subestuaries ($F = 0.89$, $P = 0.42$). For fish captured under normoxic conditions, the mean most-advanced oocyte stage in August was significantly greater than that in July ($F = 6.35$, $P = 0.01$; Fig 5). In August, the mean most-advanced oocyte stage was ~107 times more likely to be in a later stage of development, relative to the mean most-advanced oocyte stage in July. This relationship

was also observed within each subestuary for fish captured in normoxic conditions (F_{JA} = 6.35, P_{JA} = 0.01; F_{RA} = 4.37, P_{RA} = 0.04; F_{YK} = 4.85, P_{YK} = 0.03) with odds ratios indicating that the mean most-advanced oocyte stage was ~32, 5, and 7 times more likely to be in a later stage of development in August relative to July for fish captured in the James, Rappahannock, and York rivers, respectively. Under hypoxic conditions in the Rappahannock River in August, the mean most-advanced oocyte stage was ~10 times more likely to be a later stage of development relative to fish captured in July (F = 7.04, P = 0.01; Fig 6). Interestingly, the mean most-advanced oocyte stage differed between hypoxic and normoxic conditions only in fish captured in the York River in July (F = 4.85, P = 0.03; Fig 7); the mean most-advanced oocyte stage was approximately seven times more likely to be in a later stage of development in fish captured in normoxic conditions, relative to those captured in hypoxic conditions. AIC_c did not support the inclusion of the random effect of station in this model.

3.2.2 Proportion of atretic oocytes

The mean proportion of atretic oocytes in the ovaries of Atlantic croaker was significantly affected by the subestuary in which fish were captured (F = 3.82, P = 0.03), but was not significantly affected by the nesting of month within dissolved oxygen condition within subestuary (F = 1.58, P = 0.16). Fish captured in the York River were approximately five times more likely than those captured in the Rappahannock River to have a higher mean proportion of atretic oocytes in their ovaries, regardless of month or dissolved oxygen condition (F = 7.56, P = 0.01; Fig 8). There were no significant differences in the mean proportion of atretic oocytes in fish from the James and

Rappahannock rivers (F = 0.12, P = 0.73) or the James and York rivers (F = 2.09, P = 0.15). AIC_c did not support the inclusion of the random effect of station.

3.2.3 Gonadosomatic index

The mean GSI was significantly affected by month nested within dissolved oxygen condition nested within subestuary (F = 2.48; P = 0.02). The only significant difference in mean GSI for Atlantic croaker was, however, observed in the Rappahannock River for fish captured under hypoxic conditions in July and August. More specifically, fish captured in August had a mean GSI 1.6 times that of fish captured in July (F = 5.18; P = 0.03; Fig 9). AIC_c did not support the inclusion of the random effect of station.

3.2.4 Intranuclear inclusions

The mean proportion of primary growth oocytes with intranuclear inclusions was not significantly affected by subestuary (F = 0.99, P = 0.38) or by month nested within dissolved oxygen condition nested within subestuary (F = 0.87, P = 0.51). Analyses of the effect of intranuclear inclusions on metrics of reproductive potential suggest that the mean most-advanced oocyte stage and the mean proportion of atretic oocytes were significantly affected by the proportion of oocytes containing intranuclear inclusions (F_{stage} = 4.59, P_{stage} = 0.04; $F_{atresia}$ = 6.79, $P_{atresia}$ = 0.01; Fig 10). The proportion of oocytes containing intranuclear inclusions did not affect the mean GSI (F = 0.43, P = 0.52). The mean most-advanced oocyte stage with < 25% of oocytes containing intranuclear inclusions was approximately three times more likely to be a later stage of

development than for fish with > 25% of oocytes containing intranuclear inclusions. Fish with a lower proportion of oocytes containing intranuclear inclusions (< 25%) were 4.9 times more likely to have a higher mean proportion of atretic oocytes than individuals with a higher proportion of oocytes containing intranuclear inclusions (> 25%).

4. Discussion

The mean relative expressions of HIF-1α and HIF-2α were similar in fish captured in hypoxic conditions (< 3.5 mg O_2 L^{-1}) and normoxic conditions. I also found no evidence of reproductive impairment of fish captured in hypoxic conditions. My results therefore differ from those for Atlantic croaker captured in the Pensacola Bay estuary [26] and the northern Gulf of Mexico hypoxic zone [30], which showed evidence of increased expressions of HIF-1α and HIF-2α as well as impaired reproduction.

Month of capture had the greatest effect on metrics of reproductive potential, in that (as expected) the mean most-advanced oocyte stage observed in the ovaries of fish captured in August was a later stage of development than those of fish captured in July. This is consistent with Atlantic croaker preparing to spawn in the fall and winter [69]. In the Rappahannock River, the mean GSI of fish captured in hypoxic conditions in August was also higher than that of fish captured in hypoxic conditions in July. Contrary to my expectation, there was no evidence of increased GSI in fish from the James or York rivers, nor were mean GSIs in July and August affected by dissolved oxygen condition. This is surprising considering Atlantic croaker are likely to devote a substantial amount of energy to gonadal development during summer months [77, 78]. The number of days between sampling among the subestuaries was, however, inconsistent and affected the likelihood of observing significant differences in GSI. Weather-related constraints on trawl survey operations resulted in only 16 days between July and August sampling in the James River, compared to 20-30 days in the York River, and 28-45 days in the Rappahannock River. In the Rappahannock River, normoxic stations were sampled 30 days apart and hypoxic stations were sampled 28-45 days apart; most fish sampled from

hypoxic conditions in August were captured 45 days after those captured in July. The longer time between sampling in the Rappahannock River may explain why I observed significant differences in mean GSIs for fish captured in hypoxic conditions in the Rappahannock River, but not in fish captured under normoxic conditions in the Rappahannock, James or York rivers.

The subestuary of capture did affect the proportion of atretic oocytes. Fish from the York River were more likely to have a higher proportion of atretic oocytes than fish from the Rappahannock River, although the most-advanced oocyte stage and GSI were not affected. My results suggest that the reproductive potential of fish from the York River is impaired relative to individuals from the Rappahannock River. These finding, however, contrast with previous reports that fish captured in the Rappahannock River during hypoxic events had a significantly lower mean ovarian lipid content and mean GSI compared to fish captured in the York River [31]. One potential explanation for this discrepancy is the timing of sampling. Tuckey and Fabrizio [31] compared metrics of reproductive potential of fish captured in May (i.e., prior to the development of hypoxia in the York and Rappahannock rivers), to those captured, after hypoxia developed in these areas. In contrast, I sampled only during times when hypoxia was likely to be present in the York and Rappahannock rivers (July and August). This sampling strategy may have affected my ability to detect differences in metrics of reproductive potential resulting from exposure to hypoxia.

In contrast with previous studies of relative HIF expression in the tissues of hypoxia-exposed fish [26, 30, 54], I noted relatively high variation in the relative expression of HIF-1α and HIF-2α within rivers and oxygen conditions which, when

combined with my moderate sample size, may have resulted in my inability to detect significant differences in mean relative HIF expression. There was also a general lack of evidence of reproductive impairment of fish captured in hypoxic conditions, compared to those captured in normoxic conditions. The variation in HIF expression and lack of significant reproductive impairment suggest that Atlantic croaker in the lower Chesapeake Bay likely do not remain in hypoxic areas long enough to incur the negative effects. I would, however, expect to see differences in mean HIF expression between fish sampled from the James River (which does not experience hypoxia) relative to the York and Rappahannock rivers. This is because, even if fish moved from the York or Rappahannock rivers to the James River, it seems unlikely that they could move this distance in less than 24 hours, at which point HIF expression is likely to have returned to base levels [52, 60]. To verify this conclusion, a real-time tracking study with concurrent water-quality sampling would be required.

Variation in the expression of HIF-1α and HIF-2α could also result from exposure to stressors other than hypoxia [79]. The expression of HIF-1α can increase in response to contaminants such as copper [80], or to acute or chronic exposure to cold [81-83]. To my knowledge, a survey of contaminants that may cause oxidative stress (and therefore induce the expression of HIFs) has not been performed in the lower Chesapeake Bay. Large temperature differences were observed in the James River in July, where surface and bottom waters differed by more than 6°C for 25% of the stations where Atlantic croaker were captured. Similarly, there was at least a 6°C difference between surface and bottom waters at 5% of stations in the Rappahannock River at which Atlantic croaker were captured. This substantial temperature difference between surface and bottom

waters in the James and Rappahannock rivers may have caused increases in HIF expression of fish moving through the pycnocline in these subestuaries. These results imply that the James River may be unsuitable as a control for investigating the effects of hypoxia on HIF expression in Atlantic croaker.

This is the first documentation of the presence of intranuclear inclusions in the oocytes of Atlantic croaker. Similar structures have been reported in the primary growth stages of oocytes from spotted snakehead (*Channa punctata*) and walking catfish (*Clarias batrachus*) in response to exposure to ammonium sulfate [73], mercurial fungicide [74], and lead nitrate [75]. In these cases, intranuclear inclusions led to atresia. I found a significant relationship between the proportion of primary growth oocytes containing intranuclear inclusions and the proportion of atretic oocytes in the ovaries of Atlantic croaker, suggesting the presence of intranuclear inclusions may lead to oocyte atresia [73-75]. Additional research is necessary to determine the relationship between intranuclear inclusions and oocyte atresia. If such inclusions do indeed lead to atresia, then higher rates of atresia may have been evident after our July-August sampling period, reducing the reproductive potential of this Atlantic croaker population.

Atlantic croaker are present throughout Chesapeake Bay from May to October [64]. Hypoxia is widespread during summer throughout large portions of the mainstem of Chesapeake Bay and its subestuaries [49, 84] and occurs during periods of gonadal development. Atlantic croaker move to their spawning grounds in the lower Chesapeake Bay and coastal shelf waters in August and September. Spawning typically takes place from September to December [85-88], although some spawning may occur earlier [77]. The timing of my sampling coincided with gonadal development and the majority of

oocytes were in primary or secondary growth stages, making it difficult to assess the degree to which exposure to hypoxia impacts reproduction.

To better assess the impact of hypoxia exposure on the reproductive potential of the Atlantic croaker population inhabiting Chesapeake Bay during summer, sampling should occur throughout Virginia and Maryland waters from May-October. One of the difficulties with this approach is that Atlantic croaker become less likely to encounter hypoxic conditions as they migrate towards the mouth of Chesapeake Bay as they are less likely to encounter hypoxic areas in the fall [63]. The relative expression of HIFs also returns to baseline levels within 24 hours of fish returning to normoxic conditions [52, 60]. This means that the utility of HIFs as biomarkers of hypoxia exposure would be reduced, highlighting the need for another method by which hypoxia exposure can be assessed, such as stable isotope analysis. Because Mn^{2+} fluxes from the sediment to the water column under hypoxic conditions [89, 90], fish inhabiting these waters incorporate more Mn^{2+} into their otoliths than do fish residing in normoxic waters. Assessment of the Mn:Ca ratio near the edge of the otolith could, therefore, be used to determine exposure to hypoxic conditions in the days and months prior to capture [91-96]. Such an approach could be used in conjunction with the relative expression of HIFs to investigate the history of hypoxia exposure for individual fish. Individual exposure histories could also be related to metrics of reproductive potential, similar to those I used and those of Thomas et al. [26] and Thomas and Rahman [30].

Controlled laboratory experiments similar to those conducted by Rahman and Thomas [52,60] should also be employed in conjunction with field-based studies because our knowledge of the effects of acute or intermittent hypoxia exposure on the expression

of hypoxia-inducible factors and metrics of reproduction in fishes is limited. Such laboratory experiments could additionally clarify the role of temperature and contaminants in the expression of HIFs and the occurrence of intranuclear inclusions. Because the time-course of expression for HIFs may not be conducive to their use as a biomarker for intermittent or acute exposure to hypoxic conditions, laboratory experiments could also be used to identify other potential biomarkers of hypoxia exposure. Protein carbonyl and insulin-like growth factor binding protein have also been identified as potential biomarkers of hypoxia exposure [60]. Similar to HIFs, however, protein carbonyl and insulin-like growth factor binding protein require chronic exposure before expression is elevated and consequently are therefore not likely to be appropriate for assessing acute or intermittent hypoxia exposure, highlighting the need for biomarkers of hypoxia exposure that become elevated more quickly than those currently known.

Effective management of Atlantic croaker now and in the future depends on understanding the conditions that lead to reductions in reproductive potential as this is obviously a critical process for maintaining population size and productivity [97] and is sensitive to disruption by environmental conditions [35, 38, 98]. Atlantic croaker support valuable commercial and recreational fisheries in coastal areas of the western Atlantic Ocean, but the resilience of this species to fishing pressure will be reduced if hypoxia exposure results in decreased reproductive potential [31]. The expansion of hypoxic regions in Chesapeake Bay is likely to continue [99, 100], leading to increases in the proportion of the Atlantic croaker population chronically exposed to suboptimal conditions. It is therefore critical to improve our understanding of how acute, intermittent, and chronic hypoxia exposure affect Atlantic croaker in Chesapeake Bay.

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Tables

Table 1. Tissue-specific amplification efficiencies.

	Brain	Gill	Heart
18-S	1.90	1.91	2.03
HIF-1α	2.00	1.94	2.02
HIF-2α	1.93	1.83	2.06

Amplification efficiencies of 18-S, HIF-1α, and HIF-2α in the brain, gill, and heart samples from adult, female Atlantic croaker captured in Chesapeake Bay. All amplification efficiencies were within the range 1.8-2.2 and considered similar enough to permit use of the comparative C_t method [67].

Table 2. Oocyte staging scheme used for Atlantic croaker captured in Chesapeake Bay.

Stage	Code	Number
Primary Growth, One Nucleolus	PGon	1
Primary Growth, Multiple Nucleoli	PGmn	2
Primary Growth, Perinucleolar	PGpn	3
Primary Growth, Cortical Alveoli	PGca	4
Secondary Growth, Early/Late	SGe/SGl	5
Secondary Growth, Full-Grown	SGfg	6
Oocyte Maturation, Eccentric Germinal Vesicle	OMegv	7

Observed oocyte stages and their codes (following Grier [68]) for Atlantic croaker captured in three subestuaries of Chesapeake Bay, the James, York, and Rappahannock rivers, between 05 July and 26 August 2016.

Table 3. Correlations in the relative expression of HIF-1α and HIF-2α within and among tissues.

	Brain HIF-1	Brain HIF-2	Gill HIF-1	Gill HIF-2	Heart HIF-1	Heart HIF-2
Brain HIF-1		0.47	0.21	0.09	0.15	0.11
Brain HIF-2			0.20	0.40	0.07	0.25
Gill HIF-1				0.71	0.22	0.25
Gill HIF-2					0.13	0.31
Heart HIF-1						0.88
Heart HIF-2						

Correlations of the relative expression of HIF-1α and HIF-2α in the brain, gill, and heart of adult, female Atlantic croaker. Significant correlations ($P < 0.05$) are indicated by gray shading.

Figures

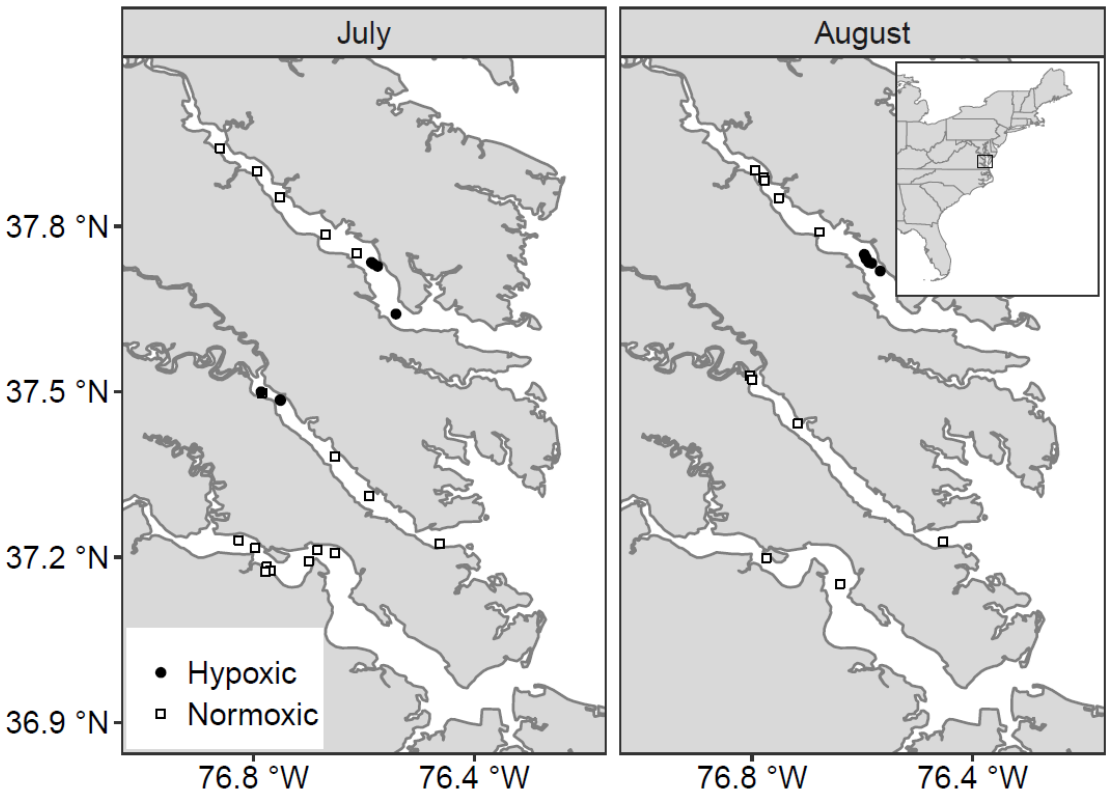

Fig 1. Study area in the lower Chesapeake Bay. The three subestuaries, from north to south, are the Rappahannock, York, and James rivers. Stations where Atlantic croaker were captured under hypoxic conditions (i.e., oxygen level < 3.5 mg L^{-1}) are indicated with filled circles and under normoxic conditions (i.e., oxygen level > 3.5 mg L^{-1}) with open squares. The inset shows the location of the study area.

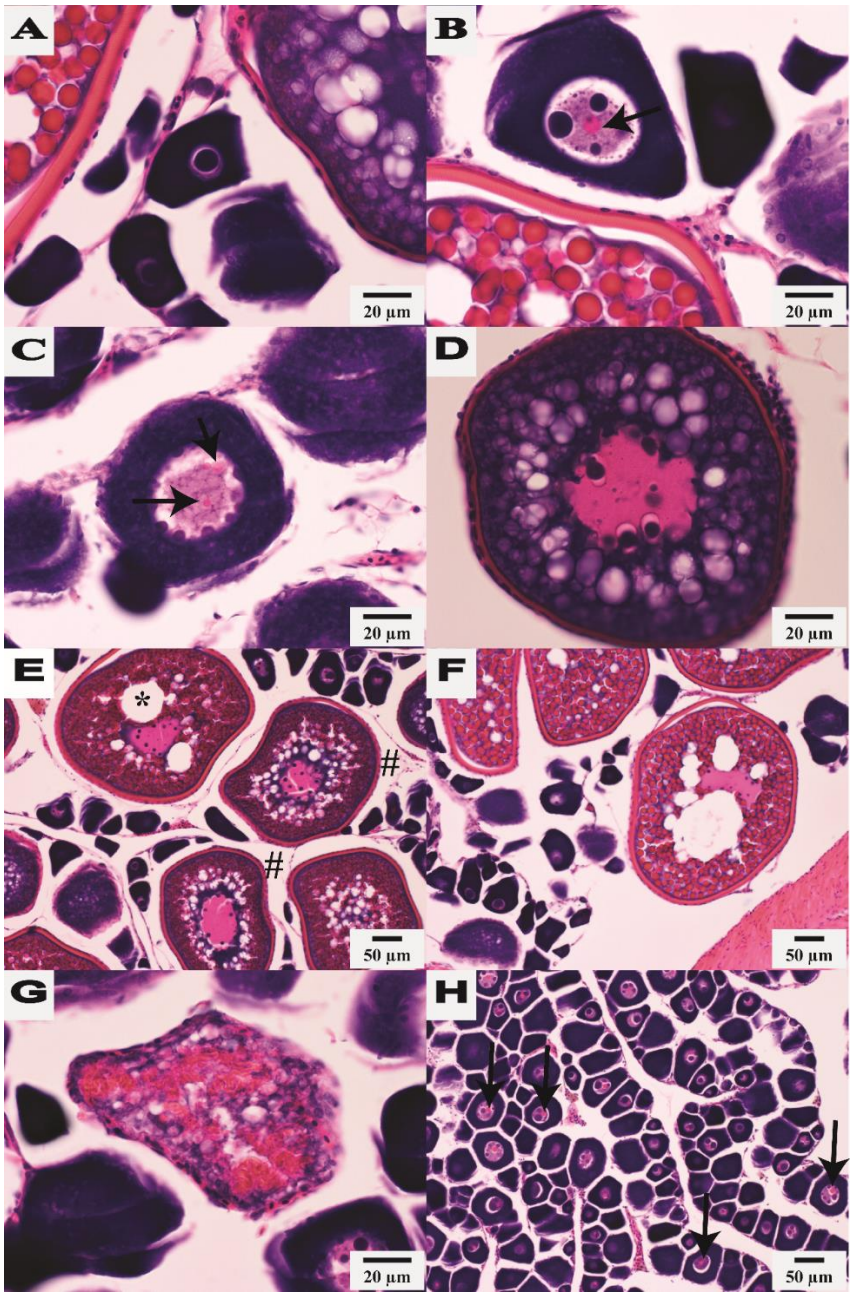

Fig 2. Oocyte stages observed for Atlantic croaker captured in the Virginia subestuaries of Chesapeake Bay during July and August 2016. Sections of oocytes were stained using hematoxylin and eosin (H&E) and terminology followed Grier (2012). (A) Primary growth, one nucleolus (PGon); (B) Primary growth, multiple nucleoli (PGmn) with intranuclear inclusion (arrow); (C) Primary growth, perinucleolar (PGpn) with intranuclear inclusions (arrows); (D) Primary growth, cortical alveolar (PGca); (E) Secondary growth, early/Secondary growth, late (SGe/SGl, #) and Secondary growth, full grown (SGfg, *); (F) Oocyte maturation, eccentric germinal vesicle (OMegv); (G) an atretic oocyte; (H) an ovary section showing greater than 50% of oocytes containing intranuclear inclusions (examples are indicated with arrows).

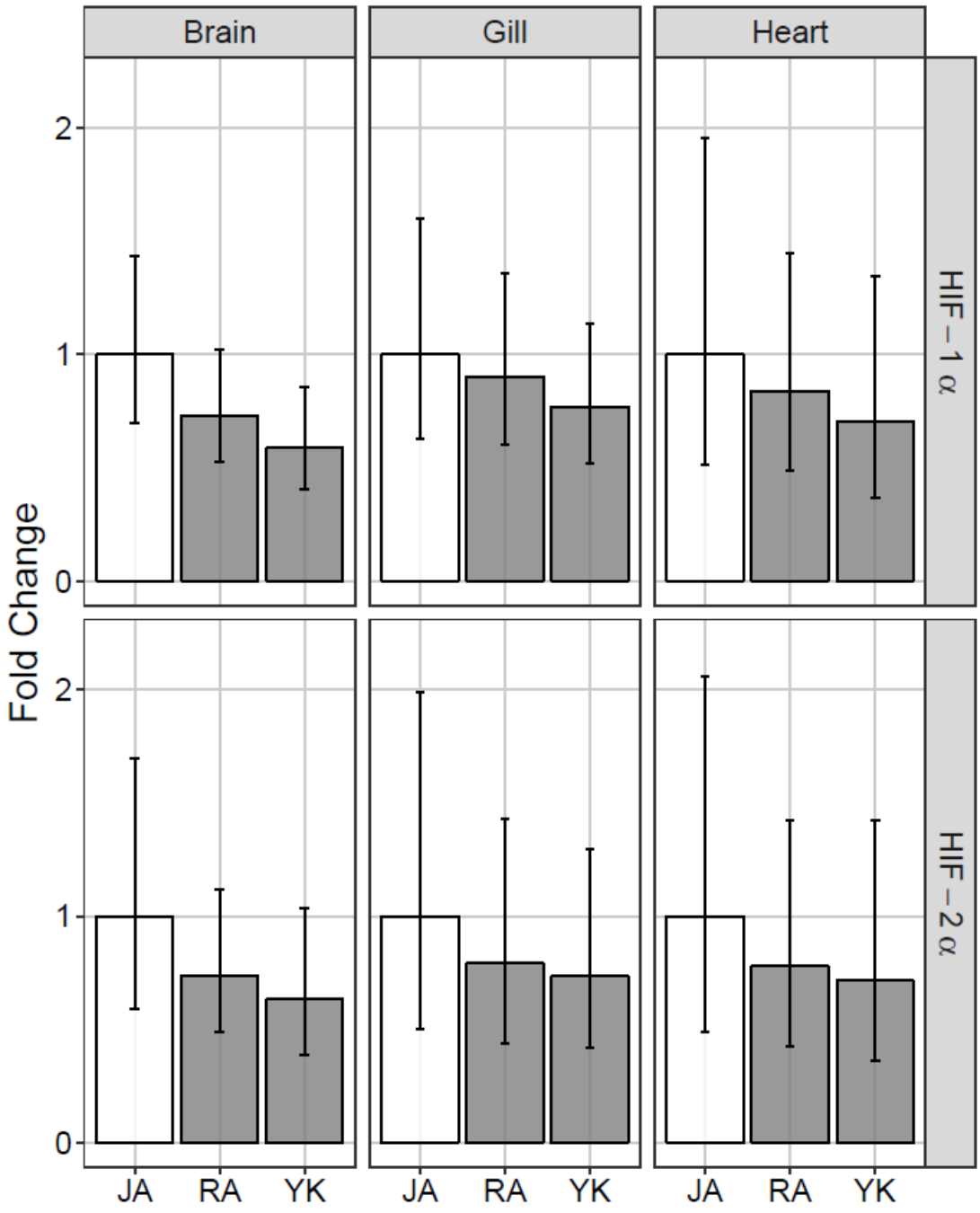

Fig 3. The mean (± 95% confidence intervals) fold change of HIF-1α (upper panel) and HIF-2α (lower panel) in Atlantic croaker. Fish were captured in the Rappahannock (RA) and York rivers (YK, gray bars) relative to fish captured in the James River (JA, white bars). Tissues are: brain (left), gill (center), and heart (right).

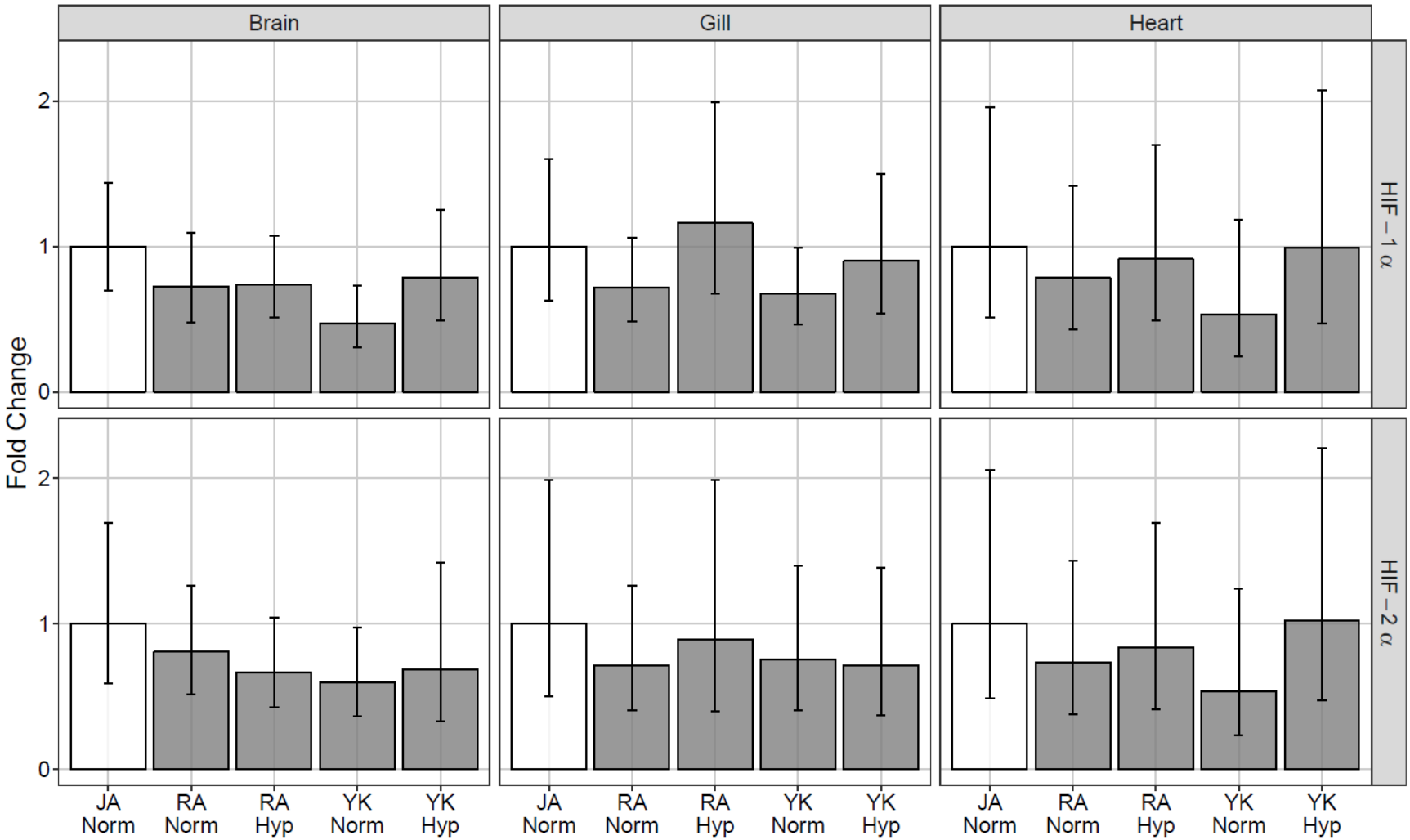

Fig 4. The mean (± 95 relative confidence interval) fold change of HIF-1α (upper panel) and HIF-2α (lower panel) in Atlantic croaker. Fold change in HIFs is examined for fish were captured under hypoxic (hyp) and normoxic (norm) conditions in the Rappahannock (RA) and York rivers (YK, gray bars) relative to normoxic conditions in the James River (JA, white bars). Tissues are: brain (left), gill (center), and heart (right).

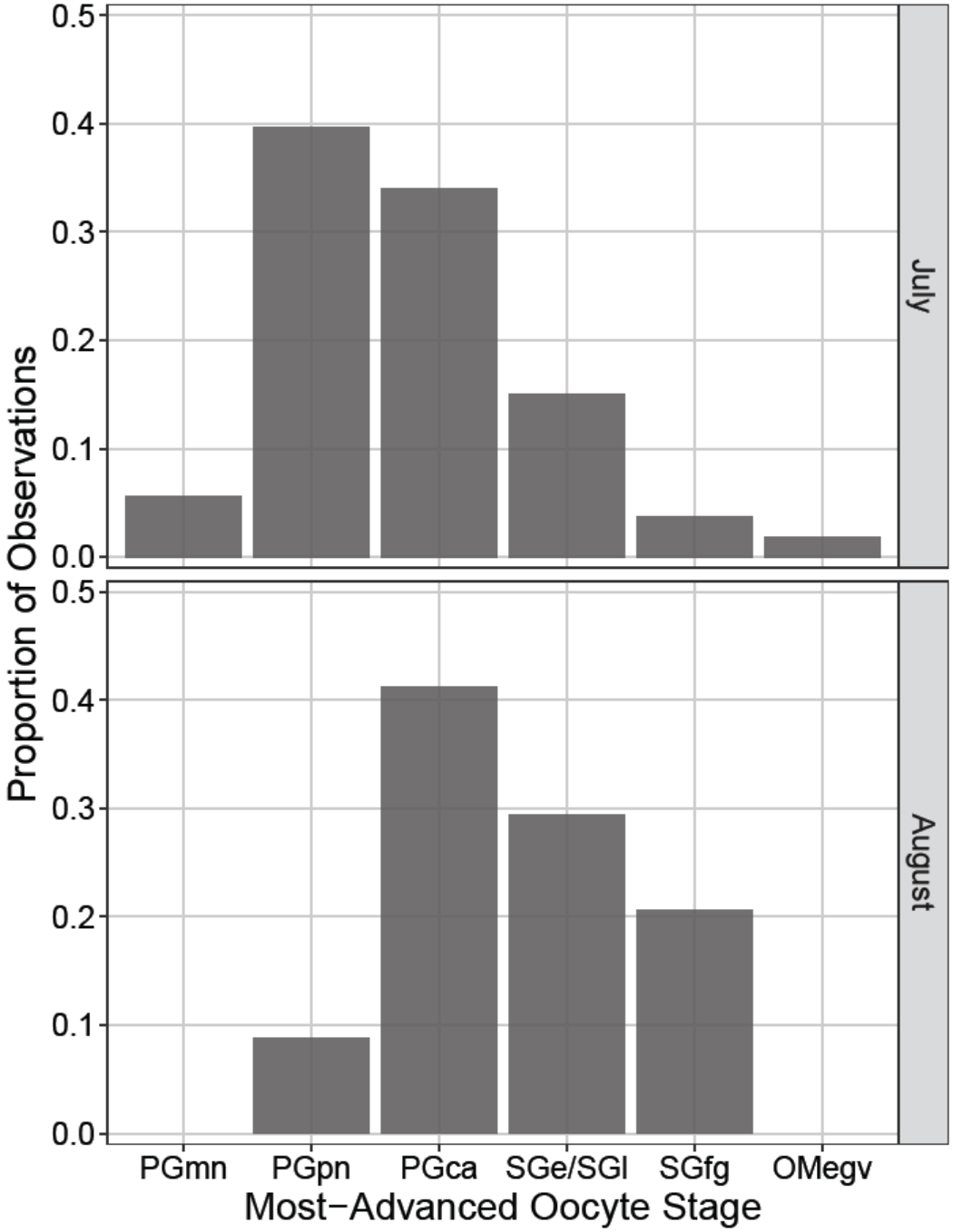

Fig 5. Proportions of each of the most-advanced oocyte stages found in the ovaries of Atlantic croaker captured in normoxic conditions in July and August. The top panel shows the most-advanced oocyte stage for Atlantic croaker captured in normoxic conditions across all subestuaries in July whereas the bottom panel shows the most-advanced oocyte stage for fish captured in August. Bars represent the proportion of ovaries for which each stage was the most-advanced oocyte stage observed from primary growth stages (left) to maturation stages (right).

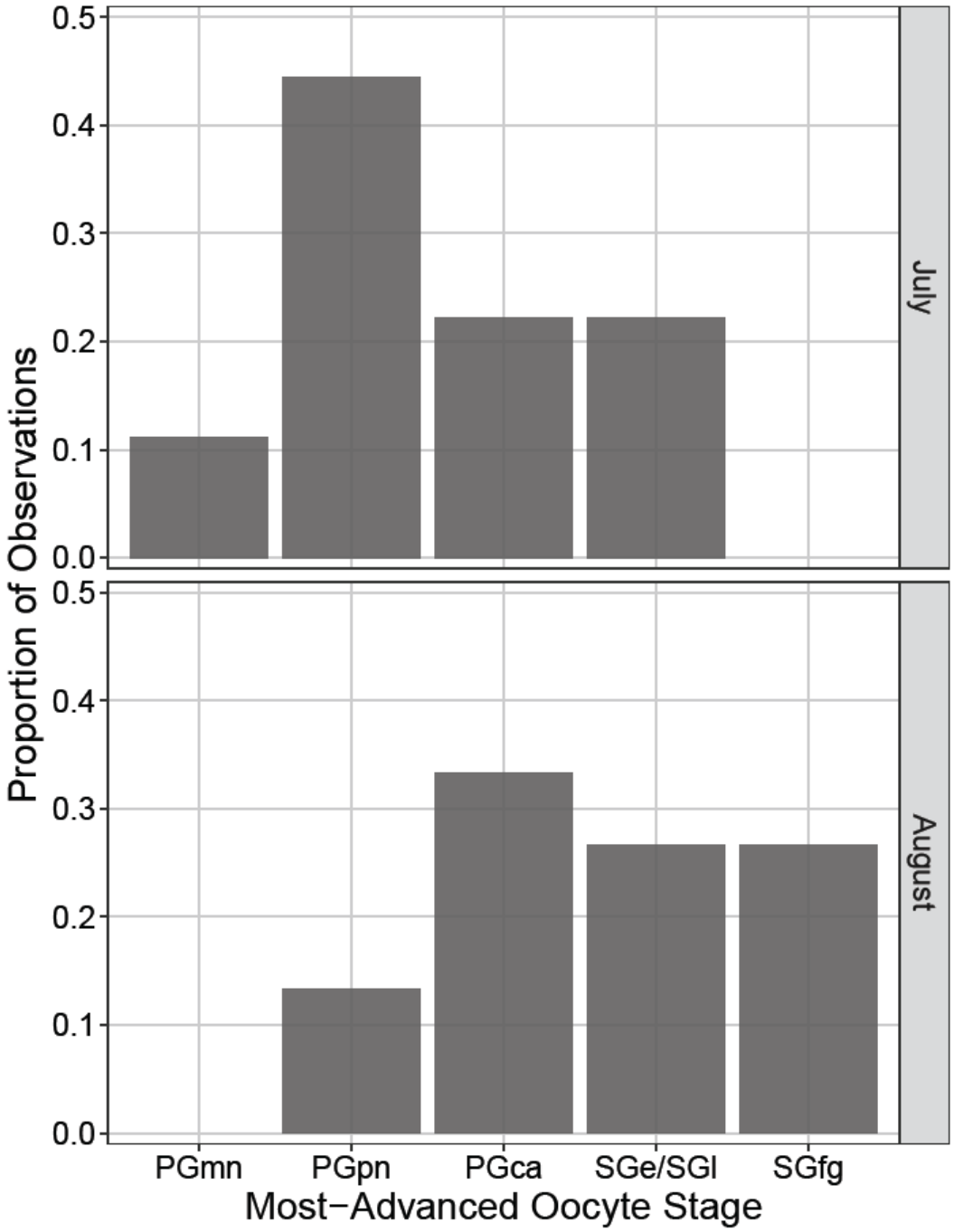

Fig 6. Proportions of each of the most-advanced oocyte stages found in the ovaries of Atlantic croaker captured in hypoxic conditions in the Rappahannock River. The top panel shows the most-advanced oocyte stage for Atlantic croaker captured in July whereas the bottom panel shows the most-advanced oocyte stage for fish captured in August. Bars represent the proportion of ovaries for which each stage was the most-advanced oocyte stage observed from primary growth stages (left) to secondary growth stages (right).

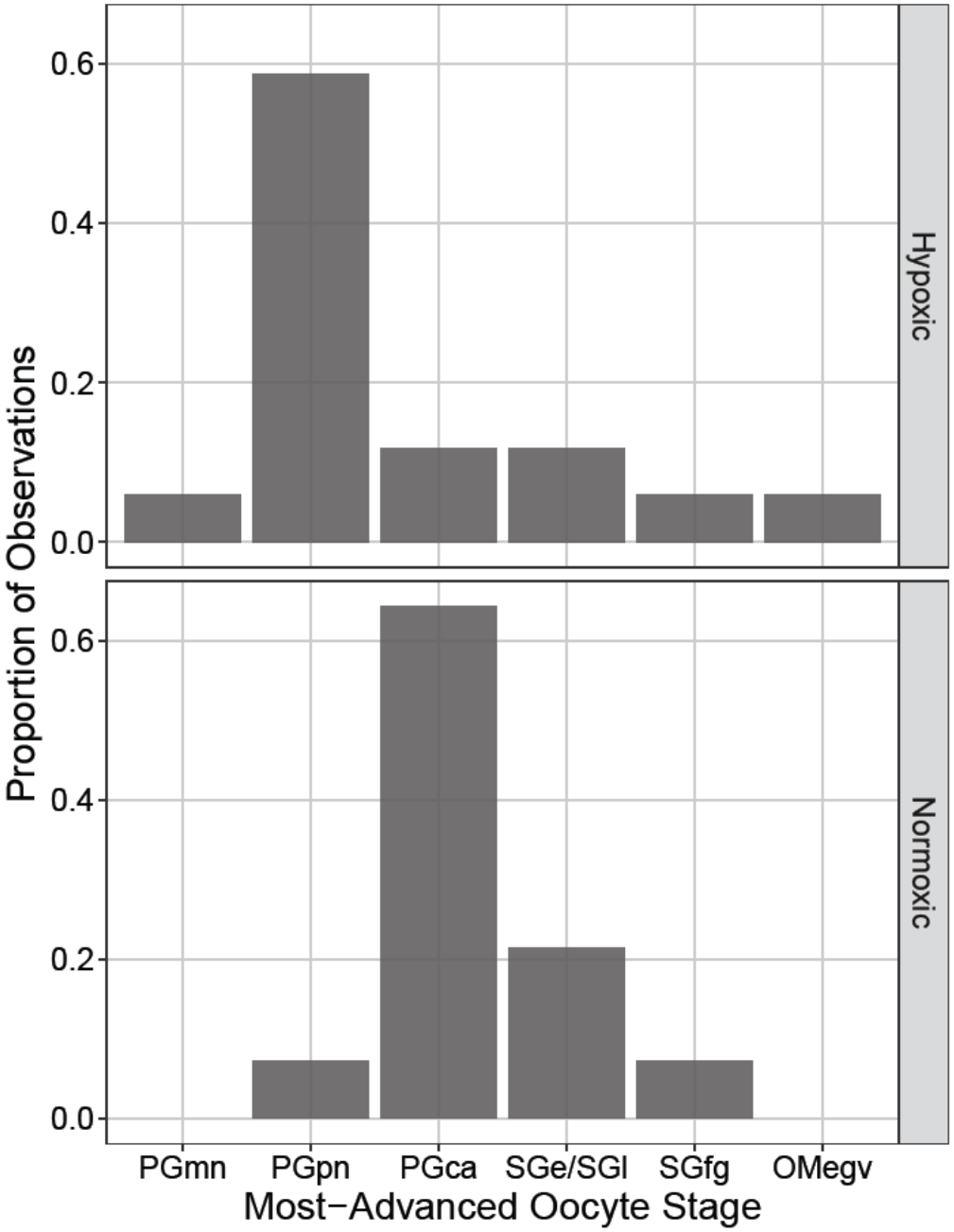

Fig 7. Proportions of each of the most-advanced oocyte stages found in the ovaries of Atlantic croaker captured in July in the York River. The top panel shows the most-advanced oocyte stage for Atlantic croaker captured in hypoxic conditions whereas the bottom panel shows the most-advanced oocyte stage for fish captured in normoxic conditions. Bars represent the proportion of ovaries for which each stage was the most-advanced oocyte stage observed from primary growth stages (left) to maturation stages (right).

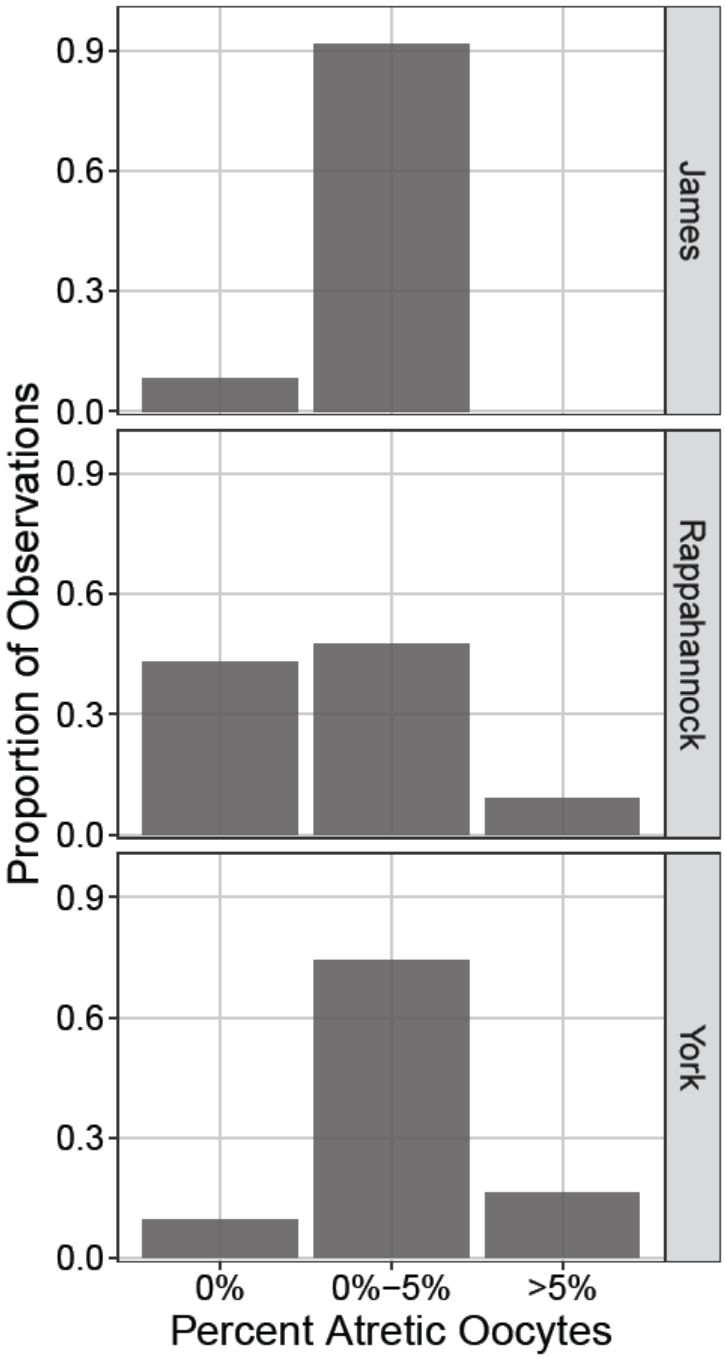

Fig 8. Percentage of atretic oocytes occurring in the ovaries of Atlantic croaker. Bars show the proportion of observations for which atretic oocytes were 0%, 0-5%, or >5% of the total observed oocytes for the James (top), Rappahannock (middle), and York (bottom) rivers.

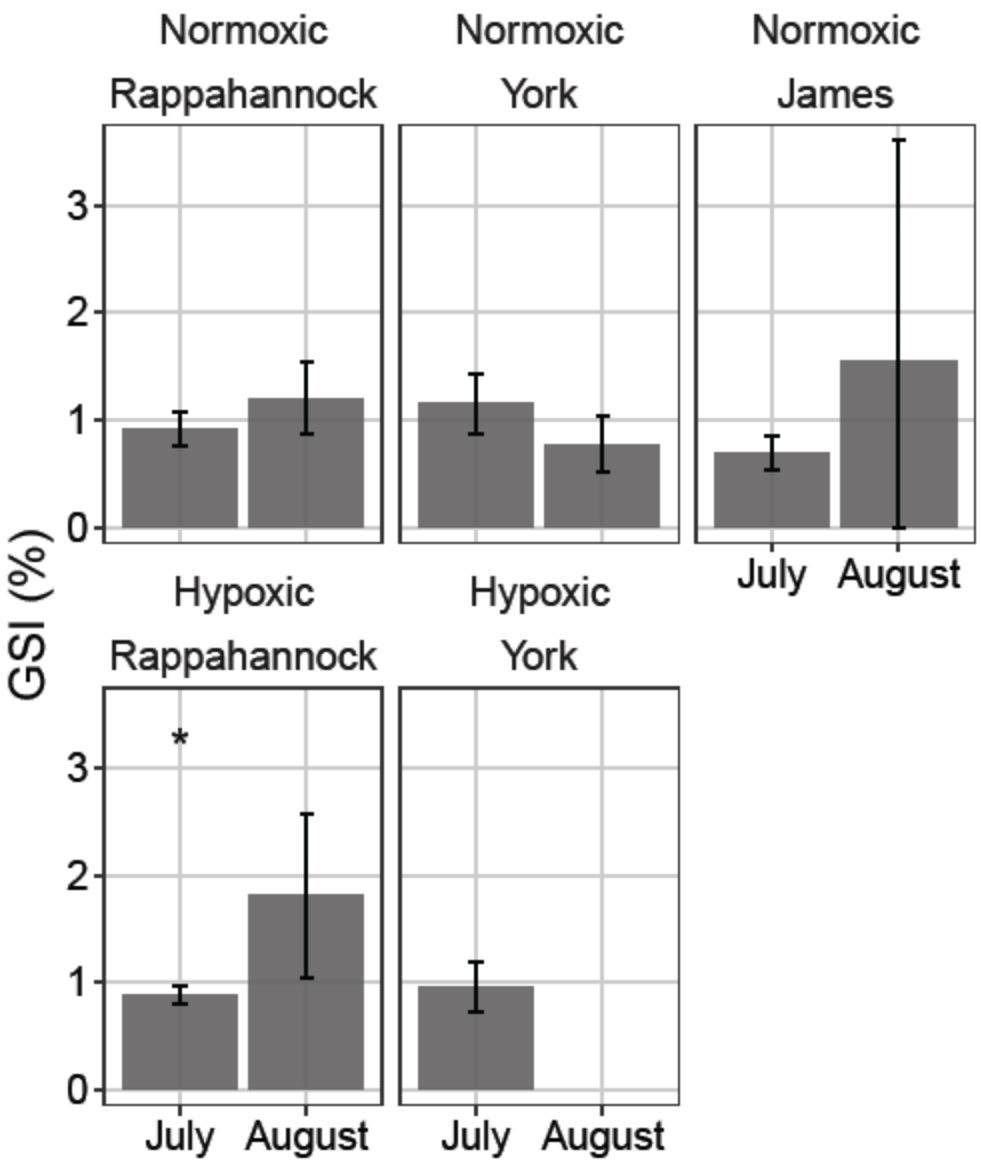

Fig 9. The mean (± 95 relative confidence interval) gonadosomatic index (GSI) of Atlantic croaker captured in July and August in the Rappahannock River (left panels), York River (center panels), and James River (right panels) in normoxia (upper panels) and hypoxia (lower panels).

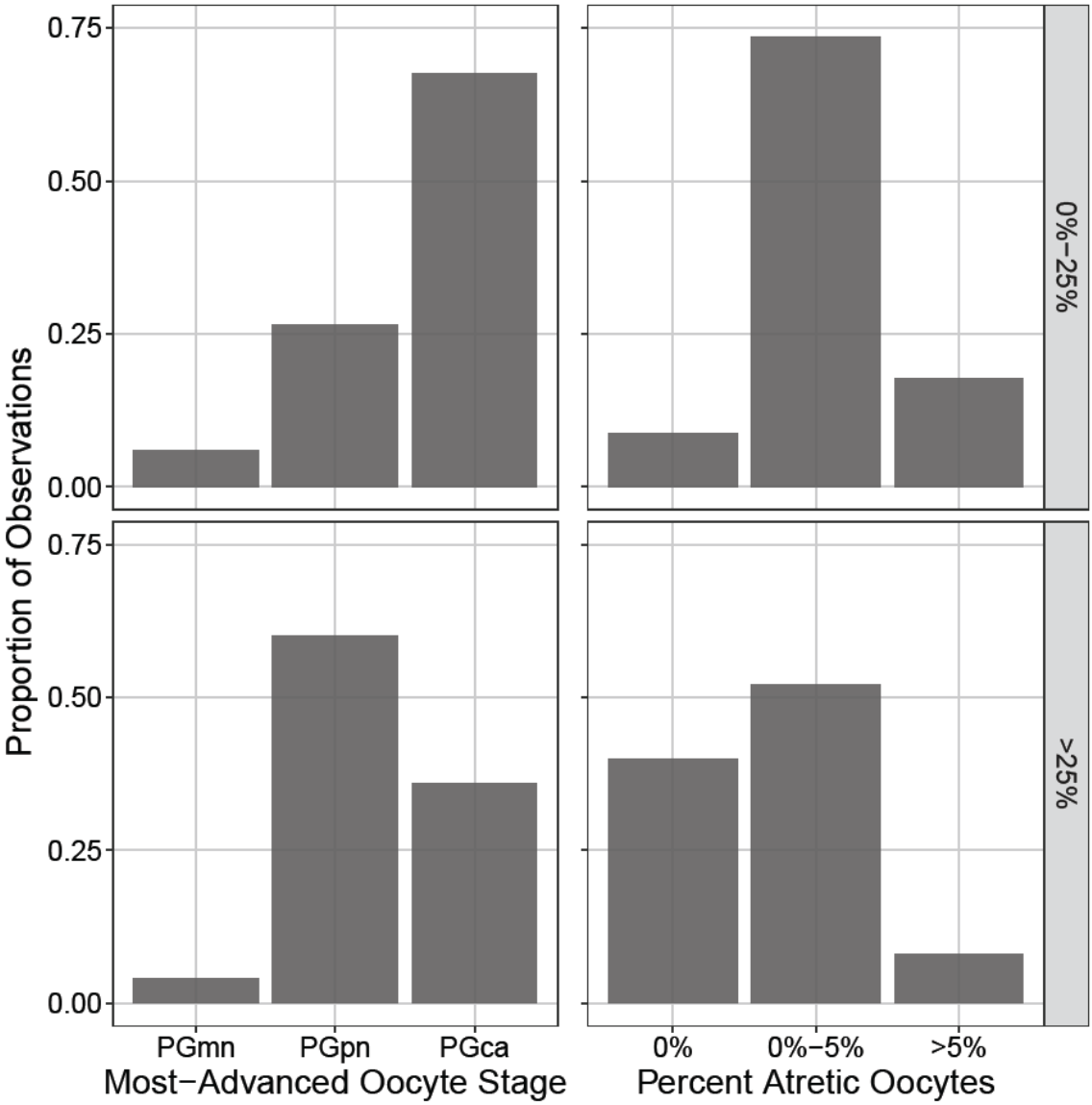

Fig 10. Intranuclear inclusions in Atlantic croaker oocytes. The left panels show the proportion of Atlantic croaker ovaries for which the most-advanced oocyte stage was Primary Growth, multiple nucleoli (PGmn), Primary Growth, perinucleolar (PGpn), or Primary Growth, cortical alveolar (PGca) when the proportion of oocytes containing intranuclear inclusions was 0-25% (upper panel) or >25% (lower panel). Additionally, the right panels show the proportion of Atlantic croaker ovaries for which the proportion of atretic oocytes was 0%, 0-5%, or >5% when the proportion of oocytes containing intranuclear inclusions was 0-25% or >25%.

CHAPTER 4

Modeling the Distribution of Two Demersal Fishes in a Dynamic Seascape: Atlantic Croaker and Spot in Chesapeake Bay

Abstract

Anthropogenic activities have led to increases in water temperatures and in the frequency and severity of hypoxic conditions in estuarine and coastal systems worldwide. These environmental conditions have been linked to population-level effects, such as changes in species-specific movements and distributions. Physiology (i.e., the ability to maintain internal homeostasis in the face of fluctuating conditions) can be considered the transfer function that links environmental conditions to individual behavior and therefore to population-level effects, however; few studies have incorporated species-specific physiological abilities into population models. I developed individual-based models (IBMs) for Atlantic croaker and spot that include physiological constraints to investigate the effects of temperature and ambient oxygen levels on the distribution of fishes in Chesapeake Bay. I used three movement submodels (random walk, kinesis, and restricted-area search) motivated by the effects of temperature and hypoxia on metabolic scope. Monthly distributions from the IBM were validated against observations from the VIMS Juvenile Fish Trawl Survey (hereafter "trawl survey") from 1988-2014. Simulated fish consistently occupied warmer, better oxygenated waters than fish captured by the trawl survey. Simulations indicated that the majority of fish would be distributed in the lower portion of Chesapeake Bay and with smaller proportions in the York and Rappahannock rivers. These results were not supported by trawl survey observations and suggest that temperature may not be the most important driver of Atlantic croaker and spot distributions in Chesapeake Bay during summer, although climate-change models often focus on potential impacts of temperature change alone. My results suggest that other factors, such as prey availability and predator avoidance, may contribute to the

spatial distributions of Atlantic croaker and spot in Chesapeake Bay. I contend that to better understand the movement of individual fish and population distributions, both additional abiotic factors (e.g., water clarity) and biotic factors (e.g., predator and prey abundance) need to be considered along with the more commonly used abiotic factors (temperature, salinity, and dissolved oxygen). This approach could aid the development of movement models capable of predicting the distribution of fish populations under future climate scenarios.

1. Introduction

Anthropogenic activities drive directional changes in temperature that have resulted in increased water temperatures throughout the world's oceans (IPCC, 2014). Agricultural runoff and increases in non-point source pollution resulting from increased urbanization of coastal areas have contributed to eutrophication of estuarine and coastal systems, as well as an increase in the number and severity of hypoxic events (Diaz and Rosenberg, 2008; Rabalais et al., 2010; Howarth et al., 2011; Breitburg et al., 2018). In fishes, elevated temperature and hypoxia exposure negatively impact individuals and populations through changes in aerobic metabolic scope (Schurmann and Steffensen, 1997; Claireaux and Lagardère, 1999; Lapointe et al., 2014), increased disease prevalence (Keefer et al., 2008; Karvonen et al., 2010), increased mortality rates (Breitburg, 1992; Craig et al., 2001; Pollock et al., 2007), decreased gonadal growth rates and reproductive potential (van der Kraak and Pankhurst, 1997; Rahman and Thomas, 2007; Thomas and Rahman, 2009; Tuckey and Fabrizio, 2016), and decreased somatic growth rates (Hrycik et al., 2017). Degradation of environmental conditions due to warming and eutrophication have also been linked to changes in the spatial distribution of fishes in estuarine and marine ecosystems (Perry et al., 2005; Sabatés et al., 2006; Brady and Targett, 2013; Buchheister et al., 2013).

Spatial shifts of fish populations are generally considered to be driven by environmentally-mediated changes in aerobic metabolic scope (Pörtner and Knust, 2007; Hare et al., 2012; Deutsch et al., 2015; Kleypas, 2015). Albeit with a few exceptions (Cucco et al., 2012; Marras et al., 2015), studies explicitly linking the impacts of changing environmental conditions to population-level effects such as changes in spatial

distribution are lacking. Because physiology is the transfer function that links environmental conditions to fish behavior (Pörtner and Farrell, 2008; Denny and Helmuth, 2009; Chown et al., 2010; Horodysky et al., 2015) and, by extension to their distribution, effective management of fishes relies on understanding how (and how well) the physiological abilities of individual fish allow them to withstand changing environmental conditions (Cooke et al. 2016). Because individuals within a population may react differently to changes in environmental conditions, population models need to incorporate variation in individual responses (Kearney, 2006; Fabry et al., 2008; Horodysky et al., 2015; Riebesell and Gattuso, 2015; Cooke et al., 2016; Koenigstein et al., 2016; Townhill et al., 2017).

Individual-based models (IBMs) provide researchers with a tool to simulate processes at multiple levels of biological organization, such as the individual, population, and community (Huston et al., 1988; Judson, 1994; Grimm, 1999). IBMs allow researchers to investigate the potential impacts of biotic and abiotic conditions on growth, reproduction, movement, and mortality of individuals and the entire population because many individuals are modeled. IBMs have been used to examine effects of sea-level rise and changes in temperature, habitat availability, salinity, oxygen concentrations, and prey densities on growth, mortality, and movement (Humston et al., 2000; 2004; Goodwin et al., 2006; Fulford et al., 2011; 2014; 2016; Rose et al., 2013b; 2018b). IBMs have also been used to examine these effects on population growth (Humston et al., 2004; Rose et al., 2013b; 2018b; Fulford et al., 2014).

Spatially-explicit IBMs require movement algorithms to simulate fish movement within a dynamic seascape. Movement algorithms are used because few studies have

gathered environmental data at high spatial and temporal resolutions simultaneously with the movement of individuals (but see Childs et al., 2008; Fabrizio et al., 2013; 2014). Movement algorithms are often based on the assumption that fish will move towards areas with optimal conditions or sets of conditions, and avoid suboptimal conditions (Humston et al., 2000; 2004; Rose et al., 2013a; 2018a; Watkins and Rose, 2013; 2014; 2017). The first assertion is likely incorrect in that it assumes either that fish know where optimal conditions exist and in which direction to move to locate optimal conditions, or that they are capable of sensing a gradient in environmental conditions such that they can determine if they are moving in a direction towards more favorable conditions or away from them. In some cases, optimal conditions can be determined based on the observed environmental conditions in which a school of fish is located (Humston et al., 2000); in other cases, more mechanistic bioenergetics models have been used to determine environmental conditions at which growth is maximized (Humston et al., 2004; Rose et al., 2013a; 2013b; 2018a; 2018b). The latter approach assumes the direct effects of the environment on the aerobic metabolic scope of fish (first described by Fry, 1947), but studies do not necessarily directly employ this concept.

Aerobic metabolic scope is the difference between the maximum metabolic rate and the minimum metabolic rate necessary to maintain homeostasis (standard metabolic rate) (Fry, 1947). Metabolic scope represents, therefore, the energetic state space within which all aerobic processes (e.g., movement, reproduction, growth) must occur (Fry, 1971; Claireaux and Lefrançois, 2007). Metabolic scope can be reduced by exposure to suboptimal environmental conditions either through limiting the maximum metabolic rate (e.g., dissolved oxygen) or increasing the standard metabolic rate (e.g., temperature,

salinity) (Fry, 1947; 1971; Neill et al., 1994; Horodysky et al., 2015). Environmental conditions can therefore limit processes such as individual growth and reproductive potential (which are important to population productivity) through reductions in metabolic scope (Claireaux and Lefrançois, 2007). I argue (as have others, Kelsch and Neill, 1990; Neill and Bryan, 1991; Neill et al., 1994) that individual fish are likely to move away from areas with conditions that reduce metabolic scope, but remain in areas where conditions optimize metabolic scope (and therefore their growth and reproductive potential). In aggregate, such movements of individuals can affect the spatial distribution of the population. I contend, however, that the effects of environmental conditions on the metabolic scope of individual fish, and the subsequent impacts on population distributions, have not been adequately investigated in an individual-based context.

The goal of this portion of my dissertation is to develop individual-based, dynamic-seascape models for adult Atlantic croaker (*Micropogonias undulatus*) and spot (*Leiostomus xanthurus*) occupying Chesapeake Bay. The effects of temperature and hypoxia on metabolic scope were used to motivate movement of fish such that fish would remain in areas with environmental conditions that maximize their metabolic scope. Three movement submodels were considered: completely random movement (random walk), reactionary but undirected movement (kinesis), and directed movement (restricted-area search, RAS). The random walk submodel was uncoupled from the physiological effects of temperature and hypoxia on Atlantic croaker and spot and thereby provided a scenario to evaluate the effectiveness of incorporating physiological constraints into the kinesis and RAS submodels. Individuals in the kinesis submodel slowed down and generally maintained direction when exposed to environmental conditions that increased

metabolic scope, whereas individuals in the RAS submodel used a gradient approach to move to areas that maximized metabolic scope.

Adult Atlantic croaker and spot were chosen for this study because they are seasonal residents of Chesapeake Bay. Both species typically inhabit estuarine waters from late spring to fall (May – October) when they leave the estuary to spawn in waters of the continental shelf (Haven, 1959), although some Atlantic croaker may spawn in the lower Chesapeake Bay (Barbieri et al., 1994). Adult Atlantic croaker and spot are absent from Chesapeake Bay in winter (December – March) (Murdy and Musick, 2013) and return to the estuary from their wintering grounds on the continental shelf between mid-April and May. In addition to a well-studied life history, the metabolic rates of adult Atlantic croaker and spot are well-known (Horodysky et al., 2011; Chapter 2). Furthermore, relative abundance and spatial distributions have been monitored monthly for these species from 1988 to 2014, which allows for validation of model results for a period in which temperatures and the frequency and severity of hypoxic conditions have increased in Chesapeake Bay.

This multi-decadal time series of the distribution of Atlantic croaker and spot, together with observations on temperature and dissolved oxygen conditions during the time of capture provides a unique opportunity to evaluate the performance of the three movement submodels. I assessed the performance of the IBMs by:

(1) comparing environmental conditions experienced by simulated individuals to those in which fish were captured by the VIMS Juvenile Fish Trawl Survey and

(2) determining the effectiveness of IBMs in reproducing the observed spatial distributions of Atlantic croaker and spot in Chesapeake Bay.

Based on the results from Chapter 2, I hypothesized that individuals that moved according to the RAS submodel would occupy areas with the highest temperatures and dissolved oxygen concentrations and would more effectively avoid areas of low dissolved oxygen concentrations when compared with individuals in the kinesis and random walk submodels. Additionally, because fish movements are likely reactionary to environmental conditions in their immediate vicinity, I contend that the kinesis submodel is likely to most accurately reflect fish movement. I therefore propose that simulated individuals moving according to the kinesis submodel would aggregate in conditions that match those in which adult Atlantic croaker and spot are most frequently captured by the trawl survey (assuming equal gear vulnerability under all conditions), and that this submodel would most accurately reflect the distribution of adult Atlantic croaker and spot in Chesapeake Bay.

2. Methods

2.1 Model overview

The individual-based models followed the spatial distribution of individual adult Atlantic croaker and spot on a 162 km x 152 km spatial grid of 1 km^2 cells representing the lower Chesapeake Bay and its major subestuaries, the James, York, and Rappahannock rivers (Figure 1). Each grid cell in the study area had associated environmental conditions (temperature and dissolved oxygen concentration) that were dynamic at a 24-hr time step (details are given in section 2.4.2). The study period extended from May to October, 1988 – 2014. I introduced 10,000 simulated individuals into two areas near the mouth of Chesapeake Bay on May 1 of each model year to reflect the entry of these fishes into estuarine waters (Figure 1). Two areas were used to broaden the initial distribution of individuals in the simulation.

Individuals moved on an hourly time step during the simulation period, and the position of each individual was determined following its movement within the study area at each time step. At the end of the model simulation for each model year, individuals were removed from the study area before a new cohort was introduced at the beginning of the next model year, thus, each year could be treated as a separate trial. Annual variations in the severity of hypoxia may result in the displacement of fish to areas with suboptimal environmental conditions, or may expose fish to low dissolved oxygen (DO) conditions. The approach also enabled investigation of the effects of the severity of hypoxia on:

(1) the spatial distribution of fishes,

(2) the percentage of observations where individuals inhabited areas below their hypoxia tolerance, and

(3) the environmental conditions in which simulated fish were captured.

Alterations in these factors were likely most apparent for years when hypoxic conditions contrasted. I therefore selected two years with the largest volume of hypoxic water (km^3), 2007 and 2011, and two years with the smallest volume of hypoxic water, 2008 and 2014 (Scavia et al., 2017). These were designated as "severe" and "mild," respectively, to investigate the effects of the severity of hypoxia on fish distribution.

Individual movement in the kinesis and RAS submodels in response to DO conditions was determined by comparing ambient DO concentrations with estimates of the DO concentration below which fish could not maintain homeostasis ($mean_{Atlantic\ croaker}$ = 1.36 mg L^{-1}; $mean_{spot}$ = 1.86 mg L^{-1}). The latter was used as a proxy for hypoxia tolerance (Muuszea et al., 1998; Nilsson and Östlund-Nilsson, 2004; Mandic et al., 2009). Hypoxia avoidance was elicited in fish if their individual hypoxia tolerance was greater than ambient DO concentrations. Additionally, the relationship between metabolic scope and temperature was used to motivate fish movement such that fish would move towards areas that supported high metabolic scopes (i.e., > 25°C for Atlantic croaker and > 20°C for spot). Areas where the temperature maximized metabolic scope and DO concentrations were above a fish's hypoxia tolerance were considered optimal; movement submodels were parameterized to increase the time fish spent in these conditions (described in section 2.6). Each movement submodel (random walk, kinesis, and RAS) was simulated independently for each species, and models were coded in Netlogo 5.3.1 (Railsback and Grimm, 2012).

2.2 Environmental conditions

Monthly water-quality data (bottom temperatures and bottom dissolved oxygen concentrations) were provided by the VIMS Juvenile Fish Trawl Survey (hereafter "trawl survey") and long-term monitoring stations from the Virginia Estuarine and Coastal Observing System (VECOS, http://web2.vims.edu/vecos/). The trawl survey samples 111 stations each month using a random-stratified design (Tuckey and Fabrizio, 2017). Briefly, the mainstem of Chesapeake Bay is divided into three latitudinal regions which are then subdivided into six strata based on location and depth. The James, York, and Rappahannock rivers are divided into four longitudinal regions within which four depth strata are sampled. Each month, 15 stations are sampled in each of the three bay regions and 22 stations are sampled in each river (Tuckey and Fabrizio, 2017). Water-quality information was also obtained from VECOS long-term fixed stations (up to 36 stations per month) throughout the mainstem of Chesapeake Bay and the James, York, and Rappahannock rivers (Figure 2). Combined, the trawl survey and VECOS observations covered a 24,624 km^2 area between 36.8 and 38.2°N and 77.0 and 75.6°W for May to October, 1988 – 2014.

2.3 Construction of a dynamic seascape

To create a dynamic environment on spatial and temporal scales that are meaningful to fish movement, the observed bottom-water temperatures and DO concentrations were spatially interpolated to a 1 km^2 grid and temporally interpolated to daily time steps. Monthly observations from the trawl survey and VECOS were first

projected to local coordinates after which a three-step, inverse-distance weighting procedure was implemented as follows:

(1) The values of environmental parameters were used to preserve "true" local data for any grid cell that contained an observation.

(2) If only one observation occurred within a 1-km radius of the target area, the environmental data associated with that observation were used. If, however, multiple observations were within the 1-km radius, an inverse-distance weighting method (i.e, observations closer to the target cell are weighted more heavily than those further away) was used to interpolate environmental conditions for that cell.

(3) Observations from steps 1 and 2 were interpolated to the whole domain by using a 5-km search radius to inform the inverse-distance weighting for specific locations. If no observations occurred in the 5-km radius, the search radius was doubled and the process repeated.

Following spatial interpolation, monthly data were temporally interpolated to daily time steps using linear interpolation. All observations were then converted into a netCDF file and used in the IBMs.

2.4 Individual fish characteristics

Characteristics of individual fish were assigned at the start of each model year based on the results of respirometry trials with adult Atlantic croaker and spot at 10, 15, 20, 25, and 30°C (Chapter 2). To ensure that the physiological characteristics used in the IBMs accurately reflected those of wild-caught fish, I assigned lengths to the simulated

fish from the mean and standard deviation of individuals used in the respirometry trials. Fish lengths (mean ± standard deviation) were randomly assigned from normal distributions of 273 ± 20 mm and 209 ± 9 mm for Atlantic croaker and spot, respectively. Critical DO concentrations (C_{crit}; mg O_2 L^{-1}), used as a proxy for hypoxia tolerance, were also assigned to each individual in the model. C_{crit} is the DO concentration below which an individual cannot maintain homeostasis. Because the relationship between C_{crit} and temperature exhibited a significant linear, positive slope, initial C_{crit} values were assigned based on the mean and standard deviation of C_{crit} observed at 10°C during the respirometry trials (Chapter 2). C_{crit} was normally distributed with a mean and standard deviation of 1.4 ± 0.7 mg O_2 L^{-1} and 1.9 ± 0.4 mg O_2 L^{-1} for Atlantic croaker and spot, respectively. The distribution of C_{crit} for Atlantic croaker was truncated at 0.7 mg O_2 L^{-1} whereas that of spot was truncated at 1.4 mg O_2 L^{-1}, which corresponds to the lowest observed C_{crit} for each species (Chapter 2). Critical oxygen concentrations for each fish increased with increasing temperature according to:

$$(C_{crit})_{Atlantic\ croaker,t} = C_{crit} + \left((Temp_t - 10) \times 0.053\right) \quad (1)$$

and

$$(C_{crit})_{spot,t} = C_{crit} + \left((Temp_t - 10) \times 0.012\right). \quad (2)$$

In these equations, *t* indicates the current time step in the simulation, C_{crit} is the critical oxygen concentration at 10°C, *Temp* indicates the ambient temperature (°C) experienced by an individual, and 0.053 and 0.012 are the increases in C_{crit} that occur with each 1°C change in temperature for Atlantic croaker and spot, respectively. The hypoxia tolerance of individual fish therefore varied with temperature.

2.5 Movement submodels

For each movement submodel, velocities in the *x* and *y* directions [$V_{x,t}$ and $V_{y,t}$] were calculated for each hourly time step. I used these velocities to update each individual's location at each time step with the equations:

$$x_t = x_{t-1} + V_{x,t} \quad (3)$$

$$y_t = y_{t-1} + V_{y,t}, \quad (4)$$

where *t* represents the current time step. Parameters used in the movement submodels are described in Table 1.

2.5.1 Random walk submodel

A random walk submodel was used to estimate movements that are not affected by environmental conditions (Codling et al., 2008; LaBone et al., 2017). For ease of computation, movement of individual fish was parsed into *x* and *y* components that when combined, result in a single directional vector of movement. The *x* and *y* components follow Gaussian distributions with means (± standard deviations) of 2.5 ± 1.2 body lengths per second (bl s^{-1}) and 2.6 ± 1.3 bl s^{-1} for Atlantic croaker and spot, respectively. Mean swimming speeds in the *x* and *y* directions resulted in a movement vector of 3.5 bl s^{-1} for Atlantic croaker and 3.6 bl s^{-1} for spot, which correspond to experimentally-derived optimal swimming speeds for these species (Horodysky et al., 2011). The velocity (*V*) and direction (θ) of movement at time *t* were calculated with the equations:

$$V_t = \sqrt{{V_{x,t}}^2 + {V_{y,t}}^2} \quad (5)$$

and

$$cos(\theta_t) = \frac{V_{x,t}}{V_{y,t}}. \quad (6)$$

Swimming speeds were expressed in units of kilometers per hour with the equation:

$$V'_t = V_t \times \left(L \times 10^{-6}\left(\frac{km}{mm}\right)\right) \times 3600\,\frac{s}{hr}, \quad (7)$$

where V'_t is the swimming speed in km hr^{-1} and L is the length of the individual (mm).

2.5.2 Kinesis submodel

Kinesis describes the movement of individuals that sense and respond to environmental conditions in their immediate vicinity and adjust their movement based on these environmental cues (Humston et al., 2000; 2004; Watkins and Rose, 2013). The kinesis submodel used herein followed Humston et al. (2000; 2004).

Movement of individuals in the kinesis submodel was affected by temperature (T) and was decomposed into an inertial component (V_{t-1}) and a random velocity (ε). The random velocity was generated as described for the random walk submodel (section 2.5.1). The functions that describe the influence of temperature on the inertial, $f_k(V_{t-1})$, and random, $g_k(\varepsilon)$, components of movement are expressed as:

$$f_k(V_{t-1}) = V_{t-1} \times f_k(T) \quad (8)$$

$$g_k(\varepsilon) = \varepsilon \times g_k(T), \quad (9)$$

where the functions $f_k(T)$ and $g_k(T)$ describe the relationship between metabolic scope and temperature for Atlantic croaker and spot. $f_k(T)$ and $g_k(T)$ in the x and y directions were described with logistic equations because metabolic scope was shown to increase with increasing temperature following a logistic function (Chapter 2):

$$f_k(T) = \frac{L_1}{1 + e^{-s \times (T - T_0)}} \quad (10)$$

$$g_k(T) = 1 - \frac{L_2}{1 + e^{-s \times (T - T_0)}} \quad (11)$$

where L_1, L_2, and s are shape parameters that determine the response of individuals to temperature, T_0 represents the inflection point of the logistic curve, and T represents the temperature experienced by an individual. In these equations, k and T_0 were estimated by fitting a logistic curve to the metabolic scope-temperature relationship for Atlantic croaker and spot determined in Chapter 2, whereas L_1 and L_2 were chosen to control the orthokinetic (speed) response such that if $L_1 < L_2$, individuals slow down as conditions approach optimal (Humston et al., 2004). The klinokinetic (directional) response was affected by the contribution of the inertial component such that individuals would maintain their direction as conditions approach optimal (see Humston et al., 2000 for a detailed explanation).

If an individual encountered DO concentrations lower than it could tolerate ($< C_{crit}$), a hypoxia avoidance response was simulated by setting T to 1°C in equations 10 and 11. This resulted in an increase in the weight of the random component of movement relative to the inertial component, which motivated movement in a random direction.

2.5.3 Restricted-area search submodel

In movement described as a restricted-area search, individuals detect conditions within a certain distance of their position and move to their preferred conditions (Railsback et al., 1999; Giske et al., 2003; Haas et al., 2004; Watkins and Rose, 2013; LaBone et al., 2017). In the RAS submodel, an individual's swimming speed was randomly selected from a Gaussian distribution with a mean of 3.5 or 3.6 and a standard deviation of either 1.75 or 1.8 for Atlantic croaker and spot, respectively. Swimming

speed was converted from bl s^{-1} to km hr^{-1} with equation 7. Swimming speeds were then used to determine the effective search radius for that time step. All cells at the estimated search radius were considered, and the individual moved preferentially to the cell with the highest temperature. This is consistent with the observation that the metabolic scope of Atlantic croaker and spot increases with increasing temperature up to 30°C, which was the maximum temperature tested during the experiments conducted for Chapter 2.

Because some fish exploit hypoxic areas to forage (Pihl et al., 1991; 1992) and to avoid predation (Ludsin et al., 2009; Hedges and Abrahams, 2015), I wanted to ensure that simulated fish would enter areas where dissolved oxygen concentrations were less than their C_{crit}. When the DO concentration in the grid cell with the highest temperature was below C_{crit} for an individual, that individual moved to that grid cell with an arbitrarily assigned probability of 0.3.

I designed a sensitivity analysis *post hoc* whereby 100 individuals of each species moved for 1,000 time steps within a 50 x 50 km area of uniform temperature with a 20 x 20 km area at its center where the DO concentration in each grid cell was 0 mg L^{-1}, well below the lowest C_{crit} that could be assigned to an individual to determine the sensitivity of the RAS submodel to this arbitrarily assigned probability. Probabilities of moving into cells where the DO concentration was below C_{crit} ranged from 0 to 1 by 0.1. The number of time steps that each individual occupied cells below their C_{crit} was recorded and converted to a proportion. The mean and 95% confidence intervals were then calculated for each probability. The probability of fish entering cells where the DO concentration was less than their C_{crit} had a positive exponential effect on the proportion of observations below C_{crit}. The analysis revealed that a probability of 0.3 resulted in a mean of 1.4% and

1.2% of observations in cells with DO concentrations below C_{crit} for Atlantic croaker and spot, respectively. This was substantially lower than the 5.6% and 4.9% of adult Atlantic croaker and spot captured by the trawl survey in hypoxic waters from 1988-2014, respectively.

2.5.4 Boundary behavior

I set boundaries to fish movements at land masses, the Chesapeake Bay-Atlantic Ocean interface, and the northern extent of Virginia waters of Chesapeake Bay (Figure 2). I imposed a boundary at the northern extent of Virginia waters because I did not have monthly information on fish abundance in Maryland waters. I chose the Chesapeake Bay-Atlantic Ocean interface as a boundary because our simulations were focused on times when fish inhabited estuarine waters (May – October). Individuals that would have moved beyond the land or Atlantic Ocean boundaries stopped immediately adjacent to these boundaries. At the next time step, individuals were assigned a new velocity following the rules of each movement submodel. Simulated fish that moved beyond the northern extent of the study area were removed from the simulation because Atlantic croaker and spot are not restricted to Virginia waters, but often move into Maryland waters of the Bay. A larger percentage of fish (mean ± 95% CI) were removed from simulations where individuals followed the random walk submodel (Atlantic croaker: 60.1 ± 0.2%; spot: 40.9 ± 0.2%) when compared to the kinesis (Atlantic croaker: 29.0 ± 0.5%; spot: 3.1 ± 0.2%) and restricted-area search (Atlantic croaker: 0.0 ± 0.0%; spot: 0.0 ± 0.0%) submodels.

2.6 Model performance

Model output included the location of every individual on the first day of each month from June to September of each year, the ambient temperatures and DO concentrations at occupied locations, and the number of time steps that an individual occupied an area where the DO concentration was below that individual's C_{crit}. To determine differences in conditions experienced by simulated individuals as a result of the behavior imposed by different movement submodels and the severity of hypoxic conditions, I investigated the temperature and DO concentration of areas occupied by individuals at the time of sampling, as well as the proportion of observations in which individuals occupied DO concentrations below their C_{crit}s. I further divided the study area into nine regions: B1, B2, and B3 from the lower to the upper mainstem of Chesapeake Bay, and JA1, JA2, YK1, YK2, RA1, and RA2 which indicate the lower and upper regions of the James (JA), York (YK) and Rappahannock (RA) rivers (Figure 3) to facilitate comparison of the distributions of simulated individuals from each of the movement submodels. I then compared the proportion of simulated individuals in each region across movement submodels and severity of hypoxic conditions with observations from the trawl survey. Model output comparisons were conducted in R version 3.3.3 (R core team, 2017) or using the MIXED procedure in SAS version 9.3 (SAS Institute, Cary, NC).

2.6.1 Submodel comparisons

I used generalized linear models to test the hypothesis that mean temperature and DO concentration experienced by simulated individuals varied between movement

submodels and a repeated measures ANOVA to account for correlations among observations from the same individual because multiple observations (monthly from June to September) were taken for each individual. Separate models were used for each species where:

$$DO_{ij} = Submodel_j + \varepsilon_{ij} \quad (12)$$

or

$$Temp_{ij} = Submodel_j + \varepsilon_{ij}. \quad (13)$$

In these models, DO_{ij} and $Temp_{ij}$ represent the mean environmental conditions experienced by individual *i* in submodel *j* and *submodel* refers to random walk, kinesis, or RAS submodels; ε_{ij} is the random, unexplained error associated with the model. Multiple covariance structures, which included compound symmetry, first-order autoregressive, banded Toeplitz, and unstructured, were investigated and the covariance structure that best described the correlations and variances was determined with Akaike's Information Criterion (AIC) (Akaike, 1998; Logan, 2010).

I used a zero-inflated beta regression (Ospina and Ferrari, 2010; 2012) to investigate the effect of movement submodel on the proportion of observations below C_{crit}. This proportion represents the frequency with which individuals occupied areas where dissolved oxygen concentrations were below their C_{crit}. Proportion data are often difficult to analyze because they cannot easily be transformed outside the interval [0,1] and are typically skewed, which violates the general linear model assumptions of normality and homogeneity of variance (Swearingen et al., 2012). The proportion of observations below C_{crit} was zero-inflated; between 61% (Atlantic croaker) and 71% (spot) of individuals did not occupy areas with DO concentrations below C_{crit} throughout

the simulation. A zero-inflated beta regression is a mixture model where the discrete component (zeros) is modeled with a Bernoulli distribution and the continuous proportion data are modeled with the beta distribution (Ospina and Ferrari, 2012). The beta distribution is parameterized in terms of a mean (μ) and precision parameter (ϕ), where $0 < \mu < 1$ and $\phi > 0$ (Ospina and Ferrari, 2010). The mean and precision parameter are modeled in conjunction with the probability of a point mass at zero (α) (Ospina and Ferrari, 2010; 2012) through link functions in relation to linear or non-linear predictors (Ospina and Ferrari, 2010; Ospina and Ferrari 2012 provide a detailed explanation of zero-inflated beta regressions). Beta regressions of the proportion of observations below C_{crit} followed the form:

$$\begin{aligned} logit(\alpha)_{ij} &= Submodel_j + \varepsilon_{ij} \\ logit(\mu)_{ij} &= Submodel_j + \varepsilon'_{ij} \\ log(\phi)_{ij} &= Submodel_j + \varepsilon''_{ij}. \end{aligned} \tag{14}$$

In these models, the responses are the logit of the probability that the proportion of observations below C_{crit} is 0 (α), the logit of the proportion of observations below C_{crit} (μ), and the log of the precision parameter (ϕ) for individual i in submodel j. The term "*submodel*" indicates the effect of the random walk, kinesis, or RAS submodel on the responses and the ε_{ij}s represent the random, unexplained errors. Zero-inflated beta regressions for Atlantic croaker and spot were implemented in the gamlss package in R (Stasinopolous and Rigby, 2007).

2.6.2 Severity of hypoxic conditions

The effect of the severity of hypoxic conditions on the temperature and DO concentration experienced by simulated fish was assessed with generalized linear models with repeated measures. Separate models were developed for each species and included data from 2008 and 2014 as years of mild hypoxia and 2007 and 2011 as years of severe hypoxia. AIC was used to select the appropriate covariance structure (listed above). The final models for Atlantic croaker and spot were:

$$DO_{ijk} = Submodel_j + Severity_k + Submodel_j \times Severity_k + \varepsilon_{ijk} \quad (15)$$

and

$$Temp_{ijk} = Submodel_j + Severity_k + Submodel_j \times Severity_k + \varepsilon_{ijk}. \quad (16)$$

In these models, DO_{ijk} and $Temp_{ijk}$ represent mean environmental conditions experienced by individual *i*, in submodel *j*, and severity of hypoxic conditions *k*. As in equations 12 and 13, *submodel* indicates the effect of the random walk, kinesis, or RAS submodel. Additionally, *severity* represents the effect of the severity of hypoxic conditions (mild or severe). The random, unexplained error associated with the model is represented by ε_{ijk}.

To determine the effect of the severity of hypoxic conditions on the proportion of observations below C_{crit}, I used zero-inflated beta regressions where 2008 and 2014 were considered years of mild hypoxia and 2007 and 2011 were considered years of severe hypoxia. A zero-inflated beta model was used to analyze these data because 70% (Atlantic croaker) and 80% (spot) of individuals did not occupy areas with dissolved oxygen concentrations below C_{crit} in the simulations. Beta models for each species were parameterized as follows:

$$logit(\alpha)_{ijk} = Submodel_j + Severity_k + Submodel_j \times Severity_k + \varepsilon_{ijk} \quad (17)$$

$$logit(\mu)_{ijk} = Submodel_j + Severity_k + Submodel_j \times Severity_k + \varepsilon'_{ijk}$$

$$log(\phi)_{ijk} = Submodel_j + Severity_k + Submodel_j \times Severity_k + \varepsilon''_{ijk}$$

where the logit of the probability that the proportion of observations below C_{crit} is 0 (α), the logit of the proportion of observations below C_{crit} (μ), and the log of the precision parameter (ϕ) for individual *i*, in submodel *j*, and severity of hypoxia *k* are modeled in response to *submodel* (random walk, kinesis, or RAS) and the *severity* of hypoxic conditions (mild or severe). Again, ε_{ijk} represents the random, unexplained error in the model.

2.6.3 Validation of individual-based models

To validate the IBM output, I compared the mean and 95% confidence intervals for temperature and DO concentration at which simulated fish were captured with those calculated from trawl survey stations where Atlantic croaker and spot were captured. These comparisons were performed across all years and for years of mild and severe hypoxic conditions. The mean environmental conditions from IBM outputs and trawl survey observations were considered significantly different if the 95% confidence intervals did not overlap.

To compare the spatial distribution of simulated fish from IBMs to trawl survey observations across all years, the proportion of individuals in each region of Chesapeake Bay (Figure 3) was calculated for each movement submodel by dividing the number of individuals in a given region by the total number of individuals within the model domain

in each month. Similarly, the number of fish captured by the trawl survey in each region was divided by the total number of individuals captured each month. The spatial distribution of simulated fish was also compared to trawl survey observations for years of mild and severe hypoxia. In these comparisons, the proportion of individuals in each region of Chesapeake Bay was calculated for each movement submodel and trawl survey observations by dividing the number of individuals in a given region by the total number of individuals for the entire year. The proportion of individuals in each region were qualitatively compared between IBM output and survey observations to provide an indication of the accuracy with which the IBMs recreated the spatial distributions of Atlantic croaker and spot when only the relationship between environmental conditions and metabolism was used as motivation for movement or when fish moved randomly in the environment.

3. Results

3.1 Movement submodel comparisons

Mean temperatures and DO concentrations occupied by simulated Atlantic croaker and spot, across the entire simulation period, differed significantly among movement submodels used in the IBM (temperature: Atlantic croaker; $F = 178604$, $P < 0.01$, spot; $F = 228262$, $P < 0.01$; DO: Atlantic croaker; $F = 57538.6$, $P < 0.01$, spot; $F = 54710.8$, $P < 0.01$). For both species, fish in the RAS submodel occupied the highest mean dissolved oxygen conditions followed by those in the kinesis submodel and fish in the random walk submodel occupied the lowest mean dissolved oxygen conditions (Figure 4). This pattern was also apparent for mean temperatures occupied by simulated Atlantic croaker whereas spot in the RAS submodel occupied the highest mean temperatures followed by those in the random walk submodel; spot in the kinesis submodel occupied the lowest mean temperatures (Figure 4). These results indicate that, as expected, the RAS submodel more efficiently directed fish to areas of optimal conditions than either the kinesis or random walk submodels.

Fish rarely entered areas where the DO concentration was below their C_{crit} (Figure 5). The mean percentage of observations below C_{crit} was significantly lower for Atlantic croaker in the kinesis and restricted-area search submodels relative to the random walk submodel (kinesis: $t = -147.73$, $P < 0.01$; RAS: $t = -217.30$, $P < 0.01$); similar results were obtained for spot (kinesis: $t = -286.63$, $P < 0.01$; RAS: $t = -144.05$, $P < 0.01$). The results of the zero-inflated beta regression for Atlantic croaker indicate that, on average, only 3.2% of observations were below C_{crit} for the random walk submodel, 1.4% of observations were below C_{crit} for the kinesis submodel, and 0.8% for the restricted-area

search submodel (Figure 5). For spot, the mean percentage of observations below C_{crit} was 2.8% for the random walk submodel, whereas the mean percentage of observations below C_{crit} were 0.5% and 0.9% for the kinesis and restricted-area search submodels, respectively (Figure 5). For Atlantic croaker, these results are as expected; fish in the RAS submodel were effective at avoiding DO concentrations below C_{crit}. Conversely, the results for spot are somewhat unexpected because individuals in the kinesis submodel entered areas with DO concentrations below C_{crit} less frequently than those in the RAS submodel.

3.2 Effects of the severity of hypoxic conditions

The mean DO concentrations occupied by simulated Atlantic croaker and spot were significantly affected by the interaction between movement submodel and the severity of hypoxic conditions (Atlantic croaker: $F = 143.79$, $P < 0.01$; spot: $F = 352.30$, $P < 0.01$). For both species, the random walk submodel resulted in fish occupying mean DO concentrations that did not differ between years of mild and severe hypoxia; whereas fish in the kinesis submodel occupied areas that had lower mean DO concentrations in years of mild hypoxia relative to years of severe hypoxia. Those fish in the RAS submodel occupied areas that had lower mean DO concentrations in years of severe hypoxia when compared with years of mild hypoxia (Figure 6).

The interaction between movement submodel and the severity of hypoxic conditions significantly impacted the mean temperatures occupied by simulated Atlantic croaker and spot (Atlantic croaker: $F = 1070.41$, $P < 0.01$; spot: $F = 1595.21$, $P < 0.01$). The mean temperatures occupied were lower in years of severe hypoxia for both species

in the random walk submodel, and for spot in the kinesis submodel (Figure 6). The mean temperatures occupied by Atlantic croaker did not differ between years of mild and severe hypoxia for those fish in the kinesis submodel. Atlantic croaker and spot in the RAS submodel occupied lower mean temperatures in years of mild hypoxia than in years of severe hypoxia (Figure 6).

The interaction between movement submodel and the severity of hypoxic conditions also resulted in significant differences in the mean percentage of observations below C_{crit} (Figure 7). For all submodels, the mean percentage of observations below C_{crit} was lower for Atlantic croaker and spot in years of mild hypoxia, than in years of severe hypoxia (Figure 7). Additionally, in years of mild hypoxia, the random walk submodel resulted in the highest mean percentage of observations below C_{crit} for both species (Atlantic croaker: 1.8%, spot: 1.5%), followed by kinesis (Atlantic croaker: 0.8%, spot: 0.2%), and RAS (Atlantic croaker: 0.2%, spot: 0.0%). Interestingly, this pattern did not hold in years of severe hypoxia when the RAS submodel resulted in the highest proportion of observations below C_{crit} (Atlantic croaker: 6.4%, spot: 9.2%), followed by random walk (Atlantic croaker: 4.0%, spot: 3.3%), and kinesis (Atlantic croaker: 1.6%, spot: 1.4%).

3.3 Validation of individual-based models

For both Atlantic croaker and spot, simulated fish in all movement submodels occupied areas of higher mean DO concentration than observed in the trawl survey (Figure 4). The mean temperature occupied by Atlantic croaker captured in the trawl survey was also lower than that of simulated fish in all movement submodels. The mean

temperature occupied by spot captured in the trawl survey was, in contrast, higher than the mean temperatures occupied by simulated fish in the random walk and kinesis submodels, and lower than that of fish in the RAS submodel (Figure 4).

Atlantic croaker and spot occupied higher mean DO concentrations relative to survey observations regardless of the severity of hypoxia in nearly all movement submodels (Figure 6; Table 2). The one exception was the random walk submodel for spot, for which the mean DO concentration experienced by simulated individuals in years of severe hypoxia did not differ from trawl survey observations (Figure 6; Table 2). Mean DO concentrations experienced by Atlantic croaker and spot likewise did not differ between years of mild and severe hypoxic conditions from trawl survey observations, (Figure 6; Table 2).

The random walk submodel most accurately reflected the temperatures at which Atlantic croaker were observed in years of both mild and severe hypoxic conditions when compared with trawl survey observations. The output from the kinesis and RAS submodels indicated that simulated individuals occupied warmer waters than Atlantic croaker captured in the trawl survey (Figure 6). Unlike Atlantic croaker, simulated spot in the random walk and kinesis submodels occupied lower temperatures than individuals captured in the trawl survey. The temperature output from the RAS submodel was, in contrast, most similar to trawl survey observations (Figure 6). The mean temperature occupied by fish in the RAS submodel did not differ from that of survey observations under mild hypoxia.

The proportion of individuals that occupied each region of Chesapeake Bay differed markedly between simulated and actual fish for both Atlantic croaker and spot

(Figures 8 and 9). The RAS submodel most notably resulted in larger proportions of individuals in the lower and middle regions of Chesapeake Bay (areas B1 and B2), and smaller proportions of individuals in the York and Rappahannock rivers relative to trawl survey observations. In years of severe hypoxia, all movement submodels resulted in an increase in the proportion of individuals in regions B1 and B2 relative to years of mild hypoxia regardless of species (Figure 10). This is generally consistent with trawl survey observations, although larger increases occurred in the IBMs. Decreases in the proportion of individuals captured in the Rappahannock River by the trawl survey were also apparent in years of severe hypoxia relative to years of mild hypoxia. Similar changes were not apparent for simulated fish (Figure 10).

4. Discussion

My results show that IBMs with ecophysiological constraints failed to effectively reproduce the spatial distributions of Atlantic croaker and spot observed in the VIMS Juvenile Fish Trawl Survey. IBMs for both species consistently resulted in higher proportions of fish in the lower regions of the mainstem of the Chesapeake Bay, and lower proportions of fish in the York and Rappahannock rivers, compared with trawl survey observations. Differences in species distributions between IBMs and trawl survey observations were most apparent for the RAS submodel. This is likely due to the RAS algorithm aggregating individuals at local temperature optima. Fish in this movement submodel were likely to find these optima quickly and to remain near those areas for extended periods. Individuals were, therefore, less likely to occupy areas far from their initial location (the lower Chesapeake Bay). Simulated individuals in the random walk and kinesis submodels were somewhat more likely to move to areas outside the lower Chesapeake Bay, but these submodels still resulted in large discrepancies in the spatial distribution of fish when compared with trawl survey observations. One explanation for discrepancies in the spatial distribution between simulated fish in the random walk and kinesis submodels and those fish captured by the trawl survey is the large percentage of fish lost to Maryland waters in the simulations. The individuals lost in these simulations were those that moved away from their starting location. They may have, therefore, been more likely to enter the subestuaries if they had not been lost. If this had been the case, spot in the kinesis submodel might be expected to better reflect the spatial distribution of fish captured by the trawl survey, because few were lost to Maryland waters during the course of the simulation. The spatial distribution of spot in the kinesis submodel was,

however, similar to that of other simulations and differed markedly from trawl survey observations.

Discrepancies in the spatial distribution of individuals from simulations and survey observations likely affected the differences in DO concentrations experienced by simulated and actual fish. In all submodels, the mean DO concentration in which fish were captured by the trawl survey were lower than those occupied by simulated individuals. Hypoxia is most severe in July and August in the lower Rappahannock River, whereas it is mild and only periodically occurs in the lower York River (Tuckey and Fabrizio, 2016). The larger proportion of individuals captured in the York and Rappahannock rivers by the trawl survey likely decreased the mean dissolved oxygen concentration to which fish were exposed. Simulated fish did not use these areas of the model domain as frequently and therefore the mean DO concentration to which simulated individuals were exposed was greater than for fish captured by the trawl survey.

For simulated fish, the RAS submodel consistently aggregated fish in areas of high temperature and normoxia. Mean temperatures and DO concentrations were highest for simulated fish in the RAS submodels, regardless of species or the severity of hypoxic conditions. This outcome is consistent with the known sensitivity of this type of movement submodel to complex environments (Humston et al., 2004; Watkins and Rose, 2013; 2014; 2017). Individuals in the random walk and kinesis submodels, in contrast, occupied areas of similar mean temperatures and mean DO concentrations although these conditions tended to be slightly higher in the kinesis submodels compared with the random walk. These results are as expected because the RAS submodel uses a gradient response to direct the movements of individuals towards preferred conditions, in contrast

to the reactionary (kinesis) or lack of response (random walk) to environmental conditions used by the other movement submodels (Humston et al., 2004; Watkins and Rose, 2013; 2014; 2017).

Contrary to my expectations, avoidance of specific areas by simulated fish depended on the severity of hypoxic conditions. When the proportion of observations below C_{crit} was analyzed across all years, or when years of mild hypoxia were considered, the random walk submodel performed the worst in predicting hypoxia avoidance, followed by kinesis, and RAS submodels. When years with severe hypoxic conditions were considered, however, the RAS submodel performed the worst in predicting hypoxia avoidance, followed by random walk, and kinesis submodels. It is likely that this resulted from the aggregation of individuals in the RAS submodel at local temperature optima. Simulated individuals in the RAS submodel are directed towards areas of high temperature and can also enter areas where DO concentrations are below their C_{crit}. It is likely, therefore, that simulated individuals became trapped in areas of optimal temperature but low dissolved oxygen concentration. This effectively increased the percentage of time steps that simulated fish were in conditions below their C_{crit} in the RAS submodel. It is also possible that the relatively coarse one-hour time steps used in these simulations resulted in a large percentage of observations spent below C_{crit}, as suggested by Rose et al. (2018b). If this occurred, however, increases in the percentage of observations below C_{crit} in the kinesis submodel would likely occur as well. It is likely that the gradient approach used to move individuals towards optimal temperatures, coupled with a higher prevalence of DO concentrations below C_{crit}, led to this result because the fraction of observations below C_{crit} only increased in the RAS submodel in

years of severe hypoxia. The choice of 0.3 as the probability that individuals in the RAS submodel moved to areas where DO concentrations were below C_{crit} also impacted how frequently fish entered these areas. The sensitivity analysis suggested that the probability of fish entering areas where the DO concentration was less than C_{crit} used here (0.3), was too low for both Atlantic croaker and spot. When used in the simulations, this probability also resulted in a lower incidence of Atlantic croaker and spot entering areas where DO concentrations were less than C_{crit} across all years and years of mild hypoxia. It also resulted in a higher incidence of simulated fish entering areas where DO concentrations were less than C_{crit} in years of severe hypoxia. These results suggest that, either the rate at which individuals encountered DO concentrations below C_{crit} differed between the sensitivity analysis and the simulations, or that factors other than temperature and DO are involved in an individual's decision to enter hypoxic areas. Additional research is necessary to determine a more appropriate mechanism to control the entrance of fish into areas where DO concentrations are below C_{crit} in the RAS submodel in either case.

Taken together, differences in the environmental conditions occupied by simulated and actual fish, and differences in the proportion of individuals occupying different regions in the study area, suggested that the effect of temperature on metabolic scope was not a major motivator of movement for Atlantic croaker and spot during May-Oct in Chesapeake Bay. This is in contrast to the findings of Cucco et al. (2012) for flathead grey mullet (*Mugil cephalus*). A coupled empirical-numerical model was used to reproduce spatial and temporal variation in the metabolic scope of mullet in a Mediterranean shallow-water environment in that study. Model predictions of areas supporting higher metabolic scope were also areas where fisheries catches were highest

for this species (Cucco et al., 2012). In my study, metabolic scope was also used to parameterize the movement submodels. Atlantic croaker and spot have broad temperature optima, however: maximum metabolic scope occurred between 25 and 30°C for Atlantic croaker and between 20 and 30°C for spot (Chapter 2). These broad temperature optima, combined with relatively warm bottom-water temperatures during their period of residency in Chesapeake Bay, likely decrease the importance of the effect of temperature on metabolic scope as motivation for movement. That is, the majority of bottom waters in Chesapeake Bay were optimal and simulated fish moved less than fish observed by the trawl survey. Furthermore, the lack of contrast in temperature between the main stem of Chesapeake Bay and its major subestuaries (Table 3) likely affected the number of simulated individuals that moved into these subestuaries.

The effect of temperature on the metabolic scope of fishes has been well-documented (Claireaux and Lagardère, 1999; Claireaux et al., 2000; Lefrançois and Claireaux, 2003; Claireaux and Lefrançois, 2007; Horodysky et al., 2011; Clark et al., 2013; Lapointe et al., 2014) and evidence suggests that the spatial distributions of fishes and other marine ectotherms are affected by temperature (Murawski, 1993; Perry et al., 2005; Pörtner and Knust, 2007; Nye et al., 2009; Cucco et al., 2012; Deutsch et al., 2015; Kleypas et al., 2015). The results of my simulations indicate, however, that temperature is not the most important factor motivating the movement of Atlantic croaker and spot in Chesapeake Bay during summer. This is likely because of the generally optimal thermal conditions throughout the lower Chesapeake Bay and its subestuaries. Temperature explained only a small amount of variation in the north-south movement of the center of gravity for walleye pollock (*Gadus chalcogrammus*) in the Bering Sea (Thorson et al.,

2017), which suggests that other factors are important in driving changes in species distributions. For example, in addition to temperature, salinity is known to affect the metabolism of fishes through alterations of osmoregulatory pathways (Ern et al., 2014). Indeed, Childs et al. (2008) demonstrated that salinity is an important driver of movement for the spotted grunter (*Pomadasys commersonnii*) and proposed that tidal cycle and the associated changes in environmental conditions likely drive the distribution of this species. They did not, however suggest potential mechanisms for this relationship.

Environmental gradients may be important drivers of the distribution and movement of fish, but predator avoidance and prey availability may also motivate movement. The major predators of Atlantic croaker and spot in Chesapeake Bay include clearnose skate (*Raja eglanteria*), smooth and spiny butterfly rays (*Gymnura micrura* and *G. altavela*, respectively), striped bass (*Morone saxatilis*), bluefish (*Pomatomus saltatrix*), and summer flounder (*Paralichthys dentatus*) (VIMS Multispecies Research Group, 2017). Summer flounder and bluefish are most abundant in the lower main stem of Chesapeake Bay in spring, whereas striped bass are most common in the lower salinity subestuaries and upper main stem of Chesapeake Bay (Latour et al., 2008; Bonzek et al., 2014). Although spatial distribution data in Chesapeake Bay are lacking for the elasmobranch species listed here, the relative abundance of elasmobranchs is highest in high-salinity areas (Buchheister et al., 2013), suggesting that the abundance of these species may be high in the lower regions of Chesapeake Bay. High abundance of predators in the lower regions of Chesapeake Bay in spring suggests that predation pressure for Atlantic croaker and spot may be high when they first enter Chesapeake Bay in the spring. This high predation pressure may result in rapid movement of these species

through the main stem of Chesapeake Bay and into subestuaries where predation pressure may be lower. Indeed, trawl survey observations indicate that in May a mean of 16% of Atlantic croaker are captured in the main stem of Chesapeake Bay, 17% are captured in the James River, 37% in the York River, and 30% in the Rappahannock River. Similarly, a mean of 12% of spot are captured in the main stem of Chesapeake Bay in May, compared with 21% captured in the James River, 35% in the York River, and 32% in the Rappahannock River.

Prey availability may influence the movement of Atlantic croaker and spot in subestuaries (in addition to predator avoidance), specifically in the York and Rappahannock rivers where many of these fish are captured in summer (mean percentage: $\text{Atlantic croaker}_{\text{York, July}} = 32\%$, $\text{spot}_{\text{York, July}} = 26\%$, $\text{Atlantic croaker}_{\text{Rappahannock, July}} = 15\%$, $\text{spot}_{\text{Rappahannock, July}} = 16\%$, $\text{Atlantic croaker}_{\text{York, August}} = 29\%$, $\text{spot}_{\text{York, August}} = 30\%$, $\text{Atlantic croaker}_{\text{Rappahannock, August}} = 14\%$, $\text{spot}_{\text{Rappahannock, August}} = 12\%$). To our knowledge, an assessment of the spatial variation in the abundance of macrobenthic organisms on which Atlantic croaker and spot feed is lacking for Chesapeake Bay. Indices of benthic habitat quality in Virginia indicate, however, that benthic habitats in the York and Rappahannock rivers have more environmental degradation than the Chesapeake Bay and the James River (Diaz et al., 2003). In the York River, sediment instability is the major stressor of benthic habitat (Dellapenna et al., 2001) and maintains macrobenthic communities in early stages of succession (Schaffner et al., 2001). In many temperate areas, polychaetes are often the first benthic organisms to colonize new habitat (Lu and Wu, 2000) and are, therefore, likely common in the York River. Polychaetes are also one of the most common prey organisms found in the diets of

Atlantic croaker (Nye et al., 2011; Tuckey and Fabrizio, 2016) and spot (Pihl et al., 1992) suggesting that these early successional benthic communities may provide an abundance of prey. Unlike the York River, the major stressor of the benthic community in the Rappahannock River is severe, seasonal hypoxia (Llansó, 1992). The benthic community becomes stressed as DO declines (early to mid-summer), which may result in changes in the behavior of benthic organisms, such as decreased burrowing depths and extension of siphons further into the water column. These behaviors are likely to increase the susceptibility of benthic organisms to predation by Atlantic croaker and spot (Diaz et al., 1995; Taylor and Eggleston, 2000; Seitz et al., 2003; Long et al., 2008). There is substantial evidence that Atlantic croaker congregate at the edges of hypoxic zones in the Gulf of Mexico (Craig and Crowder, 2005; Craig, 2012), presumably to forage. Both Atlantic croaker and spot are known to exploit hypoxic areas in Chesapeake Bay to feed on the stressed benthos (Pihl et al., 1991; 1992; Long and Seitz, 2008).

My study provides evidence that the distributions of Atlantic croaker and spot within Chesapeake Bay and its subestuaries are primarily determined by factors other than the effect of temperature on metabolic scope or their respective hypoxia tolerances. Such factors include combinations of biotic and abiotic conditions that vary on spatial and temporal scales smaller than those I used (e.g., hours, and 100s of meters). To better understand how individual fish behave in the wild, and the effect of these behaviors on the spatial distribution of populations, we must understand the effect of environmental conditions on individuals on spatial (meters) and temporal (seconds, minutes) scales relevant to the decision making processes of individual fish. Furthermore, we need to understand how the effects of environmental conditions manifest themselves in the

behavior of those individuals. Such knowledge could be gained by tracking individual fish movements using acoustic telemetry and by simultaneously sampling environmental conditions experienced by telemetered fish in near-real time (Szedlmayer and Able, 1993; Almeida, 1996; Taverny et al., 2002; Kelly et al., 2007; Childs et al., 2008, Fabrizio et al., 2013; 2014). There are several major challenges to this type of work, such as the time and cost of monitoring multiple individuals and the environmental conditions they experience at high spatial and temporal resolutions, while simultaneously assessing predator and prey abundance. It would also be necessary to estimate the vulnerability of prey species to capture and to determine if this is proportional to the catchability of prey species by various survey techniques. The relative abundance of predators could be sampled to determine if the study species avoid areas of high predator abundance. Additionally, for benthivores like Atlantic croaker and spot, examining prey abundance would require sampling the surface of the benthos to determine sediment type and prey density. These sampling efforts may yield important information about prey and predator abundance as potential drivers of individual fish movement. A detailed model of fish behavior in response to abiotic and biotic conditions could be created with these data using linked individual-based and statistical inferential models (Zhang et al., 2017). These movement models could provide a more accurate assessment of which environmental conditions are most important in determining individual movement and therefore the distribution of the population, and could be used to predict the effects of changing environmental conditions on the distribution of ecologically and economically important species.

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Tables

Table 1. Parameters used in the movement submodels for Atlantic croaker and spot.

Parameter	Description	Atlantic croaker	spot	Units
C_{crit}	initial critical oxygen concentration of an individual	N(1.36, 0.46)	N(1.86, 0.16)	mg O_2 L^{-1}
$C_{crit, t}$	critical oxygen concentration of an individual at time *t*	*f*(C_{crit}, $Temp_t$)	*f*(C_{crit}, $Temp_t$)	mg O_2 L^{-1}
$V_{x, t}$	individual velocity in the x direction at time *t*	variable by simulation	variable by simulation	bl s^{-1}
$V_{y, t}$	individual velocity in the y direction at time *t*	variable by simulation	variable by simulation	bl s^{-1}
V_t	total velocity of an individual at time *t*	variable	variable	bl s^{-1}
V_{t-1}	total velocity of an individual at time *t*-1	variable	variable	bl s^{-1}
V'_t	transformed total velocity at time *t*	variable	variable	km hr^{-1}
θ_t	direction of travel for an individual at time *t*	variable	variable	degrees
ε	random variate providing the stochastic component of velocity in the kinesis submodel	N(μ, ψ)	N(μ, ψ)	bl s^{-1}
μ	mean of ε	variable by simulation	variable by simulation	bl s^{-1}
ψ	variance of ε	variable by simulation	variable by simulation	bl s^{-1}
T	ambient temperature during time step	variable	variable	°C
T_0	temperature at inflection point of the logistic curves	14.9	10.3	°C
L1	maximum value in logistic curve *f*(V_{t-1})	0.7	0.7	dimensionless
L2	maximum value in logistic curve *g*(ε)	0.9	0.9	dimensionless
k	steepness parameter of the logistic curves	0.1	0.2	dimensionless

Table 2. Mean dissolved oxygen concentration occupied by Atlantic croaker and spot in the random walk (RW), kinesis, and restricted-area search (RAS) submodels; as well as fish captured in the trawl survey in years of mild and severe hypoxia. LCL and UCL indicate the lower and upper confidence limits for the 95% confidence interval, respectively.

Species	Movement Submodel	Severity	Mean DO	LCL	UCL
Atlantic croaker	RW	Mild	5.87	5.86	5.88
		Severe	5.85	5.84	5.86
	Kinesis	Mild	5.90	5.89	5.91
		Severe	5.92	5.91	5.93
	RAS	Mild	6.46	6.45	6.47
		Severe	6.34	6.33	6.34
	Survey	Mild	5.16	5.08	5.23
		Severe	5.23	5.12	5.34
spot	RW	Mild	5.90	5.89	5.91
		Severe	5.91	5.90	5.92
	Kinesis	Mild	6.03	6.02	6.04
		Severe	6.11	6.10	6.11
	RAS	Mild	6.41	6.40	6.42
		Severe	6.26	6.25	6.27
	Survey	Mild	5.60	5.48	5.72
		Severe	5.80	5.69	5.91

Table 3. Mean bottom temperatures in the mainstem of Chesapeake Bay (Bay) and the James, York, and Rappahannock rivers for June to September.

Month	System	Mean Temperature (°C)
June	Bay	20.3
	James	23.7
	York	22.8
	Rappahannock	22.8
July	Bay	24.3
	James	26.8
	York	26.5
	Rappahannock	26.6
August	Bay	25.1
	James	27.0
	York	27.3
	Rappahannock	27.4
September	Bay	24.1
	James	24.1
	York	25.7
	Rappahannock	24.8

Figures

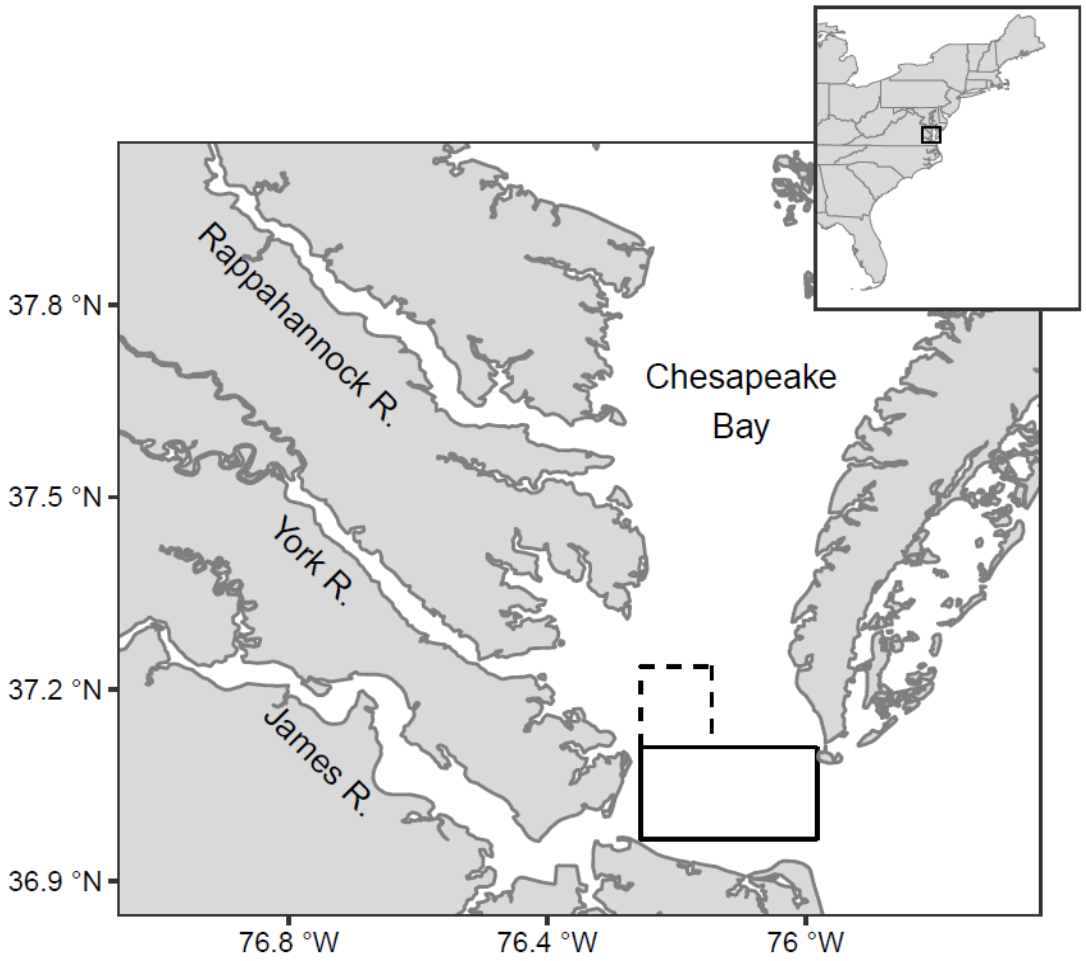

Figure 1. The study area for the individual-based models. The outlined areas were 25 km x 16 km (solid line) and 10 km x 15 km (dashed line) and represent starting location for all individuals in the simulations. Inset shows the location of the study area.

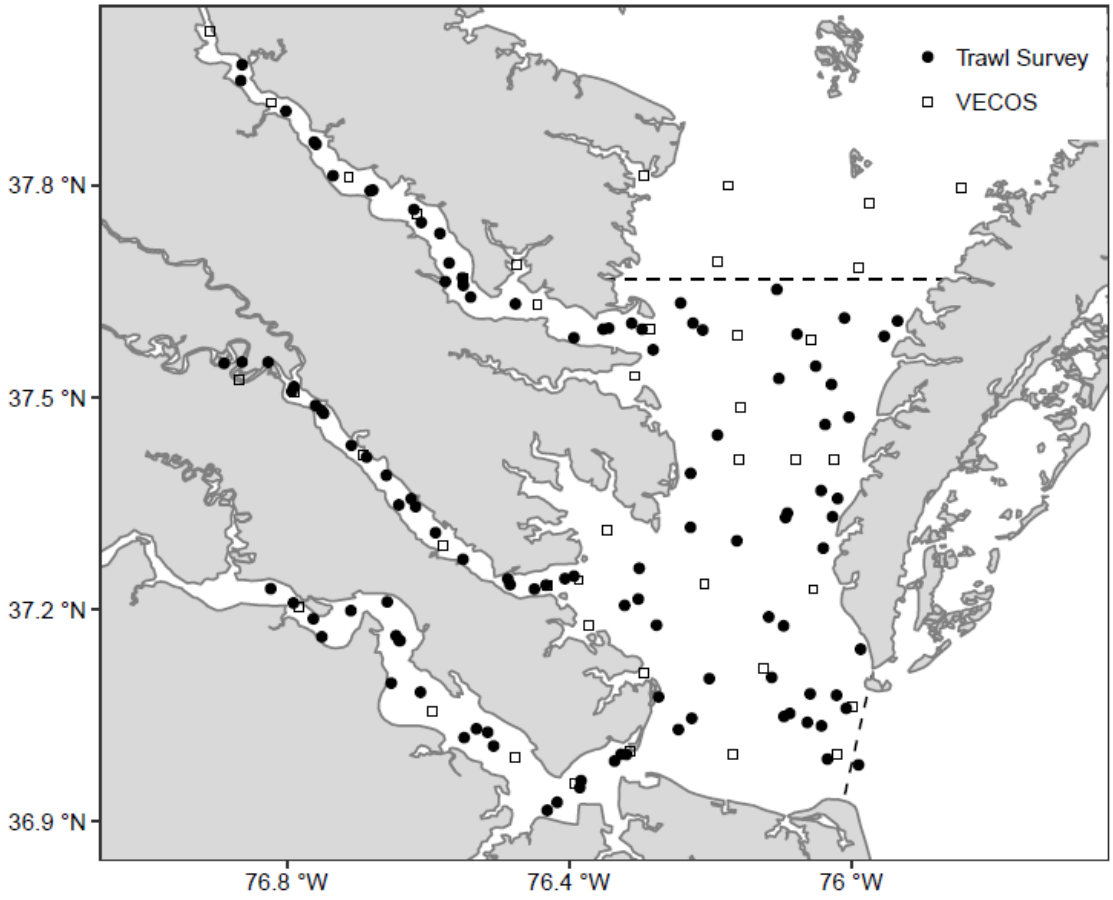

Figure 2. The locations of stations for one month of sampling (July 2011) by the VIMS Juvenile Fish Trawl survey (filled circles) which uses a random stratified design; and the Virginia Estuarine and Coastal Observing System (VECOS; open squares) which uses a fixed location sampling design. Dashed lines represent boundaries to simulated fish movement at the Chesapeake Bay-Atlantic Ocean interface and the northern extent of Virginia waters of Chesapeake Bay.

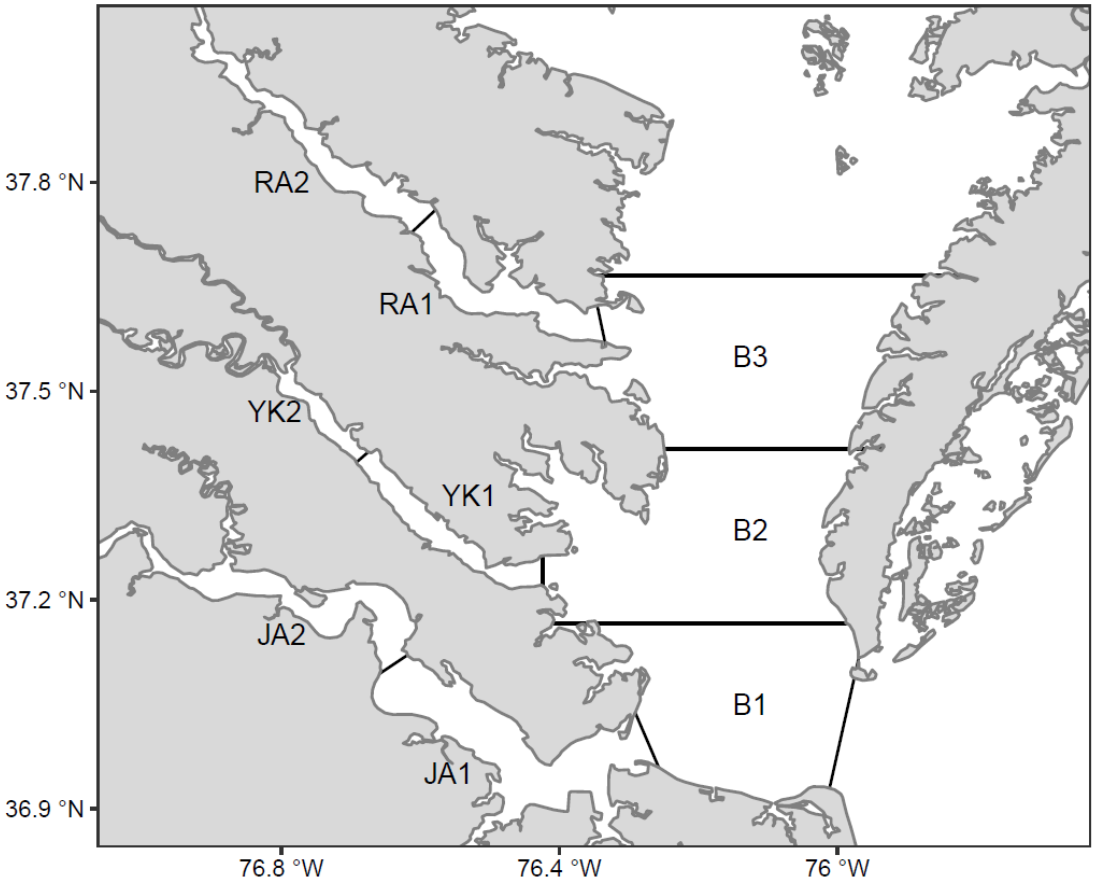

Figure 3. The regions used to compare output from the simulations to observations from the VIMS Juvenile Fish Trawl Survey. The mainstem of Chesapeake Bay is separated into three regions (B1, B2, and B3) while the James, York, and Rappahannock rivers have each been separated into two regions (JA1, JA2, YK1, YK2, RA1, and RA2, respectively).

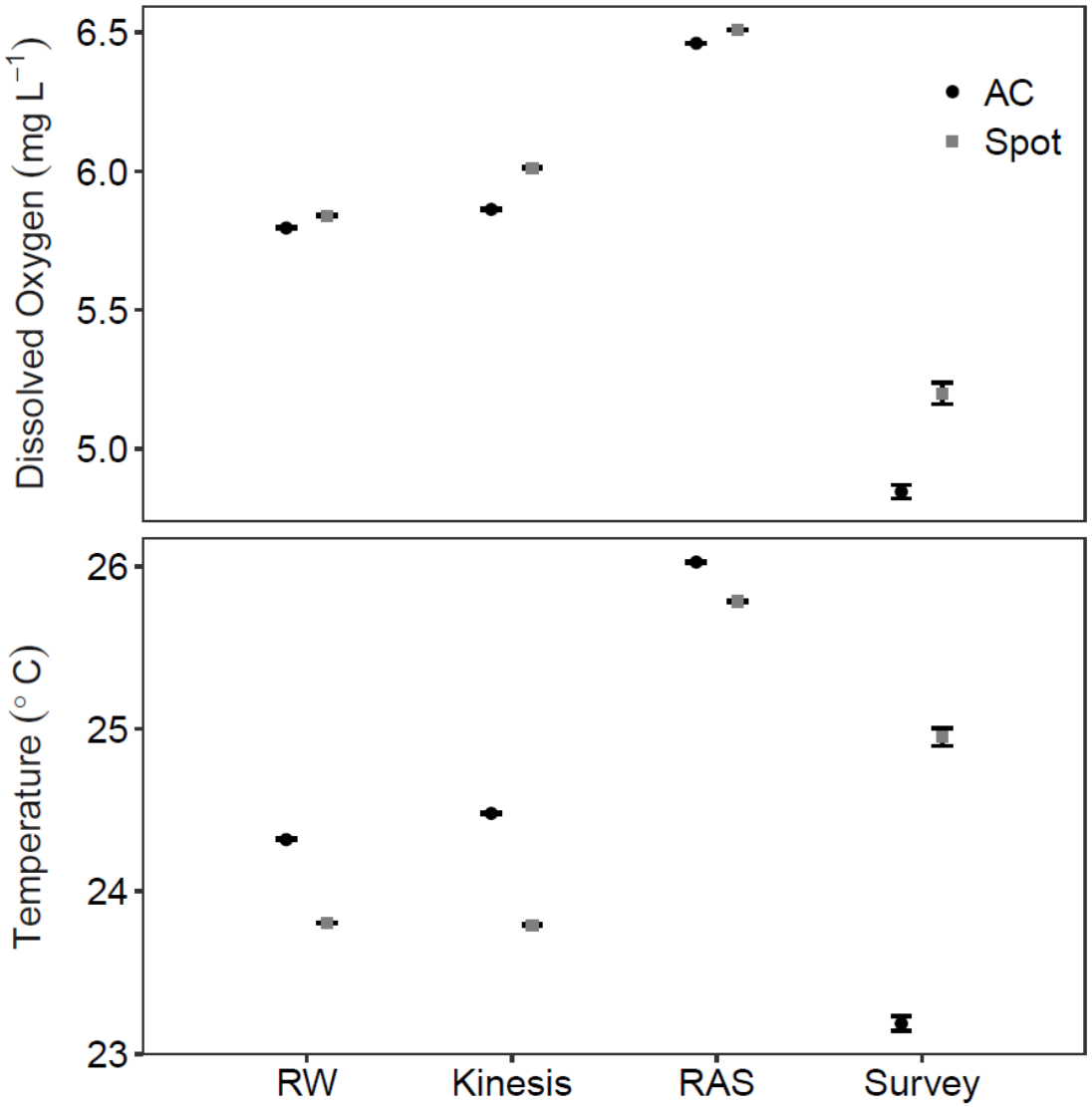

Figure 4. The model-estimated mean oxygen concentrations (top panel) and temperatures (bottom panel) occupied by simulated Atlantic croaker (black circles) and spot (gray squares) in the random walk (RW), kinesis, and restricted-area search (RAS) submodels; as well as the mean oxygen concentration at which Atlantic croaker and spot were captured in the VIMS Juvenile Fish Trawl Survey. Error bars represent the 95% confidence intervals.

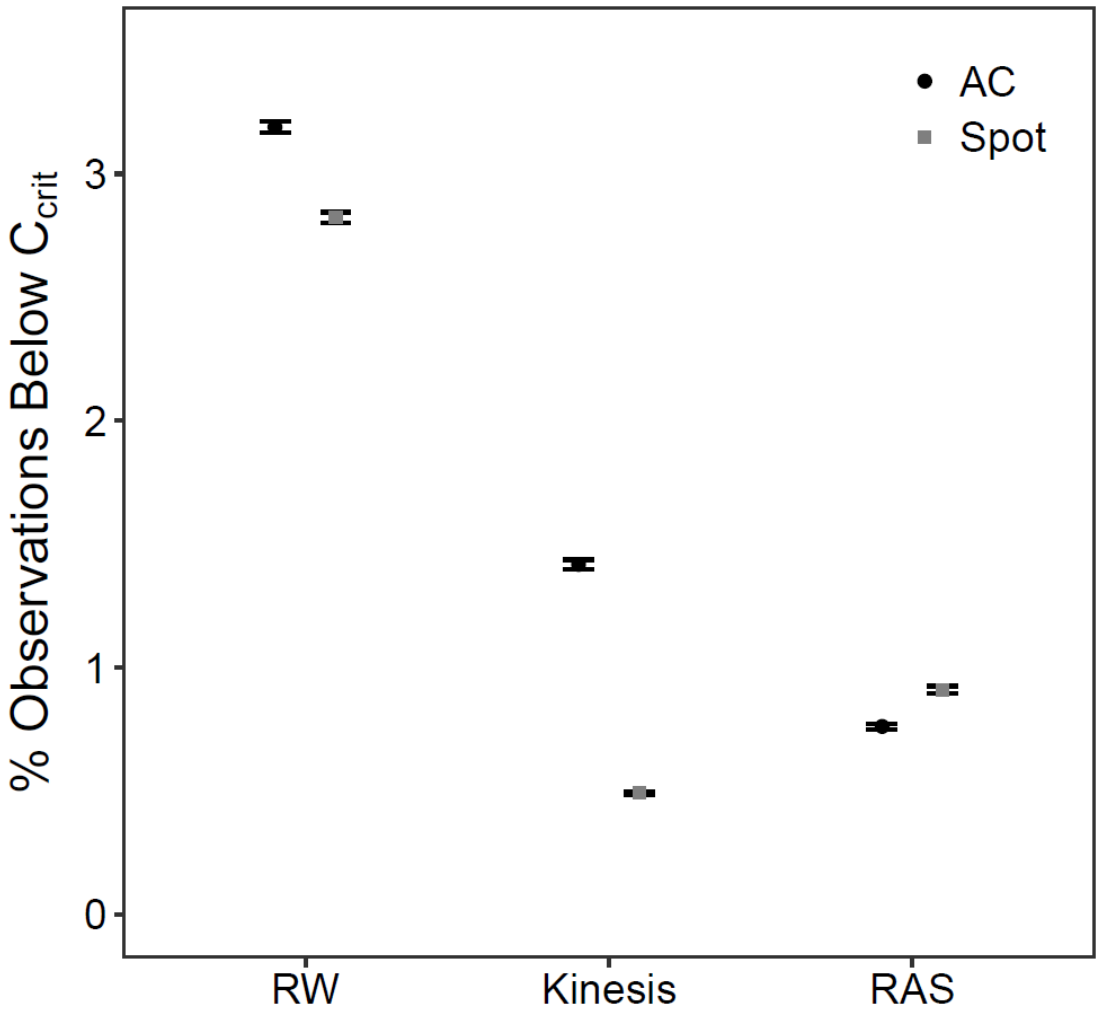

Figure 5. The model-estimated mean percentage of observations below critical oxygen concentration (C_{crit}) for simulated Atlantic croaker (black circles) and spot (gray squares) in the random walk (RW), kinesis, and restricted-area search (RAS) submodels. Error bars represent the 95% confidence intervals.

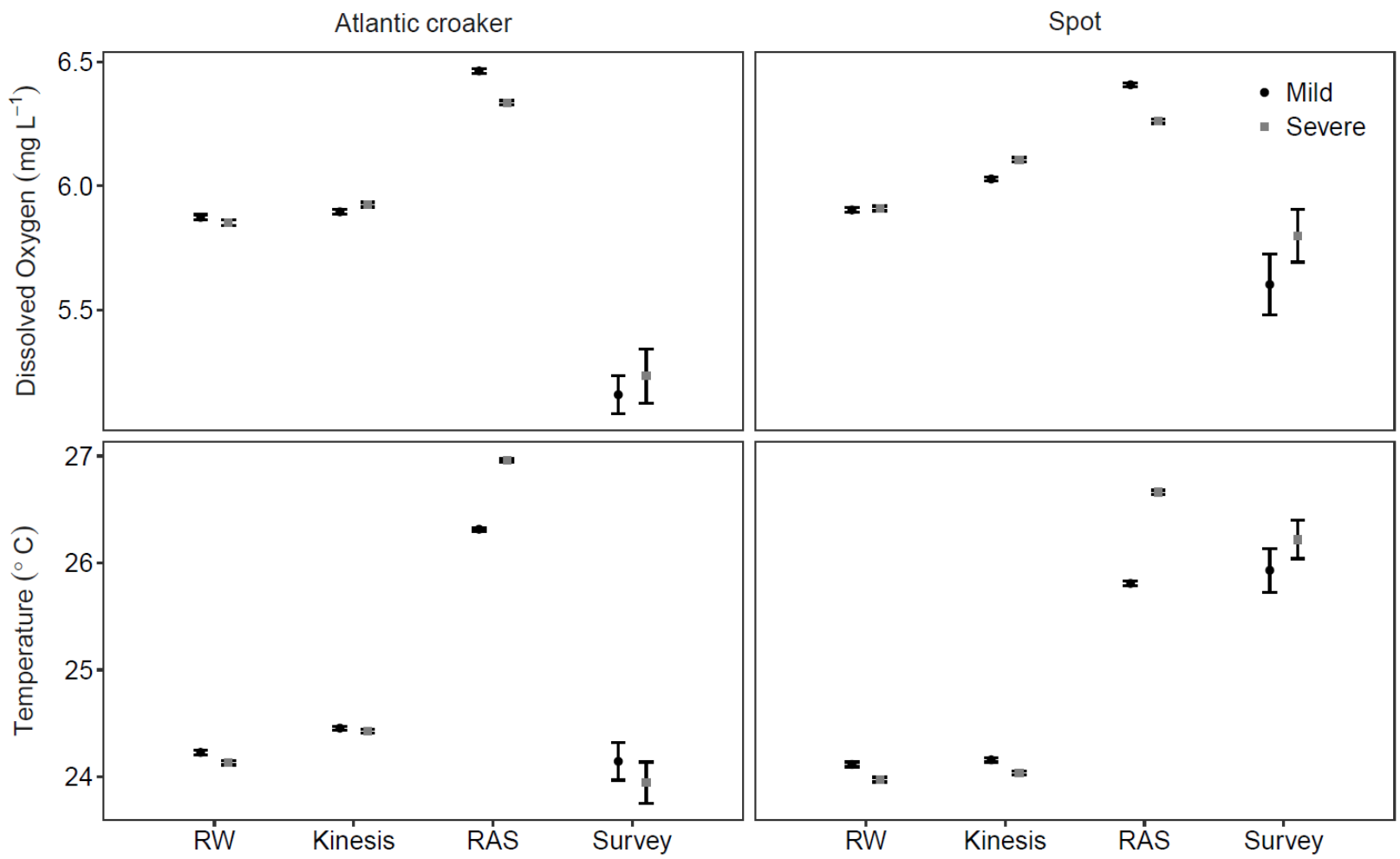

Figure 6. The mean model-estimated (±95% confidence intervals) oxygen concentrations (top panels) and temperatures (bottom panels) occupied by simulated Atlantic croaker (left panels) and spot (right panels) during years when hypoxia was defined as mild (black circles) or severe (gray squares) hypoxia. Mean oxygen concentrations are displayed for the random walk (RW), kinesis, and restricted-area search (RAS) submodels as well as the VIMS Juvenile Fish Trawl Survey.

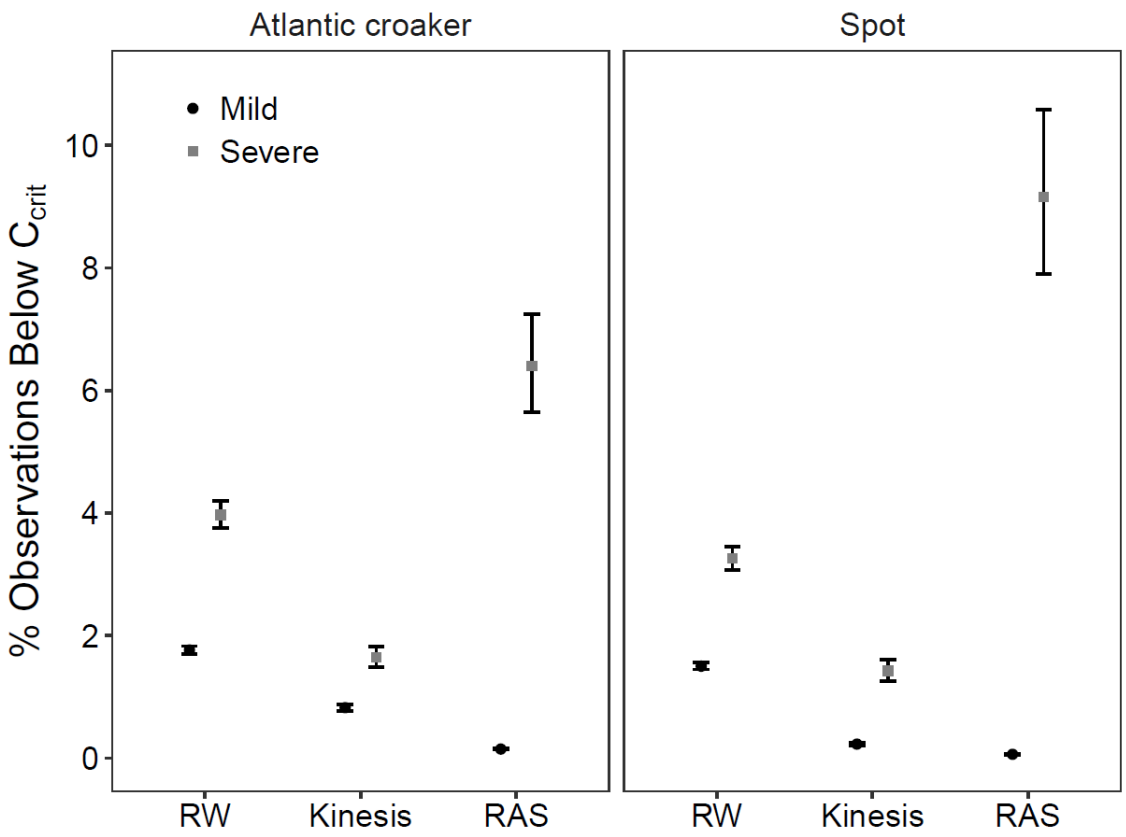

Figure 7. The mean model-estimated (±95% confidence intervals) percent of observations below critical oxygen concentration (C_{crit}) for simulated Atlantic croaker (left panel) and spot (right panel) during years of mild (black circles) and severe (gray squares) hypoxia in the random walk (RW), kinesis, and restricted-area search (RAS) submodels. Error bars represent the 95% confidence intervals.

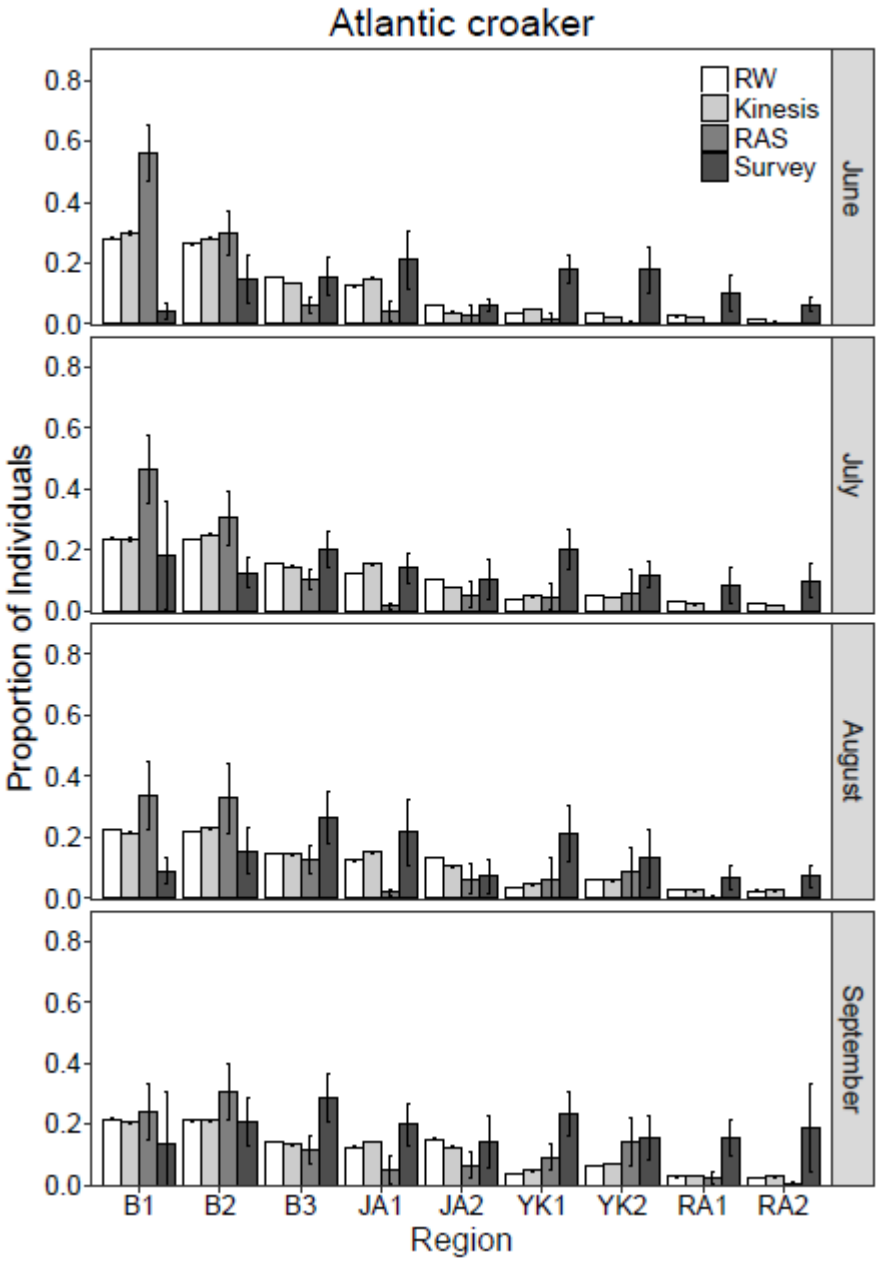

Figure 8. The mean proportion (±95% confidence intervals) of Atlantic croaker found in each region of the study area for each month of the simulation. The proportion of individuals from the random walk submodel (RW, white), the kinesis submodel (light gray), the restricted-area search submodel (RAS, medium gray), and the VIMS Juvenile Fish Trawl Survey (dark gray).

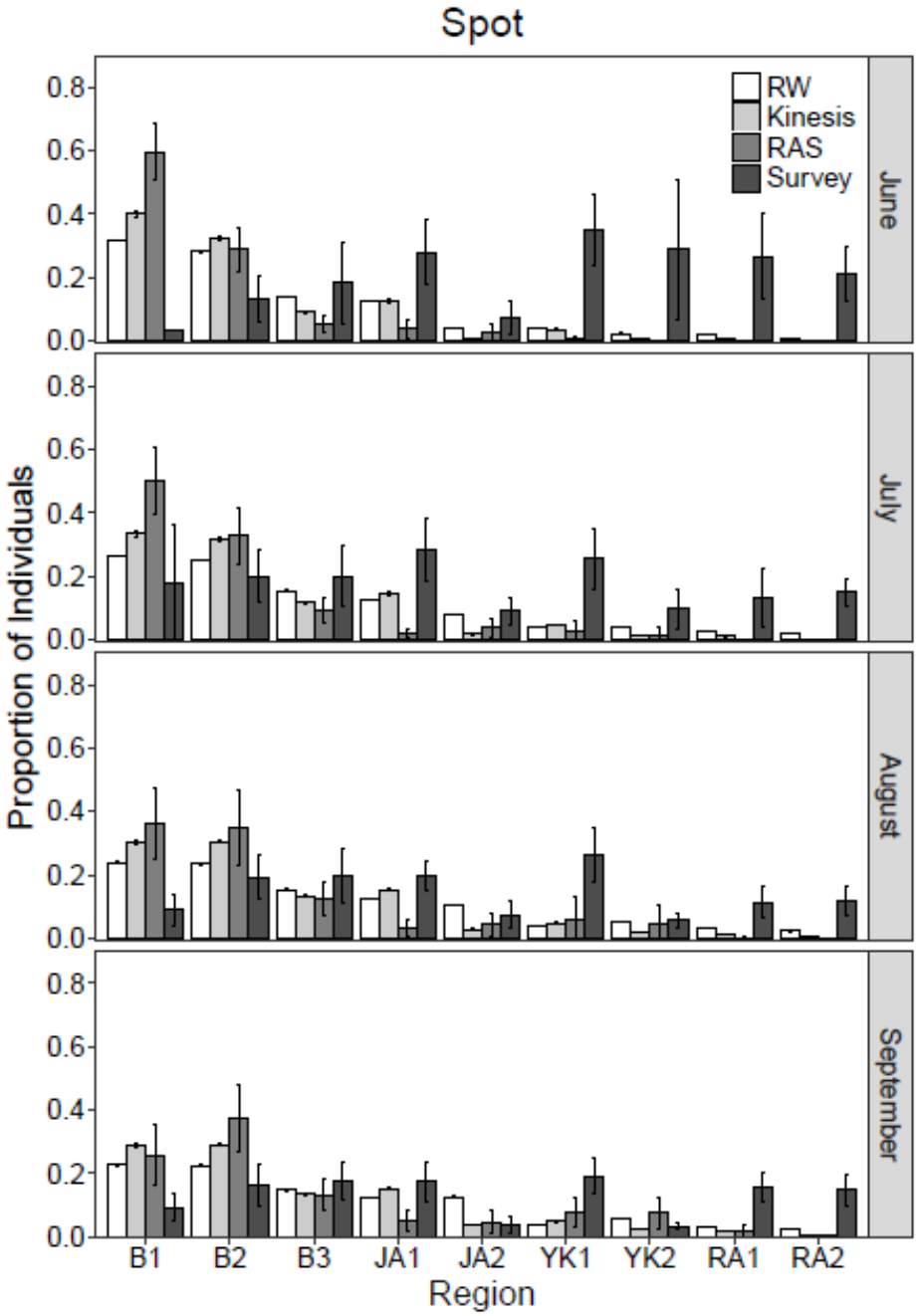

Figure 9. The mean proportion (±95% confidence intervals) of spot found in each region of the study area for each month of the simulation using the random walk submodel (RW, white), the kinesis submodel (light gray), the restricted-area search submodel (RAS medium gray), and the VIMS Juvenile Fish Trawl Survey (dark gray).

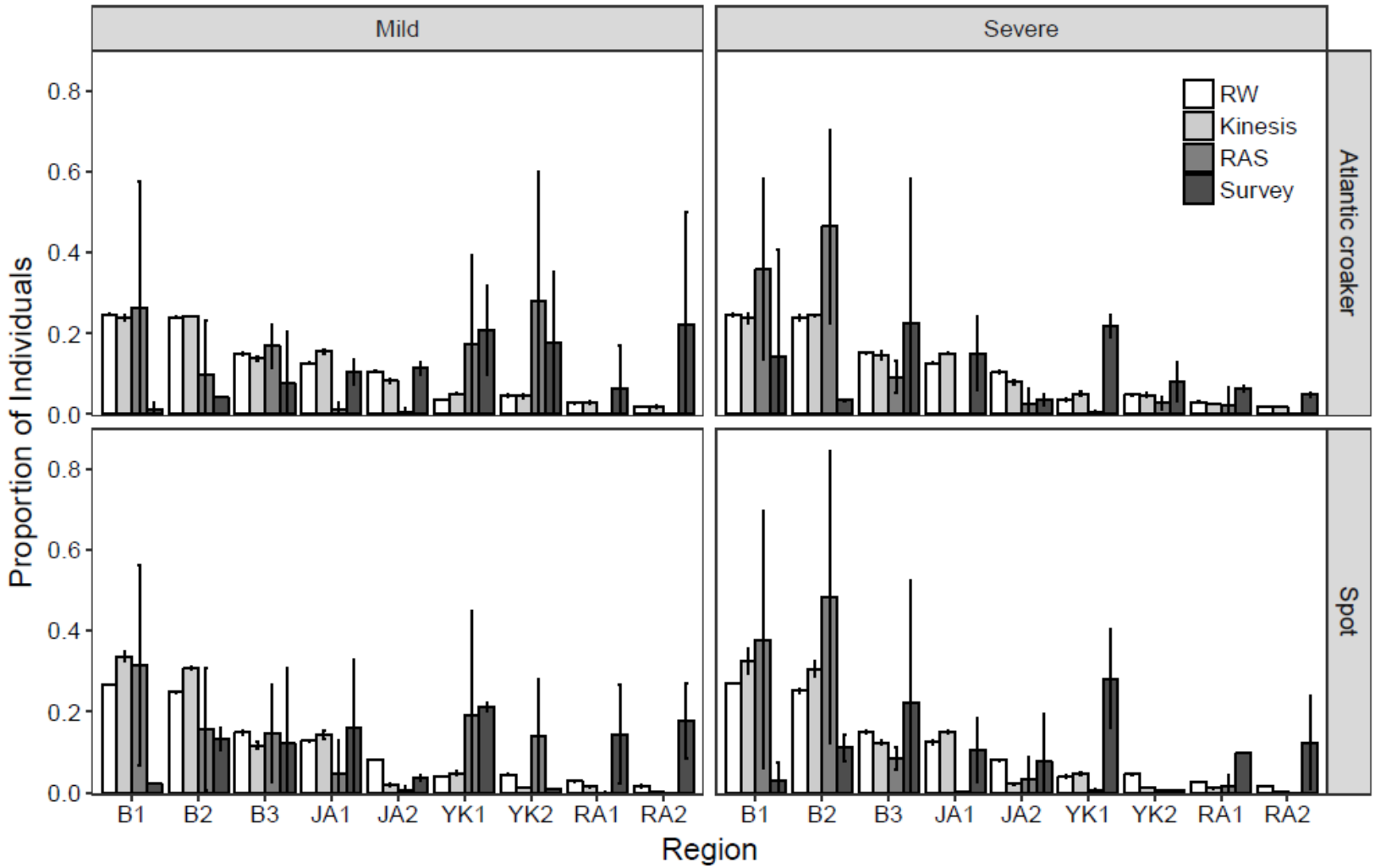

Figure 10. The mean proportion (±95% confidence intervals) of Atlantic croaker (top panels) and spot (bottom panels) found in each region of the study area in years when hypoxia was defined as mild (left panels) or severe (right panels) for the random walk submodel (RW, white), the kinesis submodel (light gray), the restricted-area search submodel (RAS, medium gray), and the VIMS Juvenile Fish Trawl Survey (dark gray).

CHAPTER 5

Summary and Concluding Remarks

Because hypoxia is one of the most widespread, deleterious processes occurring in aquatic environments (Diaz and Rosenberg 2008) and temperature has pervasive effects on the physiology of ectothermic organisms (Schulte 2015; Whitney et al. 2016), directional changes to these environmental conditions are likely to result in negative impacts to fish at the individual and population levels. To determine the effects of temperature and hypoxia on individual Atlantic croaker and spot in Chesapeake Bay, I examined the relationships between temperature and metabolic scope and hypoxia tolerance (Chapter 2) as well as the relationship between hypoxia exposure and reproductive potential (Chapter 3). Furthermore, to examine population effects, I developed an individual-based model of fish distribution incorporating the effects of temperature on metabolic scope and hypoxia tolerance into individual movement (Chapter 4).

The respirometry trials conducted for my research provided the first estimates of metabolic scope and hypoxia tolerance for Chesapeake Bay fishes across a broad range of temperatures common to this estuary. The results of the respirometry trials were used to parameterize movement submodels within an individual-based, dynamic-seascape model of fish distribution where the effect of temperature on metabolic scope was used to inform the movement of individuals in the model and dissolved oxygen concentrations below the critical oxygen concentration elicited an avoidance response. This is the first use of an individual-based model with laboratory-derived physiological constraints to assess fish distribution. This project is also unique in that long-term observations of the distribution of Atlantic croaker and spot as well as the environmental conditions in which

they were captured were available from the VIMS Juvenile Fish Trawl Survey to validate simulation results.

The distributions of Atlantic croaker and spot in Chesapeake Bay differed substantially between movement submodels and survey observations. A greater proportion of simulated fish occupied the lower regions of Chesapeake Bay relative to survey observations. This result is interesting because environmental conditions are dynamic both spatially and temporally in temperate estuaries and can have a substantial impact on the physiology and behavior of an individual. Because population responses are made up of the responses of individuals to their environment, the ability of models to accurately reflect the distribution of fish is important when considering the effects of environmental conditions on the population dynamics of a species. In many individual-based models of fish population dynamics, bioenergetics models have been used to inform the movement of fish (e.g., Humston et al. 2004; Rose et al. 2013; 2018; Politikos et al. 2015a; 2015b); however, the accuracy with which these models reflect the actual distribution of the population in question is rarely verified (but see Politikos et al. 2015a; 2015b). The use of spatially-explicit population dynamics models that have not been verified with survey data may lead to biased population assessments which may result in ineffective or counterproductive management measures (Cooke et al. 2016). Therefore, validation of model-predicted fish distributions should be performed whenever possible to ensure that environmental conditions experienced by simulated individuals accurately reflect those experienced by fish in the population of interest. Without validation of fish distributions, results of population dynamics models should be interpreted cautiously.

Differences in the spatial distribution of simulated and actual fish also suggest that during summer in Chesapeake Bay, metabolic scope and hypoxia tolerance are likely not the major drivers of Atlantic croaker and spot distribution. In the movement submodels used here, temperature is the only environmental factor with an explicit effect on metabolic scope, although critical oxygen concentration is used to elicit an avoidance response to low dissolved oxygen conditions. Because exposure to hypoxic conditions (Capossela et al. 2012; Lapointe et al. 2014) and changes in salinity (Ern et al. 2014) are known to affect the metabolic scope of fish, incorporation of the effects of these environmental factors, in combination with the effects of temperature, on metabolic scope into the movement submodels may result in more accurate distributions of simulated individuals. To determine the combined effects of varying temperatures, dissolved oxygen conditions, and salinities on the metabolic scope of individuals, additional respirometry trials must be conducted with multiple levels of temperature, dissolved oxygen concentration, and salinity in a factorial design.

If the spatial distributions of fish in the individual-based models were improved through the incorporation of the effects of salinity and dissolved oxygen conditions on metabolic scope, the individual-based model could be expanded to incorporate the population dynamics of Atlantic croaker and spot in Chesapeake Bay, similar to models of Atlantic croaker from the Gulf of Mexico (Rose et al. 2018). For instance, based on the results of my research, the effects of hypoxia exposure on the reproductive potential of Atlantic croaker could be incorporated into an individual-based model to investigate the

potential effects of annual variations in environmental conditions on the reproduction of Atlantic croaker in the Virginia subestuaries of Chesapeake Bay.

Oxidative stress associated with exposure to hypoxic conditions (DO concentrations < 3.5 mg L-1) did not result in increased relative expression of hypoxia-inducible factors or reductions in reproductive potential in the Virginia subestuaries of Chesapeake Bay. This contrasts with the results of previous studies of Atlantic croaker in the Gulf of Mexico (Thomas et al. 2007; Thomas and Rahman 2012). However, these differences likely stem from the nature of the hypoxic zones in these areas. The Gulf of Mexico hypoxic zone covers a vast portion of the Louisiana continental shelf (exceeding 22,000 km^2 in some years, Rabalais et al. 2002; Turner et al. 2008) whereas hypoxia in the Virginia subestuaries of Chesapeake Bay is largely limited to the deep channels adjacent to shallower, well-oxygenated waters (Officer et al. 1984; Hagy et al. 2004), allowing fish to move between hypoxic and normoxic conditions. To incorporate the effects of hypoxia exposure on the reproduction of Atlantic croaker in Chesapeake Bay into an individual-based model similar to that developed by Rose et al. (2018) we need to better understand the relationship between hypoxia exposure and reproduction in this species throughout Chesapeake Bay. Laboratory experiments to investigate the effects of intermittent hypoxia exposure on Atlantic croaker reproduction in a controlled setting and additional sampling efforts throughout the lower Chesapeake Bay from May to October would improve our understanding of the effects of hypoxia exposure on the reproduction of Atlantic croaker in this region and could be used to predict the impact of changing environmental conditions on this population under different climate-change scenarios.

Because I was unable to obtain sufficient samples to investigate the effects of hypoxia exposure on the reproductive potential of spot, similar efforts to those described for Atlantic croaker should be undertaken to determine if there are species-specific effects that may alter the composition of the fish community in Chesapeake Bay as hypoxia becomes more prevalent.

Additionally, compared to the Virginia waters of Chesapeake Bay, hypoxia is more widespread and severe during summer in the Maryland waters of this estuary which are also inhabited by Atlantic croaker and spot at this time. To better understand the population-wide effects of hypoxia on the reproduction of these species and to inform the management of these economically and ecologically important species under future climate scenarios, additional sampling in this region is necessary.

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