MANGANESE, MAGNESIUM, AND *MICROPOGONIAS UNDULATUS*: IDENTIFYING GROWTH AND HYPOXIA EXPOSURE HISTORIES OF FISH IN THE NORTHERN GULF OF MEXICO USING OTOLITH MICROCHEMISTRY

A Thesis

by

APRIA N. VALENZA

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This thesis meets the standards for scope and quality of
Texas A&M University-Corpus Christi and is hereby approved.

Benjamin Walther, PhD
Chair

Simon Geist, PhD
Committee Member

Karin Limburg, PhD
Committee Member

Cherie McCollough, PhD
Chair, Department of Life Sciences

May 2021

ABSTRACT

Hypoxia is a worldwide natural phenomenon that is becoming increasingly more severe as anthropogenic high-nitrogen nutrient loads enter marine systems. In the northern Gulf of Mexico (nGoMex), seasonal hypoxia occurs during the summer months due to stratification and lack of mixing events. These events have a range of ecophysiological effects on demersal and pelagic organisms, from forcing individuals out of their natural habitat to altering species niche widths and stifling growth rates. Atlantic Croaker (*Micropogonias undulatus)* is a demersal Sciaenid species abundantly found in the nGoMex. While hypoxia exposure is known to have many detrimental and sub-lethal effects on croaker for early life history stages, quantifying growth effects for the entire lifespan of croaker is critical for the understanding of how hypoxia affects metabolism and growth. Otolith chemical signatures can reflect the composition of the ambient water a fish resides in as well as the internal physiological status and growth rate. These signatures provide detail about hypoxia exposure histories and how abiotic stress affects the biology of the fish. To quantify hypoxia exposure, metabolic activity, and hypoxia corrected for growth, I used the microchemical otolith markers Mn:Ca, Mg:Ca, and Mn:Mg, to assess lifetime hypoxia exposure histories and growth responses. Age-0 croaker were revealed to have the highest Mg:Ca duration fraction, as well as the highest Mn:Ca duration fraction of all ages analyzed, indicating that a large proportion of juvenile croaker are exposed to hypoxia within the first year of life, but their growth is not directly affected by this exposure. Age-1 and Age-2 croaker that experienced high levels of hypoxia were found to have high mean Mg:Ca values. This result may indicate that hypoxia-exposed fish gain a growth advantage through enhanced foraging for benthic prey. Alternatively, differential mortality may have selectively removed

slow growing individuals from the high hypoxia exposure groups and only faster growing individuals survived this stressor. Neither Mn:Mg nor Mn:Ca showed consistent trends with Mg:Ca, possibly due to the repetitive use of Mg in the chemical metrics, or due to the fact that Mg:Ca is not the best proxy to identify growth dynamics in Atlantic Croaker. A further understanding of how hypoxia affects the growth and trophic interactions of demersal fishes is critical for managing and protecting vulnerable fishes.

DEDICATION

To my parents, Michelle and Jack, for their infinite love and support of me following my dreams, almost two thousand miles and a time zone away from home.

To my sister, Brielle, for never failing to make me laugh, and for always being incredibly supportive and interested in my work.

To my friends, you inspire and motivate me to be the best I can be.

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Additional thanks go out to the professional staff who ran Walther Lab samples including Dr. Nathan Miller at UT – Austin, and Dr. James Hogan for the use of his drying oven. thank you to A. Debose, S. Mohan and the crew of the NOAA ship RV 'Oregon II" for their assistance collecting croaker in the Gulf of Mexico. Thank you to funding from the National Science Foundation (NSF-OCE-1633020) and support from the TAMU-CC Fisheries and Mariculture Program.

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INTRODUCTION

Hypoxia, defined as dissolved oxygen concentrations (DO) below 2 mg L^{-1}, is a natural phenomenon that occurs worldwide. Hypoxic waters can be found in oxygen minimum zones as well as coastal habitats that receive significant amounts of riverine input. Rising ocean temperatures decreasing oxygen solubility, increasing rates of oxygen consumption and increasing stratification are also having an influence on oxygen minimum zones (Breitburg et al. 2018). In coastal zones, elevated nutrient concentrations of nitrogen and phosphorus due to terrestrial runoff including agricultural fertilizer, sewage, and the combustion of fossil fuels results in large phytoplankton blooms in coastal surface waters (Breitburg et al. 2018; Turner and Rabalais 1994). Once these blooms subside, the biological material produced is processed by benthic bacterial respiration, thereby consuming large amounts of dissolved oxygen. Bottom water hypoxia in coastal and shelf systems can be enhanced by thermohaline stratification of the water column during the summer. The top layer restricts any oxygen replenishment of the bottom layers, limiting the amount of oxygen present for consumption (Dagg et al. 2007; Rabalais et al. 2010; Zhang et al. 2020). Therefore, prolonged stratification driven by warming surface temperatures results in larger and stronger hypoxic zones, affecting all life that inhabits the benthic zone.

The Northern Gulf of Mexico (nGoMex) experiences natural hypoxic episodes exacerbated heavily by anthropogenic input, caused by nutrient input from the Mississippi River and the Atchafalaya watershed (Rabalais et al. 2001). While these hypoxic events occur every year, the anthropogenic influences on this system have worsened since systematic monitoring of these events began in the mid-1980s. The resulting hypoxic zone grows in spatial extent to create a so-called “dead zone”, where dissolved oxygen concentrations drop to lethal concentrations for

most living organisms (Breitburg et al. 2018). Within the last decade, the nGoMex experienced greater magnitude and duration of seasonal hypoxia, with summer 2019 having a hypoxic zone of 18,000 square kilometers (6,952 square miles) – a size approaching the land area of New Jersey, and the eighth largest zone ever recorded (LUMCON Gulf Hypoxia Program 2021). Therefore, as these events continue to grow in magnitude and severity, it is important to understand how organisms within the ecosystem are exposed to hypoxia and how they cope and respond.

Atlantic Croaker as an Ideal Test Species

The Atlantic Croaker (*Micropogonias undulatus)* is a demersal sciaenid species that is highly abundant in the nGoMex. They are characterized by their inferior mouths, sensory barbels and coarse-straining gill rakers (Overstreet and Heard 1978). Croaker undergo an ontogenetic diet shift around age 1, where they switch from eating primarily detritus, nematodes, insect larvae and amphipods to mysids and small fish species. However, due to their benthic nature, they still rely heavily on polychaetes throughout their life (Nye et al. 2011).

During the fall, Atlantic Croaker spawn in marine waters off the coast, in depths of at least 54m (177ft). Croaker are estuarine-dependent, as after the eggs hatch, larvae move into estuaries to grow and mature, and transition into juveniles. Depending on location and year, juveniles can be found in estuaries as early as September to as late as March. They remain in the estuaries through June – August, as growth is rapid in these areas, growing as much as 35mm (measured in total length, or TL) per month. When they reach a size of about 100mm, croaker emigrate to open coastal waters (Ditty et al. 1988; Hernandez et al. 2010; Petrik et al. 1999).

After one year of life, croaker have been reported to range in size between 100 – 250 mm TL, and from 200 to 310 mm TL after year two. Croaker mature early by the end of their first year of life, and many do not live past the age of two (Ditty et al. 1988). Therefore, the time they spend on the continental shelf is restricted to the fall when they are spawned, hatched, and migrate to the estuary, and in the summer months when they are mature and journey back into coastal waters to spawn. In this case, they would be interacting with hypoxia during the summer, when thermal stratification is at its highest.

Croaker are known to have high tolerances to low levels of dissolved oxygen, withstanding concentrations as low as 1 – 2 mg/L for short periods of time (Bell and Eggleston 2005; Thomas and Rahman 2009a). This makes them a useful species for understanding how hypoxia influences trophic interactions, as they are able to survive in hypoxic areas for a short period of time but may still experience adverse effects. They have therefore been used as a model species for assessing responses to hypoxia in many laboratory and field research experiments.

Atlantic Croaker are an ecologically and economically important species in the nGoMex. For instance, a total of 4.2 million pounds of croaker were commercially landed in 2017, worth roughly $5 million (NMFS 2018). Compounded onto this, croaker compose the vast majority of bycatch in shrimp trawl landings (Scott-Denton et al. 2012), and hypoxia may alter their susceptibility to trawl capture. For these reasons, there is a clear need to understand more about interactions between croaker and hypoxia stressors that affect their natural habitat.

Ecophysiological Responses to Hypoxia

The relative abilities of predators and prey to tolerate hypoxia can lead to alternative food web interaction patterns. For Atlantic croaker, their high tolerance for low dissolved oxygen concentrations can be beneficial if its prey requires a higher dissolved oxygen concentration; the prey would become more vulnerable to the croaker as they move closer to the sediment-water interface. This is known commonly as the prey stress model or PSM (Long and Seitz 2008). Therefore, hypoxia would then enhance overall consumer consumption rates (Long et al. 2014; Pihl et al. 1992) and predators might experience a net benefit through increased foraging efficiency that may offset energetic expenditures induced by hypoxic exposure stress. A different scenario is if hypoxia provides short term protection of prey from their predators, known as the consumer stress model or CSM (Long and Seitz 2008). Under these circumstances, the consumer cannot tolerate the low dissolved oxygen concentrations, but its prey can, allowing them to thrive and be sheltered from predation for a short period of time (Long and Seitz 2008). Here, if Atlantic Croaker move into the pelagic environment to escape metabolic stress due to hypoxia, they may also experience detrimental effects, like reduced growth rate due to suboptimal foraging conditions, temperatures or salinities. Finally, the spatial response of croaker to hypoxia may be individually variable and complex. If only some of the hypoxia-exposed individuals are displaced vertically, then the consumption pressure may be distributed among both benthic and pelagic prey, leading to a recently-coined model called the Distributive Stress Model or DSM (Steube et al. *In Press)*. Combined analyses of croaker otoliths and tissue stable isotopes have recently indicated that the DSM is likely, meaning that both exposure to hypoxia and its ecological impacts appear to be highly variable among individuals (Steube et al. *In Press*).

There is a range of ecophysiological effects in demersal fishes that can result from hypoxia exposure. Most commonly, the fish can simply avoid hypoxic areas (Ludsin et al. 2009). However, such a simple response can have dramatic effects. For example, if a fish moves to an area that has saturated oxygen, or 'normoxic' conditions, the result may be increased physiological and metabolic stress responses, due to increased predation and decreased resources (Essington and Paulsen 2010). Additionally, sub-optimal salinity or temperature in normoxic habitats can result in long-term shifts of niche requirements and spatial distributions (Craig and Crowder 2005; Wu 2002).

Craig (2012) found that croaker, in trying to avoid severe hypoxia, moved short distances to the edges of suboxic waters. This movement suggests that sublethal effects of hypoxia are most intense within this narrow range, as croaker still experienced dangerously low levels of DO of 1-3 mg/L (Craig 2012). Zhang et al. (2009) found similar responses to hypoxia – that low fish biomass was found in hypoxic waters, but rather that the fish were aggregating at the edges, or even immediately above hypoxic bottom waters. This movement to normoxic waters is critical in the understanding of trophic dynamics in these areas, as hypoxia reduced the availability of quality habitat for fish by reducing access to benthic bottom habitat and prey. Further, by synthesizing results from seven ecological simulation models, Rose et al. (2009) examined how low DO affected fish at the individual, population and community levels. The results from these models warn that even if hypoxia itself has small to moderate population-level effects on fish, there will be large indirect and interaction effects due to altered spatial distributions and food web interactions, for example, shifts in spatial overlap of prey and predators, and density-dependent growth due to population crowding.

Croaker have also been found to experience significant gonadal dysfunction and endocrine pathway disruption due to continued exposure to hypoxia (Thomas and Rahman 2009b; Thomas et al. 2007). These sublethal effects due to hypoxia can cause croaker to suppress ovarian and testicular growth, which in turn reduces fecundity (Thomas et al. 2007). Further, significant proportions of female croaker collected within the hypoxic region of the nGoMex showed germ cell masculinization, as well as both male and female sex organs being significantly impaired and not as developed as expected for adults (Thomas and Rahman 2012). These sublethal effects on croaker are significant enough to reduce populations.

Another sublethal effect of hypoxia exposure is how it affects growth and metabolism. Altenritter et al. (2018) found that, of all croaker sampled in one fall season, roughly one-third were young-of-the-year's exposed to hypoxia. Additionally, they found that while these Age-0 fish were smaller in length and mass, their mean conditional factor was similar to Age-0 fish that did not experience hypoxia. Further, Altenritter & Walther (Altenritter and Walther 2019) examined carryover effects of hypoxia exposure during early life stages on lifetime survival and growth. There was no evidence found that individuals that experienced hypoxia as young-of-the-year, and survived, showed any depressed somatic growth compared to individuals that never experienced hypoxia within their first year. However, the sublethal effect of hypoxia exposure on growth over the period of an individual's entire lifetime has yet to be examined.

Otolith Microchemistry to Assess Hypoxia Exposure and Growth Rate Effects

In order to track and measure hypoxia exposure in fish, it is important to develop and use the correct marker for each specific kind of ecological question. One very reliable marker is the Hypoxia Inducible Factor 1α (HIF-1α), in Atlantic Croaker. Research in the nGoMex has shown

that croaker upregulate the HIF-1α transcription factor as a molecular response to the physiological stress of experiencing dissolved oxygen concentrations below 2.0 mg/L (Thomas and Rahman 2009a; Thomas and Rahman 2012). However, this molecular marker is only a good indicator of hypoxia exposure on very short time scales, as HIF-1α expression levels decrease back to normal within 24 hours after the individual fish has been returned back to normoxic levels. Therefore, another marker must be used that is able to represent exposure histories on the order of months to years, and that can detect long term hypoxia exposure.

One way to track environmental, elemental exposure histories is with otolith microchemistry. Otoliths are 'ear stones' of fishes, composed primarily of calcium carbonate deposits, which aid fish with hearing and balance. As a fish grows, the otolith grows with it, depositing new layers incrementally, which can be counted to estimate the age of fishes. Of the three different otolith pairs that bony fish accrete within their inner ear system, the sagittal otoliths are the largest for most taxa, and therefore the type that is most commonly used for chemical analyses. Otoliths can be cross-sectioned and analyzed for chemical changes across growth increments, as certain elements present in the ambient water with similar ionic charge can replace calcium within the aragonitic deposits. The chemical composition of ambient water can be unique to particular water masses as abiotic factors like temperature, salinity, freshwater input, and dissolved oxygen can alter which elemental ions are present in higher concentrations (Bath et al. 2000; Grønkjær 2016; Thorrold et al. 1997).

Manganese (Mn) has proved to be a useful elemental "tracer" for hypoxia exposure, as dissolved Mn concentrations increase in the water column due to hypoxia-induced redox reactions. In normoxic waters, there is a consistent flux of the manganese ion from the deeper, anoxic sediment into the surficial oxic sediments where in turn, the ion is reduced into

manganese oxide, and is taken up in the anoxic sediment. This form of manganese oxide is particulate and can precipitate onto or within the oxic sediment layer. This cycle typically occurs during the fall, winter and spring months of the year. Due to oxygen depletion in the water column because of bacterial respiration during the summer, the top, oxic layer of sediment decreases in thickness. This results in a significant flux of dissolved Mn^{2+} into the water column during the summertime (Limburg et al. 2015). Because Mn is not being oxidized back to particulate manganese oxide, it may stay dissolved in the water column for days, making it available for fish to uptake and deposit onto their otoliths. Further, pronounced Mn/Ca ratios were found to be significantly correlated to extensive hypoxia exposure (Limburg et al. 2011).

Additionally, magnesium (Mg) has been found to be a valid proxy for metabolic rates and potentially growth rates, as Mg reflects metabolic activity (Limburg et al. 2018). Mg in otoliths is not strongly influenced by dissolved concentrations of Mg in the water column, but rather is strongly primarily controlled by endogenous factors such as growth and metabolism as modified by exogenous factors such as temperature (Martin and Thorrold 2005). The exact mechanism describing how Mg is incorporated into otoliths is not fully known, but what is known is that Mg is enriched in fish blood compared to the endolymph fluid which surrounds the otoliths. The Mg ion can be secreted and reabsorbed in the intestines of fish, which further suggests a physiological regulation (Woodcock et al. 2012). One hypothesis for Mg uptake in the otolith is that since the doubly hydrated Mg ion is so large and bulky, it needs to be transported into the endolymph through ion channels. Thus, with higher metabolic activity, and more active mitochondria to produce energy, more Mg are transported into the endolymph. Secondly, Mg is associated with water-soluble proteins, making its deposition onto the otolith more efficient. Therefore, higher concentrations of insoluble proteins make deposition more difficult (Limburg

et al. 2018). Since otolith accretion is hypothesized to be proportional to the metabolic rate of fish, it follows that the trace element Mg should be associated with metabolism, somatic growth, or both.

Despite the fact that Mn is an established proxy for hypoxia exposure, hypothesizing its effect on growth can be complicated as Mn incorporation into otoliths is also sensitive to growth. In order to correct for this, Mn can be ratioed with Mg (Mn:Mg), as Mg uptake is growth sensitive, but is independent of hypoxia and other environmental factors (Limburg and Casini 2018). This way hypoxia exposure and duration can be interpreted independently from any possible changes in metabolism due to exposure.

In other studies, somatic growth of croaker proved to be responsive to hypoxic conditions in some instances (Mohan et al. 2014), so there is evidence that chemical proxies of growth recorded in otoliths can be quantified to reconstruct growth histories. Increment-specific width measurements in otoliths can be coupled with Mg and Mn measurements to verify the influences of hypoxia exposure on growth and metabolism (Limburg and Casini 2018).

Otolith Microchemistry Metrics and Their Respective Uses

In order to analyze trends using the aforementioned otolith proxies, a slew of different metrics can be used depending on the type of questions being asked and the biotic or abiotic parameters being estimated with otolith chemistry. If the research question asks whether parameters such as metabolism or hypoxia exposure change with age, the chemical transects from an entire otolith must be portioned into age-specific subsets corresponding to each year of life. Once portioned, the age-specific chemical metric can be calculated. For this thesis, these metrics include the age-specific mean chemical concentration of an elemental ratio like Mn:Ca.

In addition, age-specific Mn:Ca duration fraction is the number of points within an annulus that exceed the median Mn:Ca for that specific age and cohort year divided by annulus width. Essentially this metric represents the proportional amount of time, within that annulus or year, that the fish experienced relatively high levels of Mn:Ca. This metric is also useful because it allows for the microchemistry data to be analyzed as a percentage or fraction and can provide important information about within-year trends among individuals that lived that year.

However, if the research question is asking about lifetime trends, i.e. comparing individuals of different ages but including their entire life story into the analysis, lifetime cumulative and cumulative duration fraction metrics are the best to use. Again using Mn:Ca as an example, lifetime cumulative Mn:Ca is the mean Mn:Ca times the annulus width, summed across all years an individual has lived – it is a representation of the (standardized) total Mn:Ca present in the otolith. The cumulative duration fraction is the sum of each age-specific Mn:Ca duration fraction across all years an individual has lived. Because these metrics incorporate data values from each year the individual has lived, they are the better tools to use when assessing lifetime trends.

Additionally, Dr. Karin Limburg and her research team have created and used these metrics for Mn:Ca and Mn:Mg in their otolith hypoxia studies (Limburg and Casini 2019; Limburg and Casini 2018). In an effort to be consistent and be able to directly compare results to their previous studies using different species, I used the same metrics. While these metrics have not previously been calculates for Mg:Ca, I used the same computational methods to calculate duration fraction metrics for this elemental ratio for consistency.

Previous Research

There have been many projects about the effects of hypoxia on Atlantic Croaker in the nGoMex. Mohan et al. (2014) used controlled laboratory experiments to test the incorporation of commonly used otolith chemical markers in either constant or periodic hypoxia conditions for 4 and 10 weeks, respectively, to test the influence of endogenous and exogenous controls on otolith composition. They found that otolith chemistry of croaker is minimally influenced by endogenous factors in response to hypoxic stress and that as a result Mn:Ca profiles in croaker otoliths should be strongly indicative of ambient water composition in hypoxic or normoxic waters. Mohan & Walther (Mohan and Walther 2016) used elemental profiles to quantify hypoxia and estuarine exposure histories of croaker captured in the nGoMex for the period of 2 to 3 months prior to capture. Complemented with $\delta^{13}C$ and $\delta^{15}N$ stable isotope values, hypoxic- and normoxic- grouped croaker had similar carbon and nitrogen values, suggesting limited vertical displacement to the pelagic food web, indicating the trophic resilience of croaker during hypoxic episodes. Altenritter et al. (2018) used croaker otoliths to characterize hypoxia exposure during the first year of life in an effort to explore the sublethal effects of hypoxia that may manifest after exposure. The results indicated that, in some years, up to one third of all young of the year croaker are exposed to hypoxia and survive; they were smaller in length than croaker of the same age not exposed to hypoxia but showed similar relative conditional factor values. Lastly, Altenritter & Walther (2019) tracked carryover effects of hypoxia exposure during the early years of life on survival and growth. By using geochemical signatures, they found no evidence that traits of survivors changed from one year to the next, and that growth was not depressed in fish exposed to hypoxia in the first year of life. These findings indicate that croaker are resilient to hypoxia experienced early in life, and that sublethal carryover effects from the

first year of life do not manifest in reduced survival or growth for the remainder of the lifespan of a hypoxia-exposed fish.

For the otolith microchemical analyses done for Altenritter et al. (2018) and Altenritter & Walther (2019), a slew of chemical ratios were recorded in addition to Mn:Ca, Ba:Ca and Sr:Ca. These ratios included Mg:Ca ratios. Additionally for these studies only the first year of life from the otolith transects was analyzed because previous laboratory experiments determined tissue turnover rates for stable isotope analysis and otolith accretion rates Age-0 croaker (Mohan et al. 2014; Mohan and Walther 2016). However, the entire otolith was ablated, and data for all years of an individual's life is available for the chemical ratios and proxies recorded. This thesis capitalizes on this prior data collection and analyzes the complete lifetime chemical transects of these specimens to assess patterns of hypoxia exposure and potential growth response that have not yet been quantified in this species.

Research Questions

This study examined potential sublethal metabolic and growth effects of hypoxia exposure on Atlantic Croaker across entire lifetimes. Sublethal effects of hypoxia exposure on croaker have been observed in gonadal dysfunction and on the endocrine level. However, sublethal effects on growth and metabolism based on otolith metrics have not been observed during the early years of life, and exposure during these years did not show a significant carryover effect on growth in later years. A new proxy for metabolism, Mg:Ca, shows promise in possibly elucidating any metabolic effects, in conjunction with established hypoxia proxies.

My first aim was to determine if Mn:Mg is a more accurate proxy for estimating hypoxia exposure than Mn:Ca in croaker. While Mn:Ca is an accepted proxy for hypoxia exposure, recent

research has shown that the use of Mn:Mg can be an improvement, as it corrects for any metabolism-related differences in Mn deposition into the otolith. Therefore, I hypothesized that Mn:Mg will correlate strongly with hypoxic volume for each age class and calendar year. My second aim is to examine whether Mg:Ca, the otolith proxy for metabolism, correlates to conditional factor (as Fulton's K) and hypoxia exposure (as Mn:Ca and Mn:Mg ratios) over an individual's lifespan. Croaker are relatively tolerant to hypoxia and it has been hypothesized that they take advantage of the low dissolved oxygen levels by preying on benthic infaunal species surfacing from deeper in the sediment due to hypoxia. In addition, most croaker in the nGoMex do not survive past the ages of 3 or 4, resulting in short lifespans. I therefore hypothesize that there will not be a strong reduction of growth or condition due to hypoxia. Instead, I expect to find no significant differences in Fulton's K or mean Mg:Ca between hypoxia exposure groups. Table 1 summarizes each otolith microchemical metrics I used to address my individual research questions.

Table 1. Important research questions asked in this study with the corresponding otolith microchemistry metrics used to examine them.

Research Question	Metrics Used
Is Mn:Mg a more accurate proxy for estimating hypoxia exposure than Mn:Ca in croaker?	• Mn:Ca Duration Fraction • Mn:Mg Duration Fraction
Does Mg:Ca correlate to Fulton's K conditional factor?	• Mean Mg:Ca • Lifetime Cumulative Mg:Ca • Cumulative Mg:Ca Duration Fraction
Does Mg:Ca correlate to hypoxia exposure?	• Mean Mg:Ca • Mn:Ca Duration Fraction • Mn:Mg Duration Fraction
Does Fulton's K conditional factor correlate to hypoxia exposure?	• Cumulative Mn:Ca Duration Fraction • Cumulative Mn:Mg Duration Fraction

MATERIALS & METHODS

Study Area & Field Sampling

To best identify offshore seasonal hypoxia induced metabolic growth differences, collections took place around and within the hypoxic zone of the continental shelf offshore of Louisiana and Texas (Figure 1). Peak hypoxia severity, in which some areas are called the "dead zone," occurs in late summer, and the hypoxic zone subsequently dissipates from September through November each year. Collections took place October 23rd until November 5th of 2014 and 2015 to allow for possible detrimental impacts of summer hypoxia exposure to be recorded in the hardened structures. The extent of bottom water hypoxia in 2014 was 13,080 square kilometers (5,052 square miles) and 16,760 square kilometers (6,474 square miles) in 2015. Both years measured within the average range and extent of season hypoxia for the previous 10 years within the nGoMex (LUMCON Gulf Hypoxia Program 2021), thereby reflecting typical offshore hypoxic conditions in the nGoMex.

Atlantic Croakers were collected by the National Oceanic and Atmospheric Administration's (NOAA) Southeast Monitoring and Assessment Program's (SEMAP) Fall Groundfish Surveys aboard the RV *Oregon II*. Sampling stations were chosen via a stratified random sampling design (Figure 1). Twenty-two stations were sampled in 2014 and twenty-one were sampled in 2015 (for a total of 43) via 12.8 m beam benthic shrimp trawl with 41-mm stretch mesh deployed for 30 min (GSMFC 2001). When present, between 10 - 50 Atlantic Croaker were collected and stored frozen at -20ºC. A total of 463 Croaker were collected during the 2014 cruise, and 962 in 2015. A subset of these fish was used for aging and chemical analyses, as detailed below.

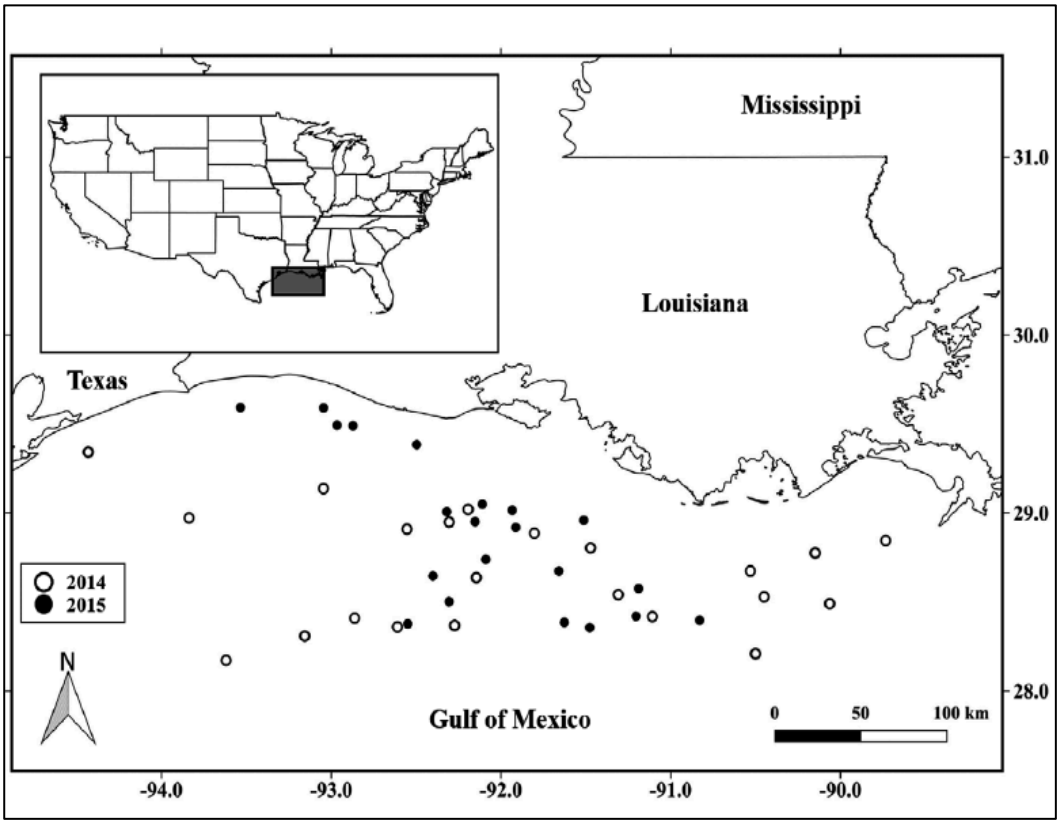

Figure. 1 Map depicting sampling stations in 2014 (open circles) and 2015 (closed circles) for NOAA's SEAMAP Groundfish Fall Surveys. Atlantic Croaker were collected from October – November of each year.

Laboratory Processing

Fully detailed methods are described by Altenritter et al. (2018). A short summary of the processing methods is described here. Collected and frozen fish were thawed, patted dry and measured for total length (± 0.5mm), and weighed (± 0.5g). Fulton's K conditional factor (K) was calculated as

$$K = 100^3 * \frac{W}{L^3} \quad (1)$$

where W is weight (g), and L is total length (mm).

The sagittal otoliths were removed, rinsed in DI water and dried. The left otolith was mounted onto a glass slide, thick-sectioned (1-2mm; Buehler Isomet low-speed saw) along the transverse plane and mounted to a glass petrographic slide with thermoplastic glue (Crystalbond). Each otolith was polished using 30-um and 3-um lapping films until the core was visible. Polished otoliths were then transferred to another slide, with 12 otoliths per slide, for laser analysis.

Otoliths were aged twice by two independent readers. Because croaker otolith ages have been validated in the nGoMex, it was assumed that one pair of opaque and translucent bands was indicative of one year of growth. Croaker were assumed to have hatched during October and November of the year before their respective year of origin (Hernandez et al. 2010; Thomas et al. 2015). Over the two sampling years, fish were analyzed by age class per year, resulting in 10 unique age classes: Age-0 fish from 2012, 2013, 2014, and 2015; Age-1 fish from 2013, 2014, and 2015; Age-2 fish from 2014 and 2015, and Age-3 fish from 2015. Sample sizes for these categories and their associated lengths, weights and Fulton's K values are reported in Table 2. Retention of these age classes was meant to facilitate comparisons between hypoxia exposure and growth between the first few years and later years of life for croaker.

Table 2. Sample size, total length, mass, and Fulton's K statistics for each age-associated calendar year. Values are means with standard deviations in parentheses.

Collection Year	Age	Sample Size	Total Length (mm)	Mass (g)	Fulton's K
2014	0	70	151 (11.1)	36.2 (9.7)	1.04 (0.09)
2014	1	49	172 (16)	52.9 (18.4)	1.01 (0.07)
2014	2	1	200 (n/a)	84.4 (n/a)	1.05 (n/a)
2015	0	85	156 (10)	36.6 (8.3)	0.94 (0.13)
2015	1	79	173 (17.3)	49.8 (17.4)	0.93 (0.07)
2015	2	30	169 (13)	46.5 (12.6)	0.95 (0.06)
2015	3	9	189 (16)	68.2 (17.7)	0.99 (0.06)

Otolith Chemical Analysis

Concentrations of manganese (^{55}Mn) and magnesium (^{25}Mg) relative to calcium (^{44}Ca) were measured using laser ablation inductively coupled plasma (ICP) mass spectrometry in the Jackson School of Geosciences, University of Texas, Austin. Laser settings are described in detail by Altenritter et al. (2018) and Altenritter and Walther (2019). Briefly, laser transects were conducted from core to edge of each otolith along the longest dorsoventral axis. Transect pathways were pre-ablated to remove surficial contamination, then re-ablated to collect elemental data across growth increments. Elements were measured using an Agilent 7500ce ICP quadrupole mass spectrometer coupled to a New Wave UP 193-FX laser. Otoliths were pre-ablated (spot size = 50 μm; scan rate = 50 μm/s) to remove surface contamination along the transect prior to the collection of data (spot size = 25 μm; scan rate = 5 μm/s). Over the roughly two weeks' worth of lasering, mean laser power was 5.37 J/cm^2 (SD = 1.70). Certified reference materials NIST-612 and MACS-3 were measured repeatedly throughout analytical sessions and used to correct for drift, assess analytical precision, and convert raw elemental counts into molar concentrations relative to calcium.

The otolith transects were inspected under microscopes and measured using the Zen Pro (Zeiss) software package. Annuli were identified as the position of the beginning of each successive opaque zone from the core to the edge of the otolith along the laser transect (Barger 1985). Annuli distances were then measured for each year present in each otolith. Calendar years were assigned to each annulus by back-calculating from the year of capture. These measured distances also allowed the chemical transects to be portioned and separated by year to calculate age- and year-specific chemical data for statistical analyses.

Otolith Exposure Indices

In order to analyze each individual's elemental concentrations, chemical transects were partitioned by annual otolith growth zones, or ages by year. The suite of metrics calculated are summarized in Table 2. The metrics used for each elemental ratio (Mn:Ca, Mg:Ca, Mn:Mg) are as follows, using Mg:Ca as example: Mean Mg:Ca (average Mg:Ca in an annulus); Cumulative Mg:Ca (mean Mg:Ca times annulus distance) represents the accumulation of Mg:Ca over the year; Lifetime cumulative Mg:Ca (the sum of the cumulative Mg:Ca's across all years an individual has lived); Mg:Ca Duration (the number of points within an annulus that exceed the median Mg:Ca for that specific age and cohort year), Mg:Ca Duration Fraction (the Mg:Ca Duration divided by annulus width), Cumulative Mg:Ca Duration Fraction (the sum of each year's duration fractions). These metrics were then calculated separately for Mn:Ca and Mn:Mg ratios. All Mg:Ca and Mn:Ca values were measured in units of μmol/mmol, and Mn:Mg values were measured in units of mol/mol.

To calculate hypoxia exposure groups using Mn:Ca ratios, croaker and their mean Mn:Ca values were grouped by age class per calendar year (i.e. Age 0's from 2012, Age 1's from 2013, etc.). Medians for each specific age class per year were calculated and used to define Mn:Ca Duration "thresholds" for each individual fish (Table 3). Then, Mn:Ca Duration Fractions were used to group fish into quartiles (less than 25%, 25-49.9%, 50-74.9%, greater than 75%). These groups are the "hypoxia exposure groups" (HEGs), where HEG-1 represents the group that was the least exposed, and HEG-4 represents the group that was the most exposed.

To calculate hypoxia exposure groups using Mn:Mg ratios, the same approach was taken as for Mn:Ca, except now individuals were grouped by their Mn:Mg Duration Fractions. The mean of Mn:Mg Duration Fraction was taken and used to group fish into "normalized hypoxia

exposure groups" (nHEGs), either nHEG-1, less than the mean, or nHEG-2, greater than or equal to the mean. This approach was used instead of the former because duration fraction for Mn:Mg were significantly skewed towards zero and not easily separable by quartiles like Mn:Ca-based exposure groups.

To calculate the Fulton's K condition groups, the Fulton's K value for each fish was sorted into quartiles (less than 25%, 25-49.9%, 50-74.9%, greater than 75%). These groups are the "condition groups" where Group 1 represents the group with the highest Fulton's K values, and Group 4 represents the group with the lowest values.

Data were inspected for normality and tested as appropriate with continuous methods. Linear regressions were used to test for significant correlations between two continuous variables. One-way and two-way ANOVAs were used to test for significant effects of age and other categorical variables on the various proxies. Post-hoc pairwise comparisons between groups followed, with the Shaffer correction, as there were always less than six groups, and the data were unbalanced.

Table 3. List of all the otolith microchemistry-related metrics used, and a brief explanation of how each was calculated or interpreted

Data/Metric	Brief Explanation
Mn:Ca (µmol/mmol)	Uncorrected proxy for hypoxia exposure
Mg:Ca (µmol/mmol)	Proxy for metabolism
Mn:Mg (mol/mol)	Normalized proxy for hypoxia exposure
Mean Mg:Ca (µmol/mmol)	Average Mg:Ca within each annulus
Cumulative Mg:Ca (µmol/mol)	Mean Mg:Ca times annulus distance
Lifetime Cumulative Mg:Ca (µmol/mol)	The sum of the cumulative Mg:Ca values across all years an individual has lived
Mg:Ca Duration	The number of points within an annulus that exceed the median Mg:Ca for that specific age & cohort year
Mg:Ca Duration Fraction	The Mg:Ca duration divided by annulus width
Cumulative Mg:Ca Duration Fraction	The sum of each year's Mg:Ca duration fraction across all years an individual has lived

RESULTS

The median values for each elemental proxy and age and calendar year combination were different and varied between years. The overall trend, however, is that for each proxy, the median value decreased from Age-0 to Age-3 (Table 4).

A one-way ANOVA found that there were no significant differences among duration fractions compared across calendar years whether the duration fraction was calculated using the uncorrected hypoxia proxy (Mn:Ca, $p=0.82$) or the normalized hypoxia proxy (Mn:Mg $p=0.99$, Figure 2). Another one-way ANOVA found that there was a significant difference in annulus widths between ages ($p<0.001$). Age-0 individuals had significantly larger annulus widths than age 1, 2 and 3 individuals ($p<0.001$ for each comparison, Figure 3). An additional one-way ANOVA also found that Fulton's K conditional proxy was significantly different between age classes ($p=0.013$). Pairwise comparisons found there was only a significant difference in Fulton's K between Age-0 and Age-1 individuals ($p=0.027$), as Age-0 individuals had larger Fulton's K values than Age-1 fish. There were no significant differences in Fulton's K between any of the other age classes ($p>0.06$, Figure 4).

Table 4. Median values of each chemical proxy calculated for each year and age. These values were subsequently used to define the duration fraction for the given calendar year and age for each annulus within all otoliths.

Mg:Ca (µmol/mmol)

Age 0				Age 1			Age 2		Age 3
2012	**2013**	**2014**	**2015**	**2013**	**2014**	**2015**	**2014**	**2015**	**2015**
195	216.7	222.6	251.4	64.3	66.4	96.9	43.1	32.88	42.44

Mn:Mg (mol/mol)

Age 0				Age 1			Age 2		Age 3
2012	**2013**	**2014**	**2015**	**2013**	**2014**	**2015**	**2014**	**2015**	**2015**
0.58	0.41	0.45	0.55	0.25	0.22	0.21	0.2	0.18	0.19

Mn:Ca (µmol/mmol)

Age 0				Age 1			Age 2		Age 3
2012	**2013**	**2014**	**2015**	**2013**	**2014**	**2015**	**2014**	**2015**	**2015**
53.01	40.15	44.96	60.7	9.93	6.81	8.81	3.57	3.01	3.37

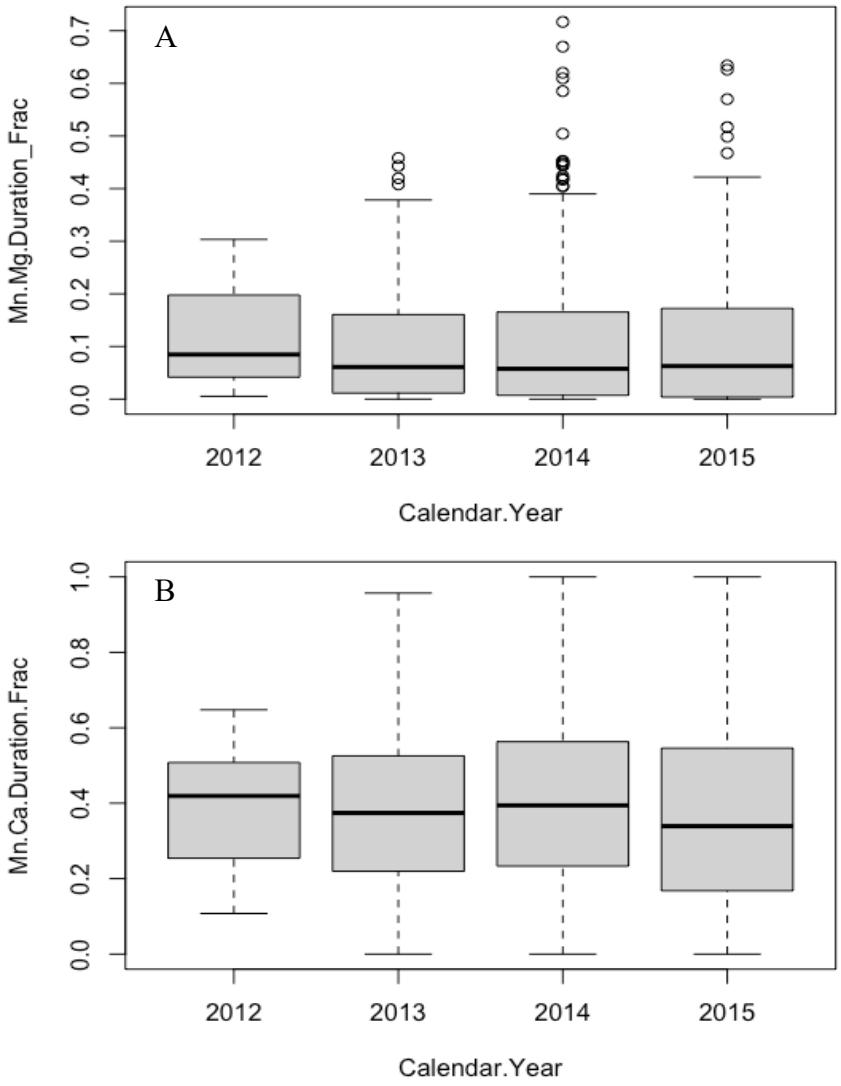

Figure 2. Mn:Mg duration fraction (A) and Mn:Ca duration fraction (B) for each analyzed calendar year. Note that y-axes ranges differ.

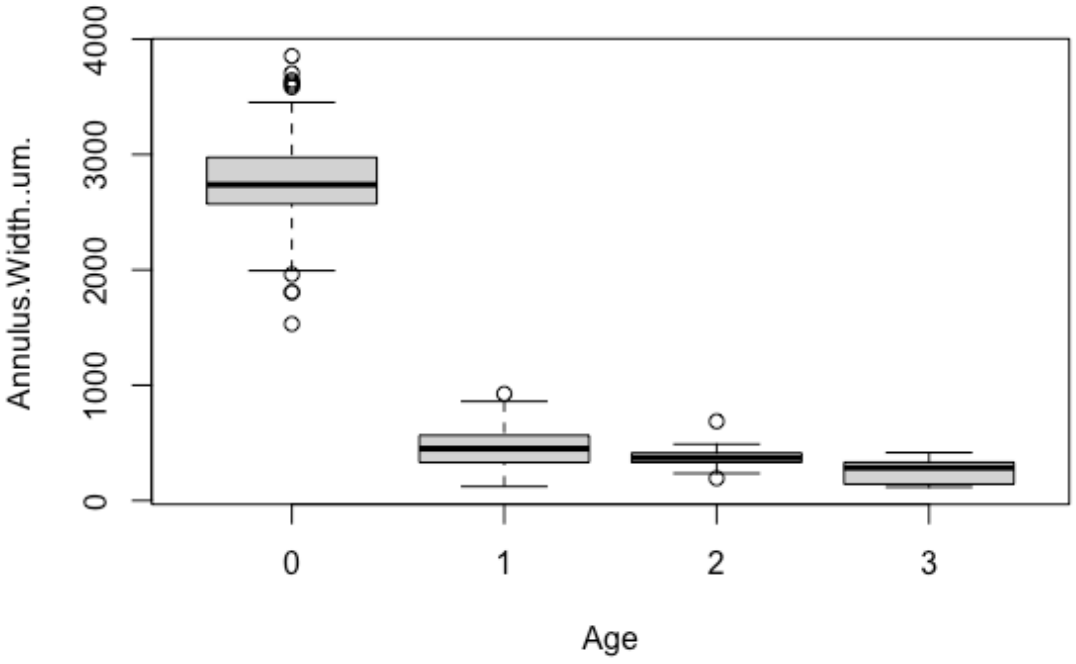

Figure 3. Annulus widths (µm) for each analyzed age.

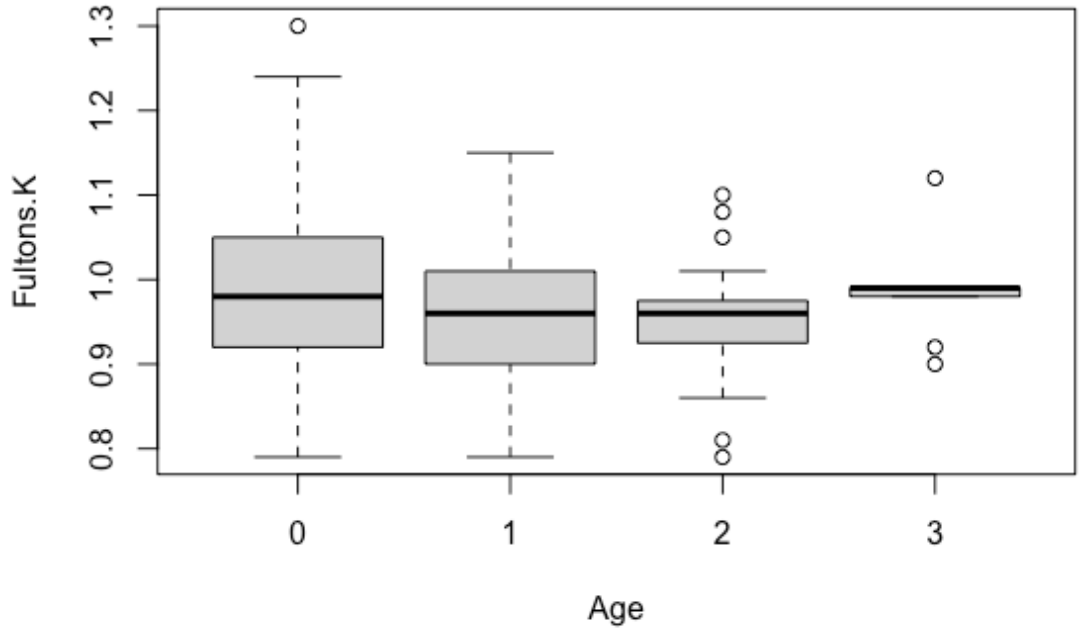

Figure 4. Fulton's K condition values for fish according to their age at collection.

Uncorrected Hypoxia Proxy (Mn:Ca)

Mn:Ca is considered an "uncorrected" proxy for hypoxia, because Mn uptake in the otolith may also be sensitive to growth rate. Here, differences in Mn:Ca were compared without accounting for potential growth rate biases (see below for growth corrected values). Fish were grouped according to quartiles of their Fulton's K values and further separated by age. Because Fulton's K is a cumulative assessment of lifetime length and mass growth, it was compared to the lifetime cumulative Mn:Ca duration fraction for each age and condition grouping.

A two-way ANOVA tested for differences in cumulative Mn:Ca duration fractions between condition groups and age. Only the main effect of age was significant ($p<0.001$); the main effect of condition group and the interaction between age and condition group were not significant for cumulative Mn:Ca duration fraction ($p=0.34$ and $p=0.16$, respectively). All pairwise comparisons were significant between each age ($p<0.001$ for each pairwise comparison). Age-0 reported the smallest cumulative Mn:Ca duration fraction, followed by Age-2, then Age-3, with Age-1 having the largest value (Figure 5).

A one-way ANOVA found that there were significant differences in Mn:Ca duration fractions between ages ($p=0.003$). Only Age-1 was significantly greater than 0 for Mn:Ca duration fractions ($p=0.0013$) (Figure 6). Another one-way ANOVA found that there were significant differences between ages and mean Mn:Ca values ($p<0.001$). Age-0 was significantly greater than Age-1, Age-2, and Age-3 ($p<0.001$ for each pairwise comparison). The other ages were not significantly different from each other ($p>0.21$ for each pairwise comparison; Figure 7).

A two-way ANOVA tested for differences in mean Mg:Ca between HEG and age. The main effect of age, the main effect of HEG, and the interaction between age and HEG were all significant ($p<0.001$). Therefore, to analyze the effect these groupings have on mean Mg:Ca,

differences were tested between HEGs for each age separately. For Age-0, there was no significant difference between HEGs for mean Mg:Ca ($p>0.0664$). For Age-1, each HEG was significantly different than one another ($p<0.027$). HEG-4, the group with the highest exposure, showed the highest mean Mg:Ca, followed by Age-3, then Age-2, and finally Age-1 with the lowest mean Mg:Ca. For Age-2, HEG-1 had significantly lower mean Mg:Ca values than HEG-2 ($p=0.042$), HEG-3 ($p=0.011$), and HEG-4 ($p<0.001$). HEG-2 an HEG-3 were not significantly different from one another ($p=0.16$). Lastly, HEG-4 had the highest mean Mg:Ca values compared to HEG-1 ($p<0.001$), HEG-2 ($p<0.001$), and HEG-3 ($p=0.0019$). For Age-3, there was no significant difference in HEG's for mean Mg:Ca ($p>0.47$) (Figure 8).

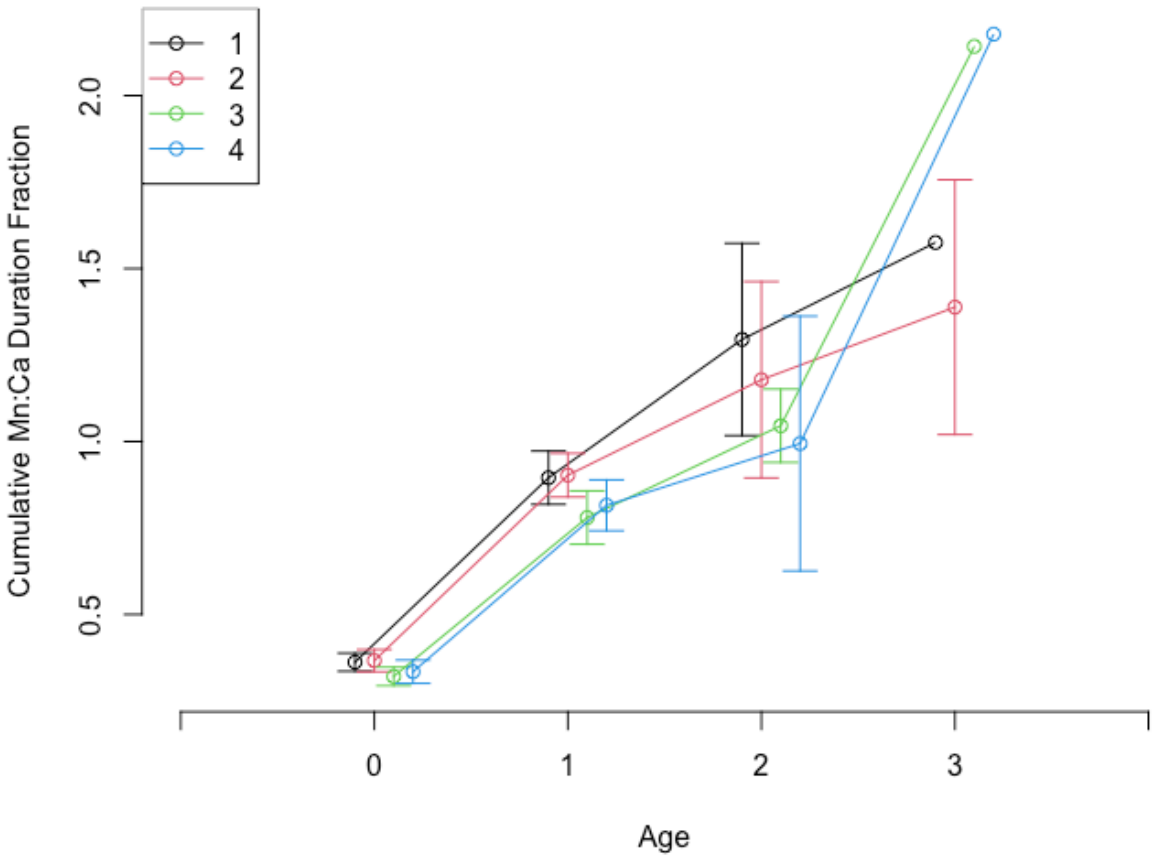

Figure 5. Cumulative Mn:Ca Duration Fractions as the fish increase in age. Fish at each age are grouped by Fulton's K condition value quartiles, where Group 1 has the highest Fulton's K values, and Group 4 has the lowest Fulton's K values.

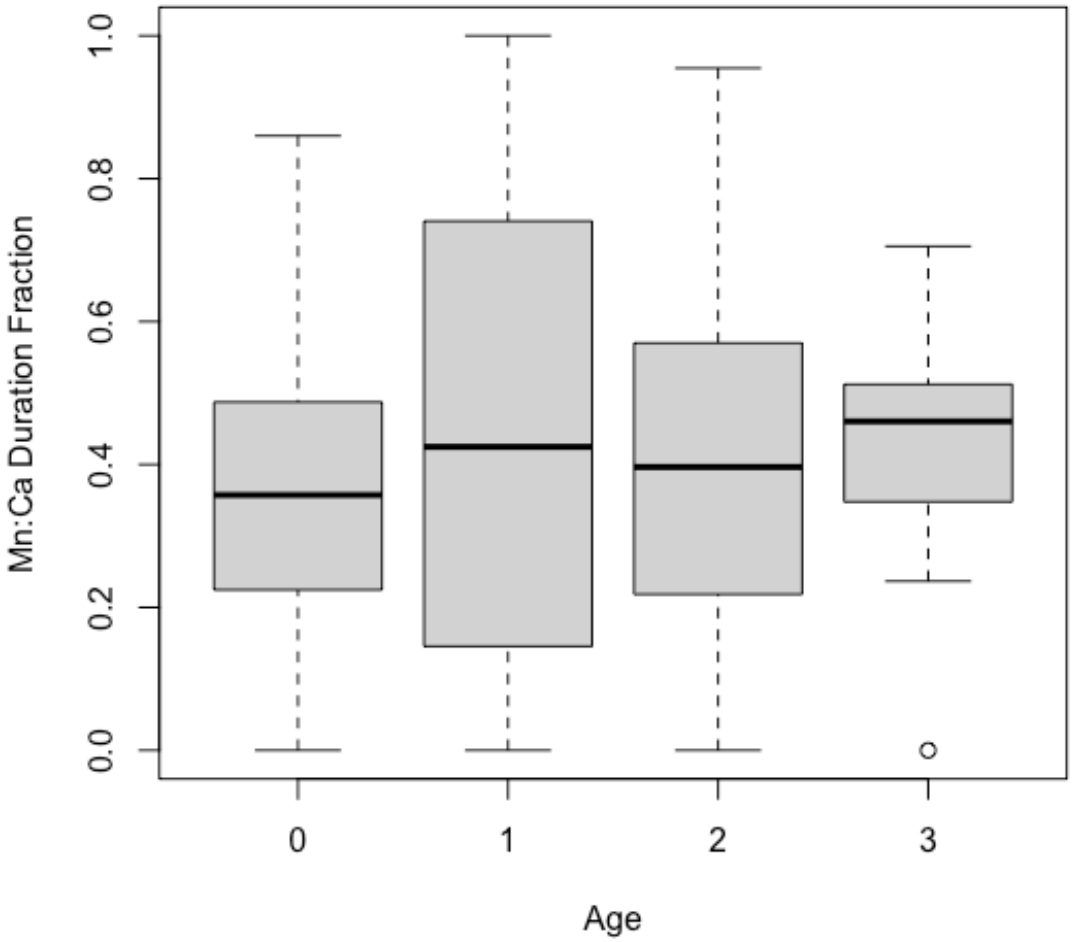

Figure 6. Mn:Ca duration fractions for each analyzed age.

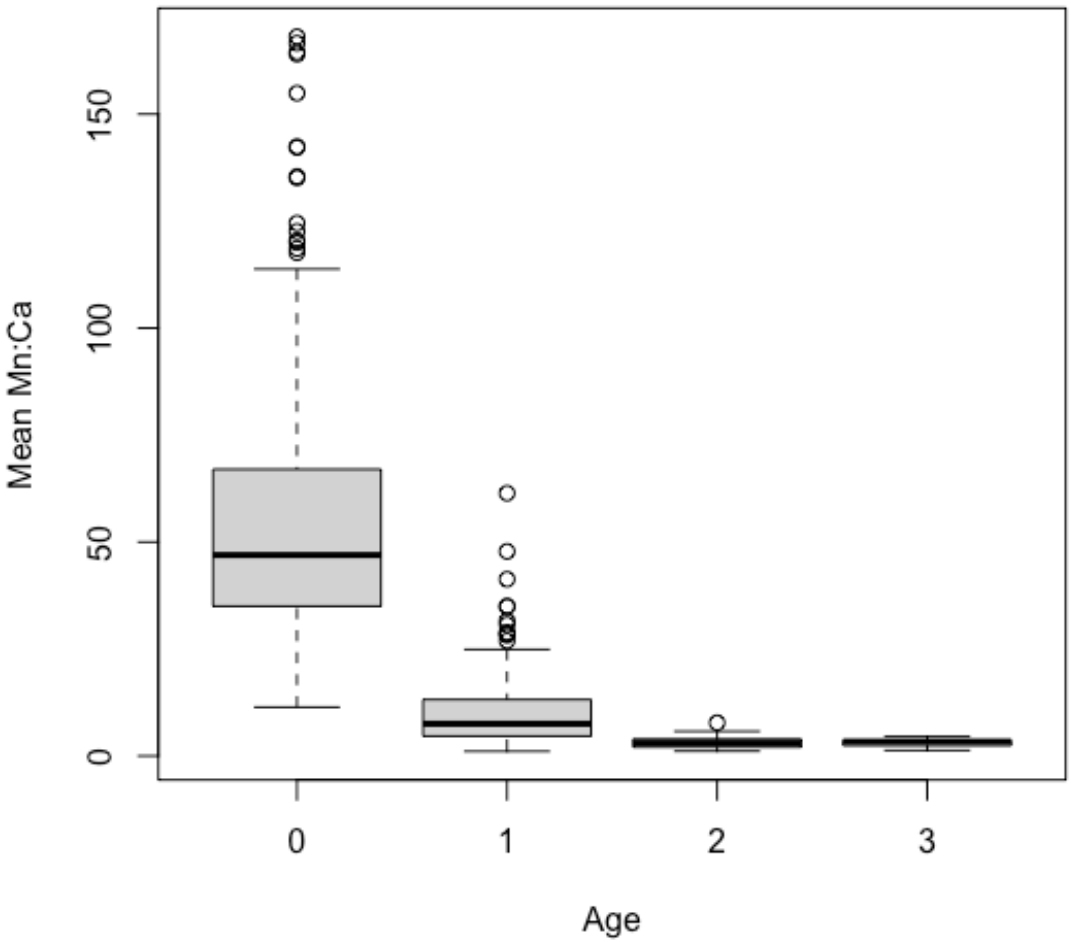

Figure 7. Mean Mn:Ca (µmol/mmol) values for each analyzed age.

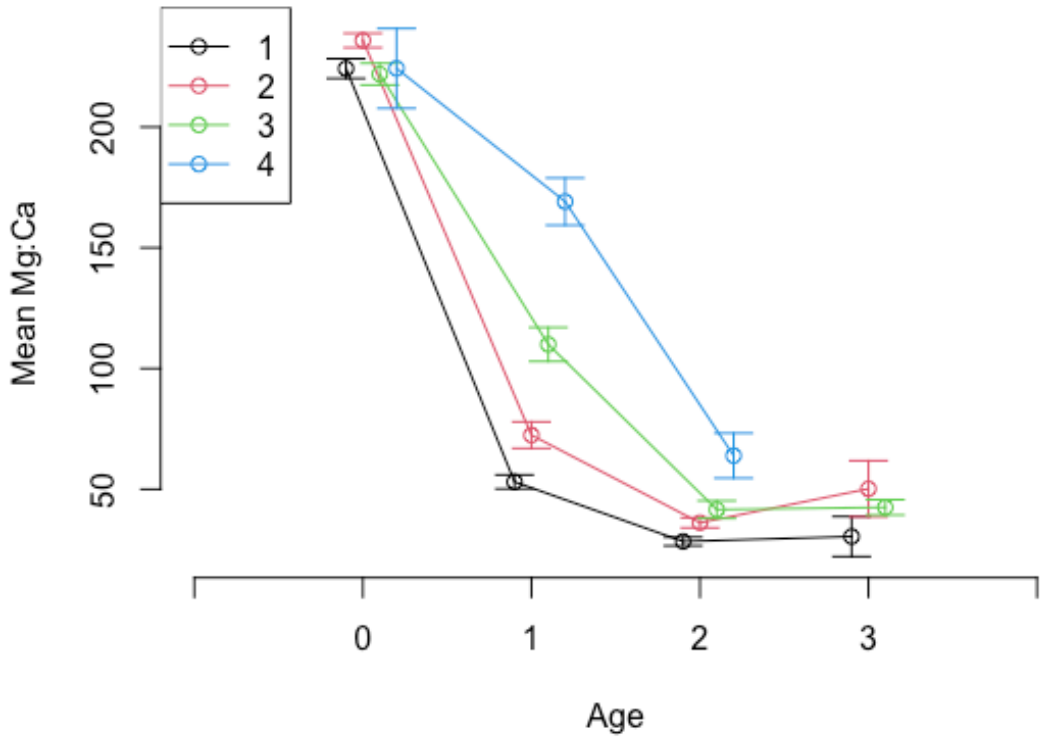

Figure 8. Mean Mg:Ca (µmol/mmol) values for each age, grouped by the uncorrected hypoxia exposure groups 1 through 4, where Group 4 has the highest hypoxia exposure and Group 1 has the lowest hypoxia exposure.

Metabolic Proxy (Mg:Ca)

In this study, Mg:Ca is a proxy for metabolism, and Mg:Ca values were examined over entire lifespans of croaker. Linear regressions were fit between mean Mg:Ca values and the corresponding annulus width separately for each year of life (Age-0 to Age-3). When looking at ages of croaker independently, mean Mg:Ca for a given year of life showed a weak, positive correlation to the corresponding annulus width for Age-0 (adjusted $R^2 = 0.12$, $p<0.001$) (Figure 9a), and almost no correlation for Age-1 (adjusted $R^2 = -0.0051$, $p=0.69$) (Figure 9b). Age-2 and Age-3 showed a slight negative correlation (adjusted R^2 for Age-2 = -0.02, $p=0.53$, adjusted R^2 for Age-3 = 0.07, $p=0.25$) (Figure 9c & d).

A one-way ANOVA found that there was a significant difference between mean Mg:Ca values by age ($p<0.001$). Pairwise comparisons found that Age-0 fish had a significantly higher Mg:Ca than Age-1, Age-2 and Age-3 ($p<0.001$ for each comparison; Figure 10).

Because the trends in annulus width versus age, and mean Mg:Ca versus age were very similar, Mg:Ca was ratioed against annulus width and plotted against age to assess if the residuals of Mg:Ca after normalization to annulus width differed across ages. This analysis was to determine if Mg:Ca values increased or decreased in a particular year of life once the lifetime decline in growth was accounted for. A one-way ANOVA found significant differences in annulus-normalized Mg:Ca values by age ($p<0.001$). Annulus-normalized Mg:Ca values for Age-1 were significantly greater than those for Age-0 and Age-2 ($p<0.001$ for both comparisons). Annulus-normalized Mg:Ca values for Age-3 were also significantly greater than values for Age-0 ($p<0.001$), and Age-2 ($p=0.018$). However, there were no significant differences in annulus-normalized Mg:Ca values between Age-0 and Age-2 ($p=0.669$) or between Age-1 and Age-3 ($p=0.669$; Figure 11).

A two-way ANOVA tested for the relationship between condition groups and age for Mean Mg:Ca values. Only the main effect of age was significant ($p=0.0015$); the main effect of condition group and the interaction term between age and condition group were not significant ($p=0.19$ and $p= 0.44$, respectively). There was a significant difference between Age-0 and Age-1, Age-2, and Age-3 ($p=0.05$, 0.012, and 0.05, respectively). There was no significant difference between any of the other ages ($p=0.32$, 0.32, 0.54 for pairwise comparisons; Figure 12).

Another two-way ANOVA tested for the relationship between condition groups and age for cumulative Mg:Ca duration fractions. The main effects of condition group and age as well as the interaction between age and condition group were all significant ($p= 0.04$, $p<0.001$, $p<0.001$, respectively). Therefore, to analyze the effect these groupings have on the cumulative Mg:Ca duration fraction, differences were tested between condition groups for each age separately. For each age, there was no significant difference in condition groups for the cumulative Mg:Ca duration fraction (Age-0 $p>0.195$, Age-1 $p= 1.0$, Age-2 $p>0.11$, Age-3 $p>0.53$; Figure 13).

A one-way ANOVA found that there was a significant difference in Mg:Ca duration fractions between ages ($p=0.03$). However, Shaffer post-hoc test did not identify significant differences between pairwise combinations of ages ($p>0.1$) (Figure 14). The data also showed no significant linear regressions between lifetime cumulative Mg:Ca values and Fulton's K values for any of the analyzed ages (Age-0: adjusted $R^2= -0.006$, $p=0.9$; Age-1: adjusted $R^2 = 0.007$, $p=0.17$; Age-2: adjusted $R^2 = -0.03$, $p=0.96$; Age-3: adjusted $R^2 = 0.31$, $p=0.07$; Figure 15).

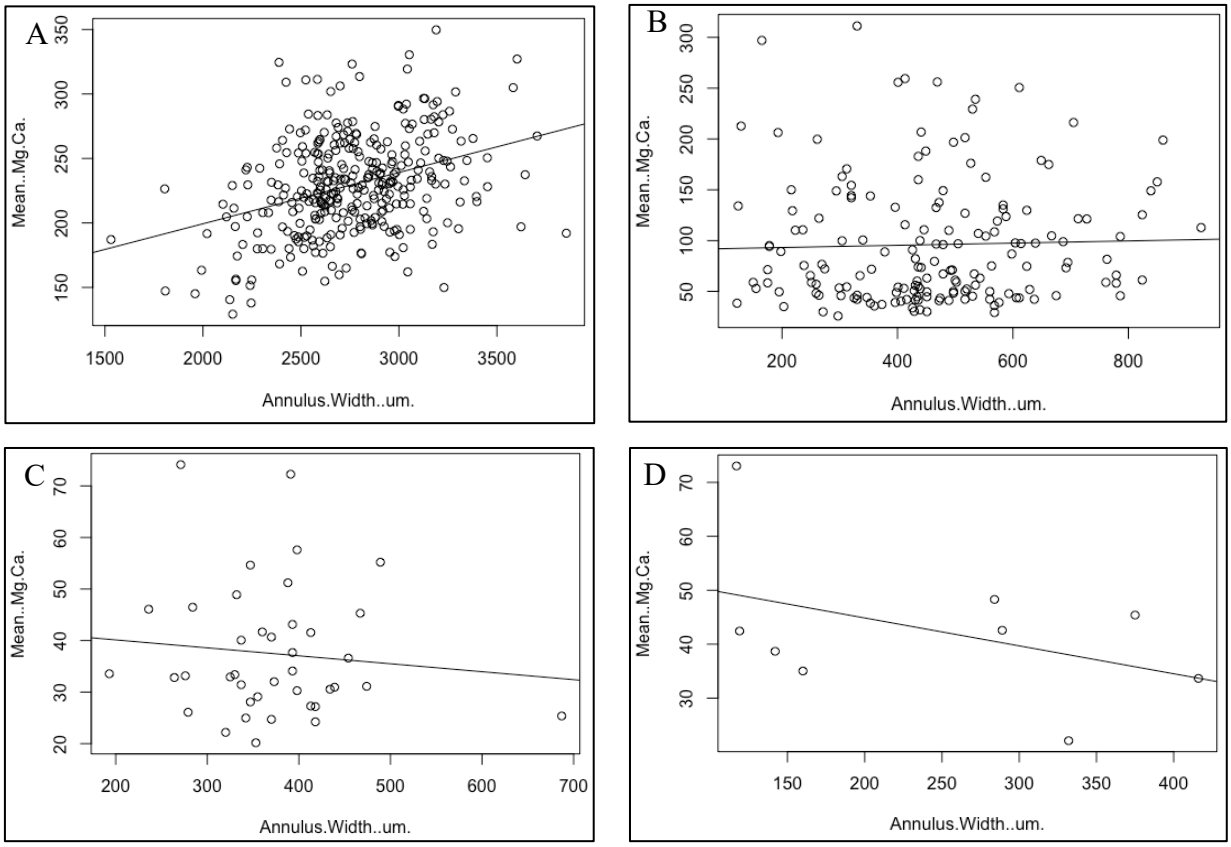

Figure 9. Linear regressions between mean Mg:Ca (µmol/mmol) and annulus widths (µm) for a given year of life. Panels show results for Age-0 (A), Age-1 (B), Age-2 (C), and Age-3 (D) individuals. Note axis values differ between ages.

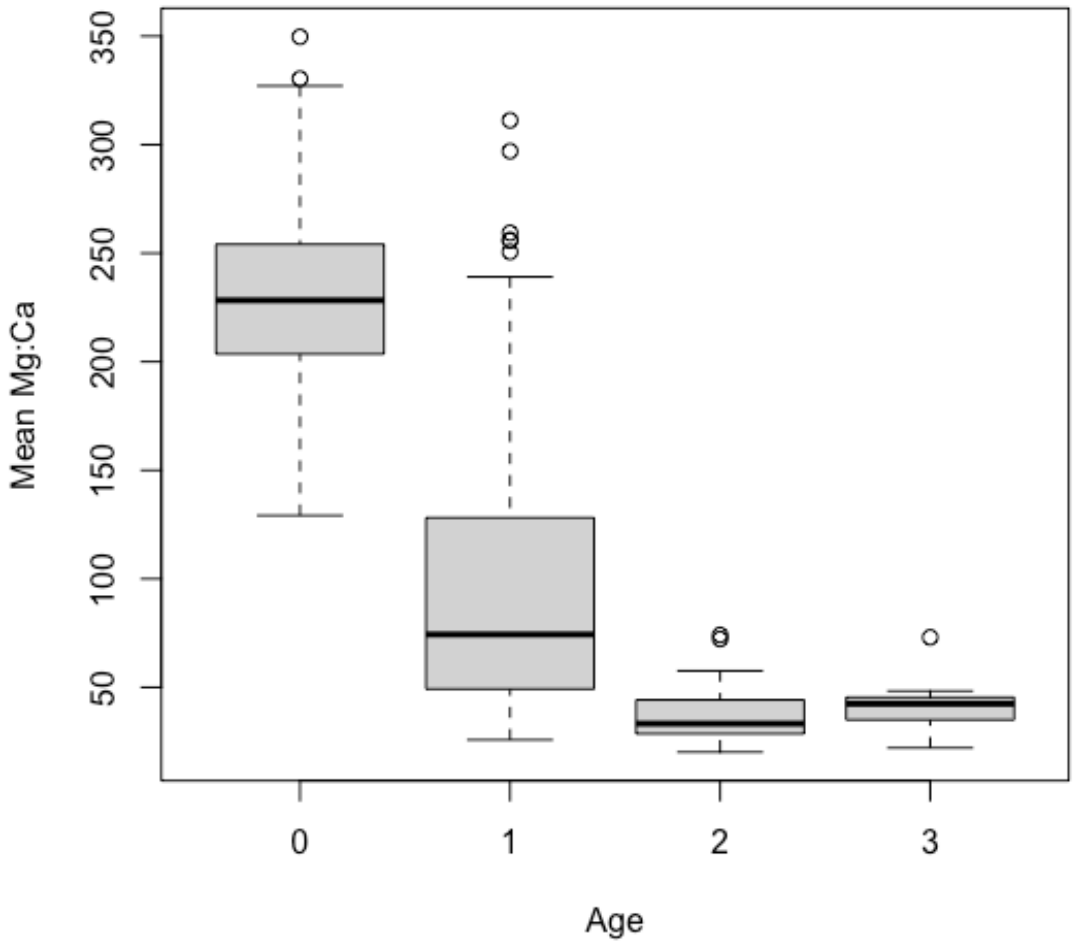

Figure 10. Mean Mg:Ca (µmol/mmol) values for each age.

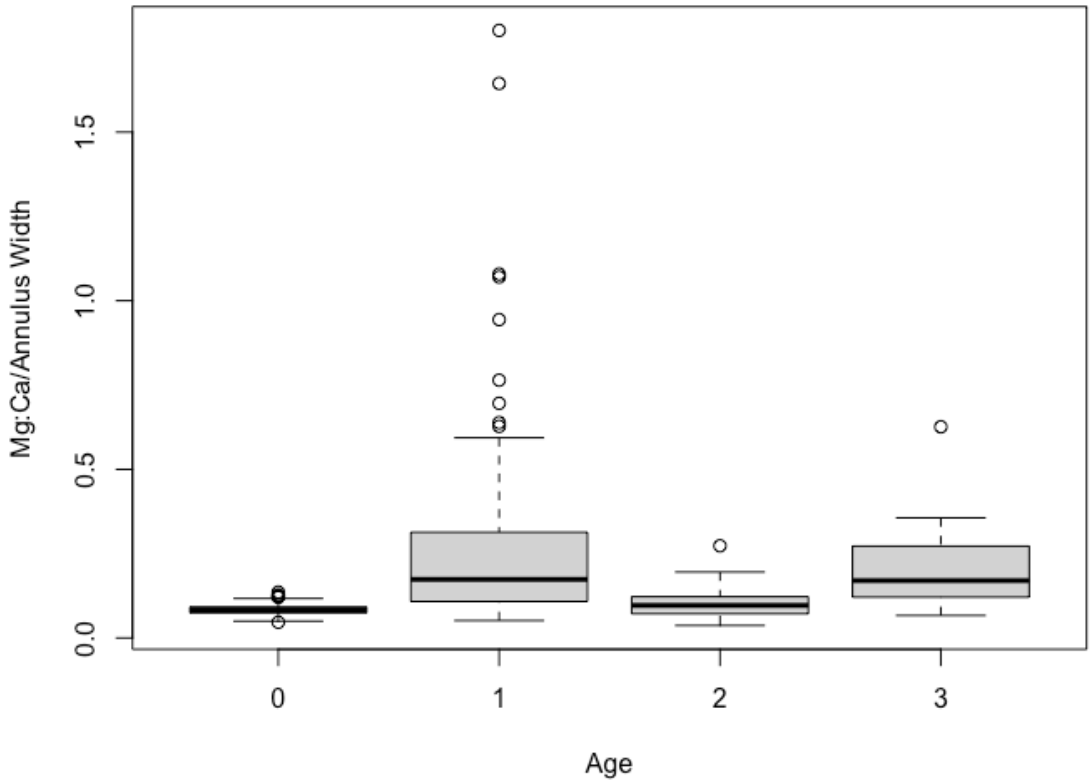

Figure 11. Annulus width-normalized Mg:Ca (µmol/mol) values for each analyzed age.

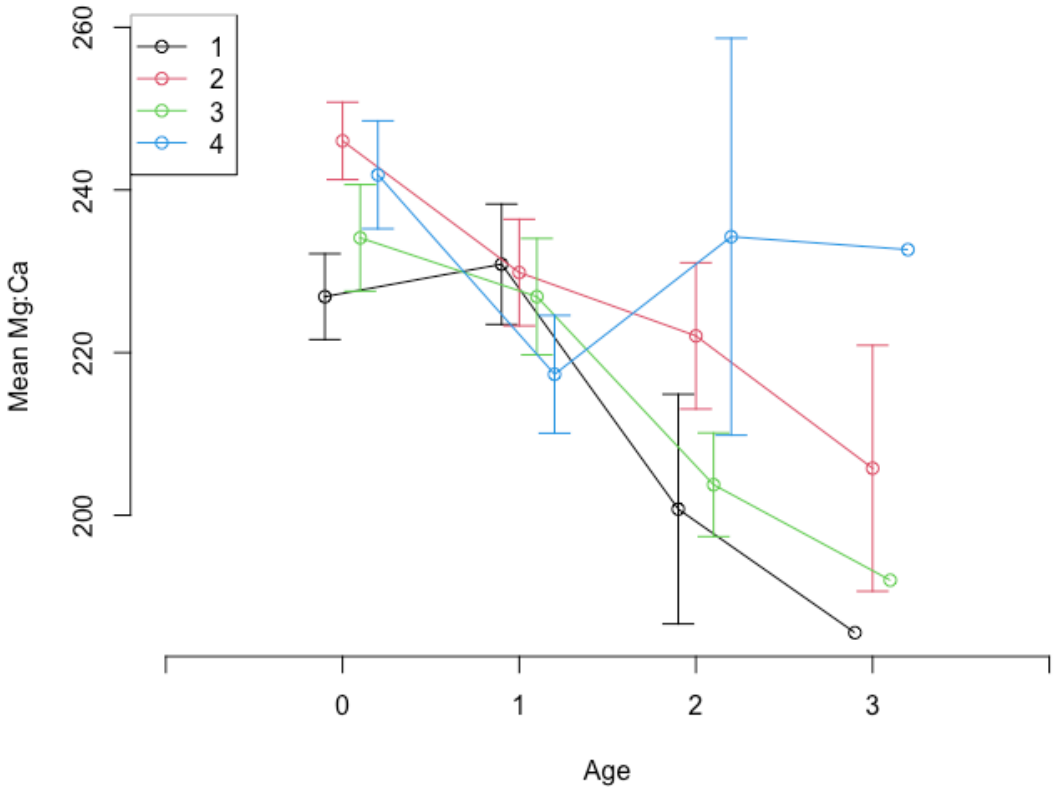

Figure 12. Mean Mg:Ca (µmol/mmol) values for each age. Individuals are grouped by Fulton's K condition value quartiles, where Group 4 has the lowest Fulton's K values, and Group 1 has the highest Fulton's K values.

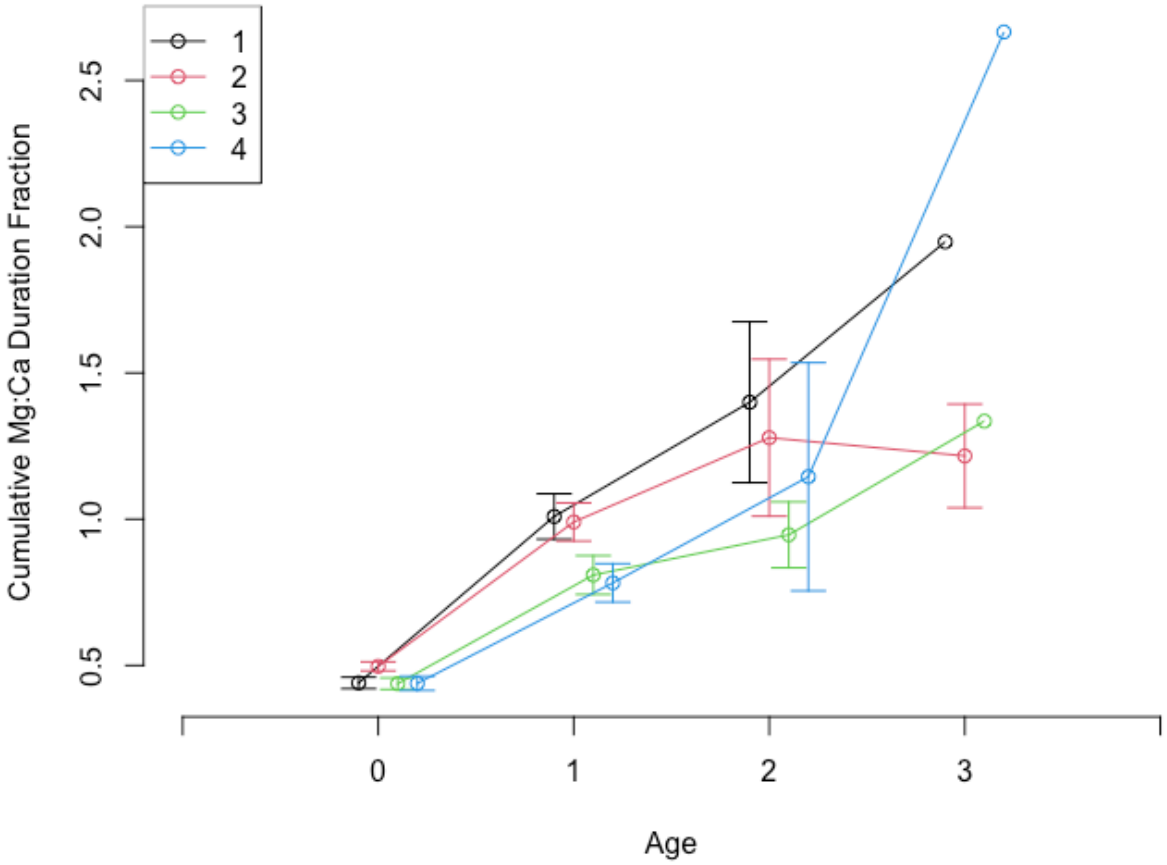

Figure 13. Cumulative Mg:Ca duration fraction values for each age, grouped by Fulton's K condition value quartiles, where Group 4 has the lowest Fulton's K values, and Group 1 has the highest Fulton's K values.

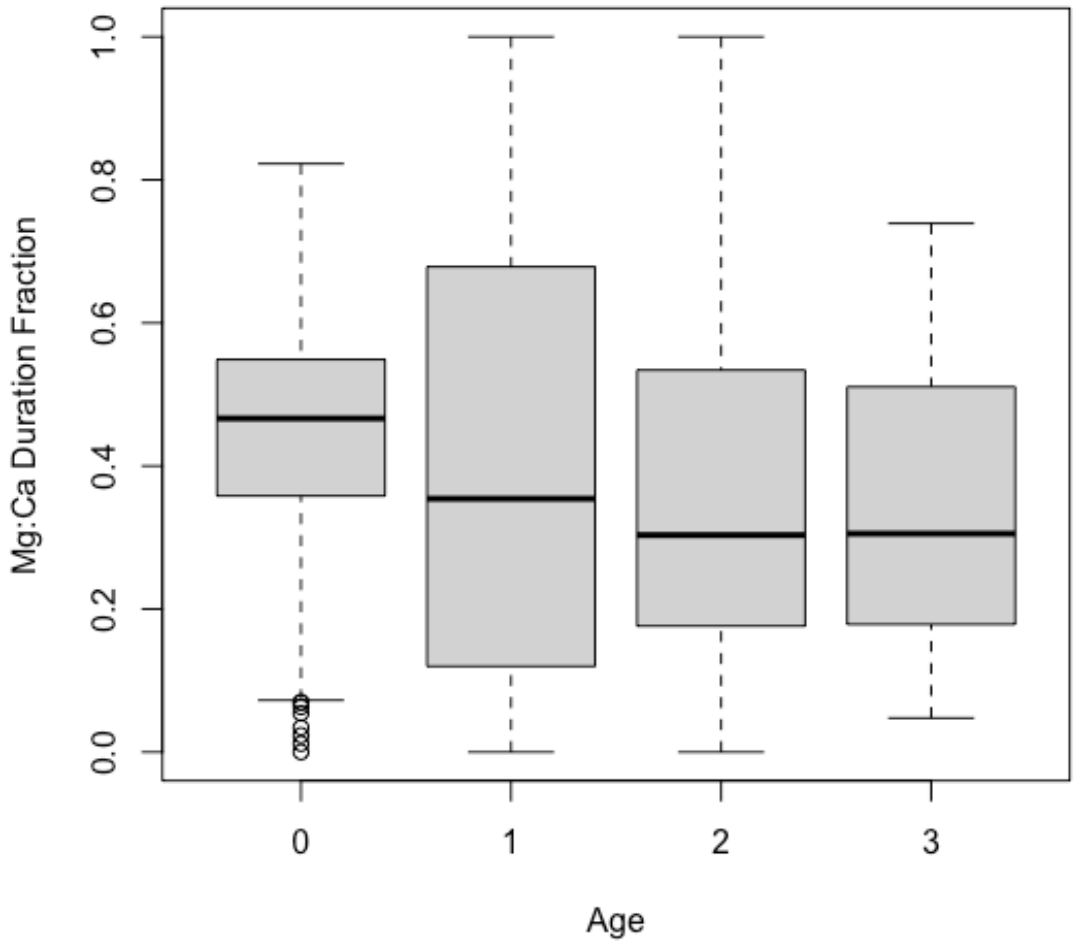

Figure 14. Mg:Ca Duration Fraction values for each analyzed age.

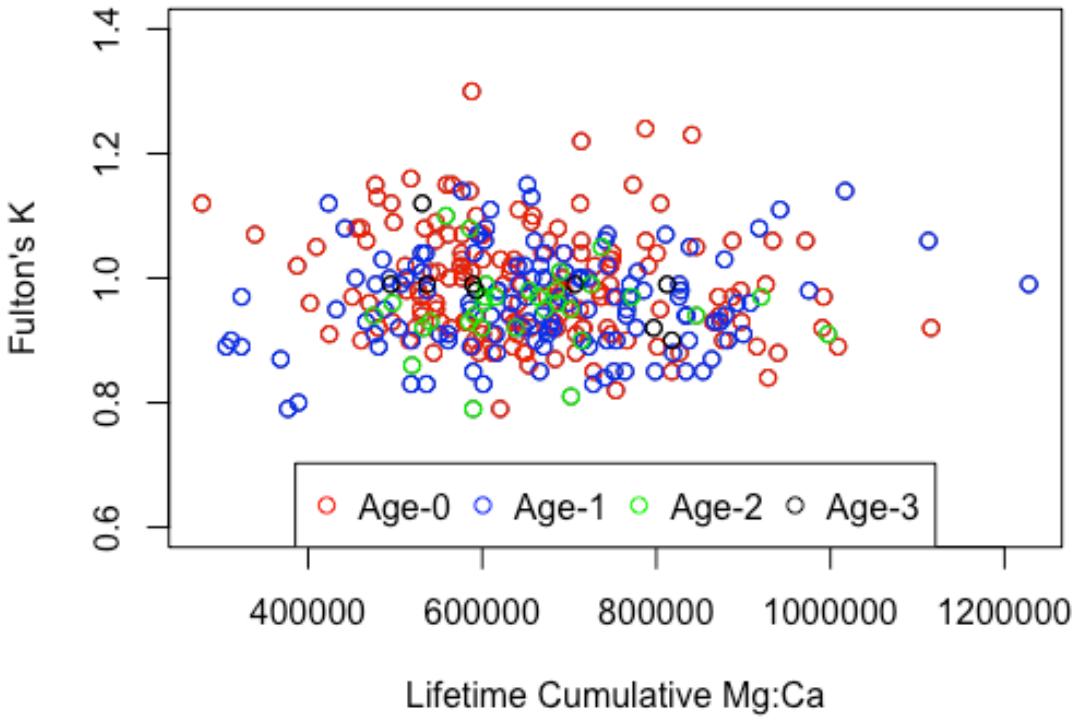

Figure 15. Values of Fulton's K versus Lifetime Cumulative Mg:Ca (µmol*µm/mol) for each age class.

Metabolism-Normalized Hypoxia Proxy (Mn:Mg)

Mn:Mg has been proposed as metabolism-normalized proxy for hypoxia exposure because while Mn uptake in the otolith is growth rate sensitive, Mg is a proxy for metabolic rate, thereby correcting for any endogenous effects on Mn uptake. When examining croaker according to their ages at collection, linear regressions between cumulative annulus width and lifetime cumulative Mn:Mg ratios found poor relationships for each age class (adjusted R^2 for Age-0= 0.009, p=0.11, adjusted R^2 for Age-1 = 0.08, p=0.0007, adjusted R^2 for Age-2 = -0.03, p=0.78, adjusted R^2 for Age-3 = 0.18, p=0.14; Figure 16). Similarly, when otolith transects were partitioned into separate annuli and corresponding mean Mg:Ca, there were no trends identified for fish regardless of their hypoxia exposure group as defined by Mn:Mg duration fractions (adjusted R^2 for all ranged from -0.49 to 0.12; p-values for all were greater than 0.001; Table 5; Figure 17).

A two-way ANOVA tested for differences in mean Mg:Ca between normalized hypoxia exposure groups and age. Only the main effects of age and nHEG were significant ($p<0.001$ and 0.0016, respectively); the interaction between age and nHEG was not significant (p=0.89). Therefore, each main effect was analyzed for differences in mean Mg:Ca values independently of each other. Age-0 had significantly greater mean Mg:Ca values than Age-1, Age-2 and Age-3 ($p<0.001$ for each comparison). There was no difference in mean Mg:Ca between Age-2 and Age-3 (p=0.77). Age-1 had the lowest values of mean Mg:Ca when compared to Age-0, Age-2, and Age-3 ($p<0.001$ for each). For nHEG, however, there was no significant difference in mean Mg:Ca between the two groups (p=0.088; Figure 18).

A two-way ANOVA tested for differences in annulus width between nHEG groups and age. Only the main effect of age was significant ($p<0.001$); nHEG and the interaction between

age and nHEG were not significant ($p=0.23$ and $p=0.31$, respectively). Therefore, nHEG had no effect on annulus width, and only differences in age were tested for significance with post-hoc comparisons. Annulus width for Age-0 was significantly greater than all other ages regardless of hypoxia exposure ($p<0.001$ for each age comparison; Figure 19).

A two-way ANOVA tested for differences in the cumulative Mn:Mg duration fractions between Fulton's K condition groups and age. The main effect of age and the interaction between condition group and age were both significant ($p<0.01$ and $p=0.019$, respectively); the main effect of condition group was not significant ($p=0.30$). Therefore, to analyze the effect these groupings have on the cumulative Mn:Mg duration fraction, differences were tested between condition groups for each age separately. For each age, there were no significant differences in condition groups for the cumulative Mn:Mg duration fraction (Age-0 $p=1.0$, Age-1 $p>0.27$, Age-2 $p>0.48$, Age-3 $p>0.15$; Figure 20).

Table 5. Adjusted R^2 and p-values for each age & nHEG grouping when mean Mg:Ca was compared to annulus width

nHEG Group	Age	Adjusted R^2	p-value
1	0	0.11	<0.001
2	0	0.12	<0.001
1	1	-0.008	0.76
2	1	-0.014	0.64
1	2	-0.06	0.97
2	2	-0.044	0.7
1	3	0.07	0.34
2	3	-0.49	0.9

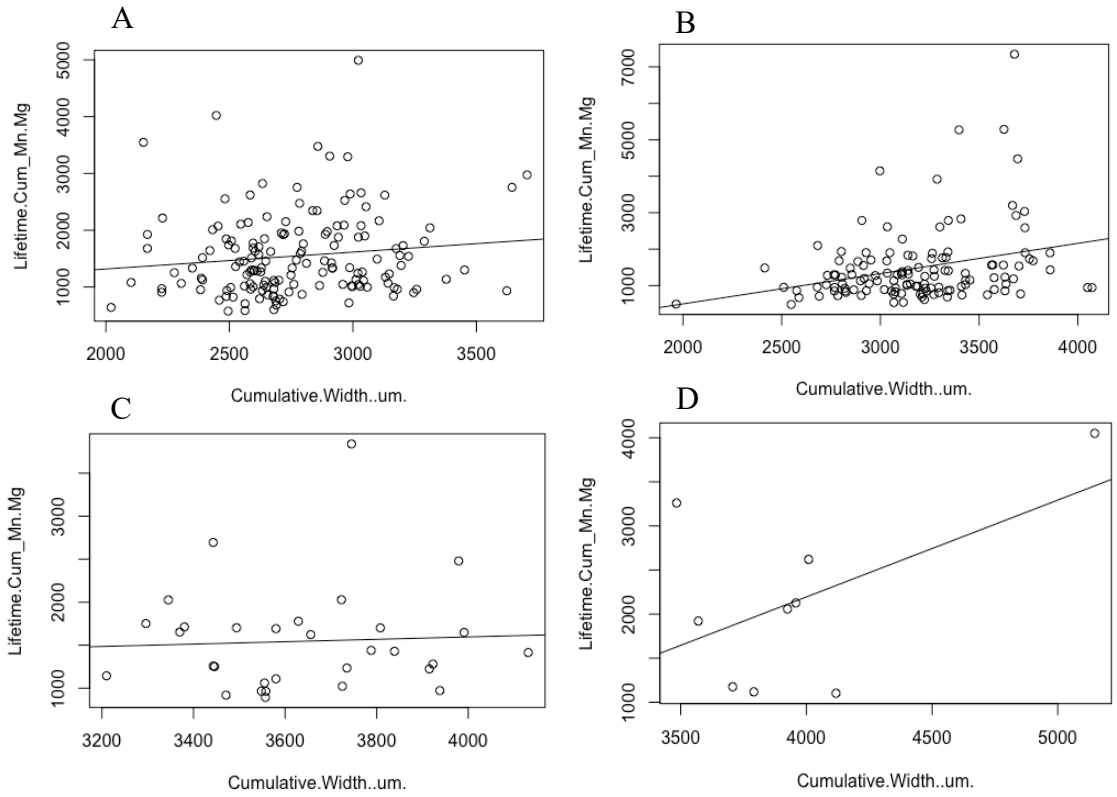

Figure 16. Lifetime cumulative Mn:Mg values (mol/mol) and cumulative annulus width (μm) for Age-0 (A), Age-1 (B), Age-2 (C), and Age-3 (D). Note the differences in axis values for each panel.

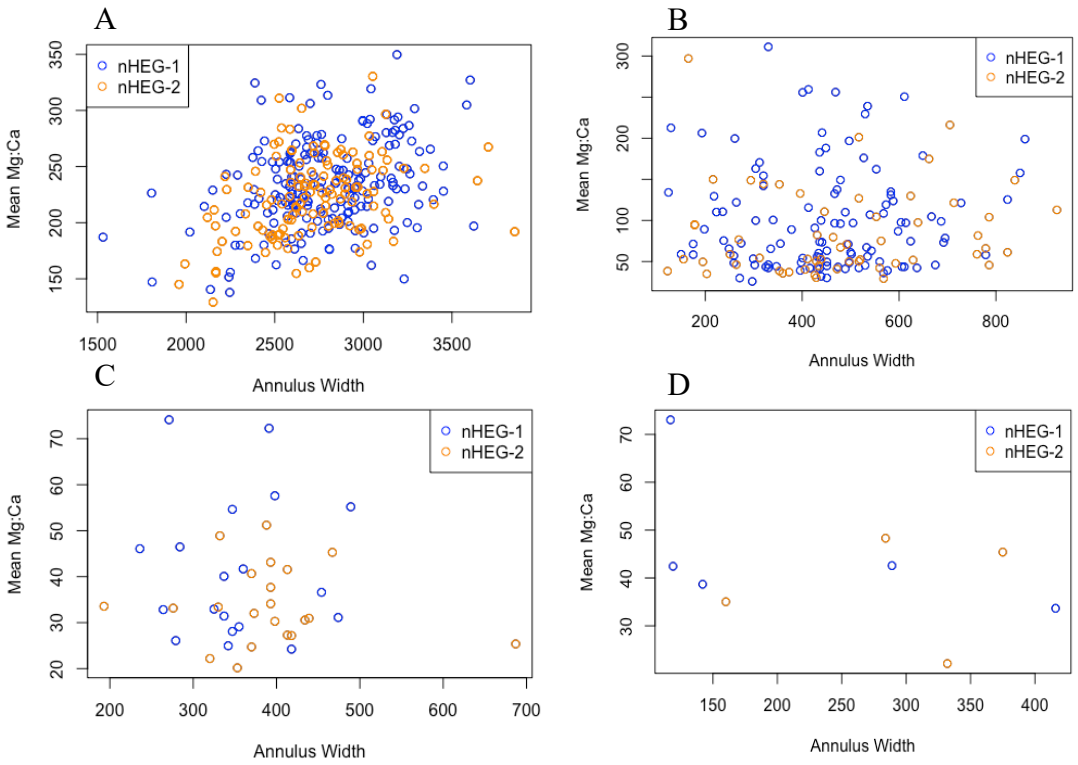

Figure 17. Mean Mg:Ca (µmol/mmol) versus annulus width (µm) for a particular year of life, grouped by nHEGs for Age-0 (A), Age-1 (B), Age-2 (C), Age-3 (D). Note that axis values differ between panels.

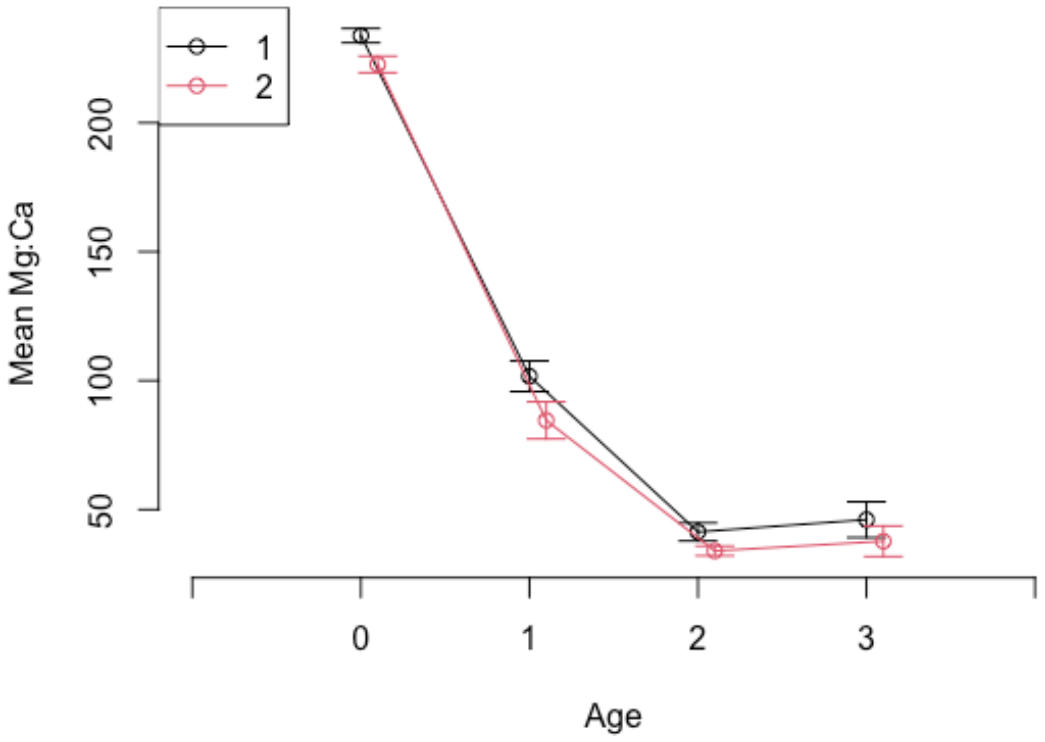

Figure 18. Mean Mg:Ca (µmol/mmol) values for each age, grouped by metabolism-normalized hypoxia exposure groups (nHEGs). Group 1 has the lowest hypoxia exposure and Group 2 has the highest hypoxia exposure.

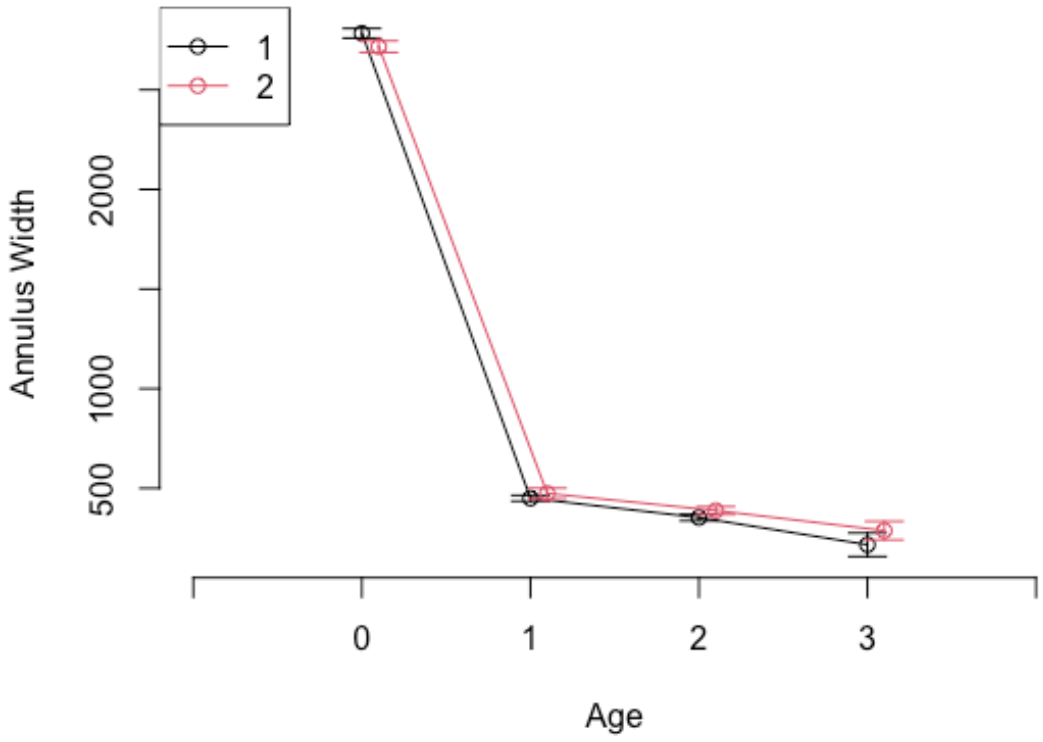

Figure 19. Annulus widths (μm) for each age, grouped by metabolism-normalized hypoxia exposure groups (nHEGs). Group 1 has the lowest hypoxia exposure and Group 2 has the highest hypoxia exposure.

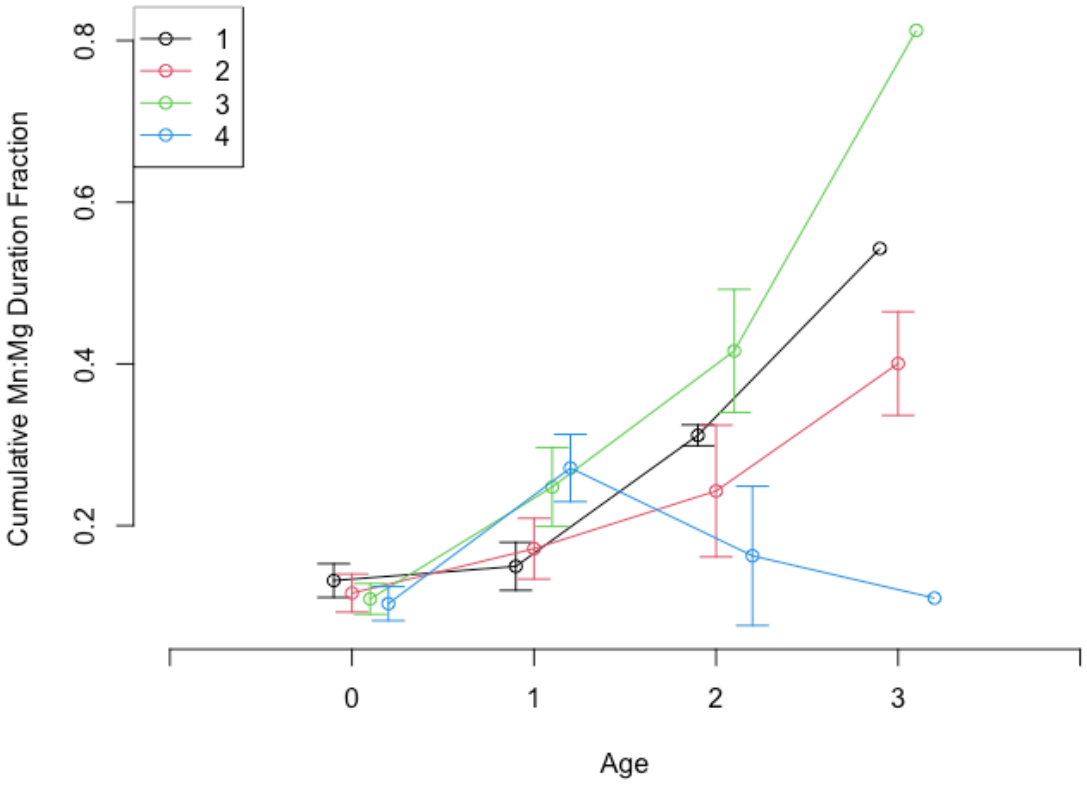

Figure 20. Cumulative Mn:Mg duration fraction values for each age, grouped by Fulton's K condition value quartiles, with Group 1 having the lowest Fulton's K values, and Group 4 having the highest Fulton's K values.

DISCUSSION

As hypoxia exposure proxies in fish otoliths, Mn:Ca and Mn:Mg have been shown to change with the hypoxic volume of Baltic Sea habitats for a given year (Limburg and Casini 2018). However, for croaker in the nGoMex neither of these duration fraction proxies varied significantly by calendar year. In other words, differences in hypoxia volume by year could not explain variation in either hypoxia exposure proxies' duration fraction. This is potentially because the actual hypoxic volume of the nGoMex during 2012 – 2015 did not vary dramatically. Between the four years encompassed by this dataset, 2012 had the smallest hypoxic volume at 7,480 sq km, followed by 2014 at 13,080 sq km, then 2013 at 15,120 sq km, and 2015 with the largest area at 16,760 sq km (LUMCON Gulf Hypoxia Program 2021). The differences in hypoxic volume at least between 2013-2015 is probably not large enough to see a statistically significant difference in exposure duration fractions when the volumes are so similar. In addition, the distribution of hypoxia in the nGoMex can be spatially and temporally patchy, and fish avoidance behavior may also decouple the actual likelihood of hypoxia exposure and concomitant growth effects from the overall measured hypoxia volume (Rabalais and Turner 2019). Thus, neither of these proxies performed well at predicting annual variable hypoxia exposure and growth impacts that each individual fish record in their otoliths. Additionally, there is also the issue of hypoxic intensity, as the intensity of the hypoxic area also directly influences the hypoxia proxies' duration fraction values. Because the hypoxic extent in the nGoMex is constantly in flux within the water column, it is difficult to say if it is the volume of hypoxia or the intensity of that zone that mainly contributes to hypoxia exposure; rather it is better to consider both as possible contributors to hypoxia exposure in fish.

Mean Mg:Ca, used here as a proxy for metabolism, was compared against two hypoxia exposure groups: Mn:Ca-defined HEG's broken up in four groups based on quartiles, and Mn:Mg-defined 'nHEG's' broken up into two groups based on the average Mn:Mg duration fraction. When Mg:Ca was compared against the four HEGs, the groups by age showed the expected trend, where the youngest individuals have the highest Mg:Ca values. A similar analysis in Baltic cod showed that fish within the highest hypoxia exposure group had the lowest Mg:Ca values across all ages (Limburg and Casini 2019). The exact opposite pattern was observed for croaker in the nGoMex, particularly for Age-1 and Age-2, where the group classified by highest hypoxia exposure had significantly greater Mg:Ca than the other groups, and the lowest hypoxia exposure group had the mean Mg:Ca values overall. This finding, while contradictory to my hypothesis, may result from the relatively high tolerance croaker have for low DO levels (Bell and Eggleston 2005; Long and Seitz 2008; Thomas and Rahman 2009a). These findings could be explained if at least some individual Atlantic Croaker withstand the low dissolved oxygen concentrations of seasonal bottom-water hypoxia to prey on benthic invertebrates that migrate closer to the sediment-water interface for higher DO levels. In turn, this enhances overall croaker consumption rates and benefits croaker growth, as seen by the higher Mg:Ca values in the highest HEGs (Long et al. 2014; Pihl et al. 1992). However, these findings did not hold true for the nHEGs, as there was no significant difference in mean Mg:Ca by group. Alternatively, slow-growing individuals may have experienced higher mortality rates and were therefore underrepresented in the samples analyzed. If hypoxia-tolerant individuals with elevated growth rates were the only ones to survive in the high hypoxia exposure group, then the counterintuitive Mg:Ca patterns I observed could have resulted. Controlled laboratory

experiments that quantify the interaction of growth and survival of hypoxia exposed croaker could help distinguish among these possible interpretations.

Similarly, mean Mg:Ca was compared against Fulton's K condition groups by age to elucidate if groups defined by a lower or higher condition showed any trend in metabolic growth by age. In this case, there was no significant difference in Fulton's K condition groups by age for either mean Mg:Ca or Mg:Ca duration fraction, meaning condition does not have an effect on Mg:Ca. This is most likely due to the fact that all croaker analyzed had high Fulton's K values, meaning that they were all of relatively good condition (see Table 1). These findings support the hypothesis that all croaker are tolerant to hypoxia, and therefore do not have large enough differences in conditional value to show significant differences in the growth proxy Mg:Ca.

For croaker, Age-0 is a year of fast growth and ontogenetic change as they exploit their environment as much as possible to grow in order to escape predators (Nye et al. 2011; Overstreet and Heard 1978). Therefore, it is no surprise that Age-0 individuals recorded high mean Mg:Ca and Mg:Ca duration fractions. But Age-0 fish also recorded the highest Mn:Ca duration fractions in their otoliths. Altenritter & Walther (2019) found that despite only a 28% increase in hypoxic volume in the nGoMex from 2014 to 2015, there was double the proportion of Age-0 fish exposed to hypoxia in 2015 than 2014, indicating that exposure is not linearly related to hypoxic volume. Therefore, these Age-0 fish, in order to survive hypoxia exposure, must initiate their hypoxia avoidance behaviors earlier than older fish, and/or make an effort to grow faster while energy expenditure can focus solely on growth, and not gonadal maturation (Altenritter and Walther 2019).

In an attempt to correct for the difference in annulus width between ages, mean Mg:Ca was normalized against annulus width, as it showed the same pattern as annulus width. The

results found that Age-1 and Age-3 had a greater mean Mg:Ca/annulus width value than Age-0 and Age-2, but there was no significant difference in Age-0 and Age-3, and Age-1 and Age-3. Notably, the variances of these annulus-normalized Mg:Ca values differed substantially among these groups such that the variance was highly compressed for Age-0 and Age-2 individuals. The reason for this age-specific variance reduction in this metabolism-normalized metric are not clear. It could be a mathematical artefact due to more individually matched variances in Mg:Ca and annulus width for a given age, particularly in Age-0 fish where annuli are by definition wider. Alternatively, the variance compression could reflect a strong relationship between annulus width and mean Mg:Ca for individual fish, particularly in the first year of life, and that means the mean Mg:Ca growth proxy is most informative during the Age-0 portion of the otolith. The sensitivity of Mg:Ca to growth as a function of age will require both future experimentation and comparisons across taxa to determine if this is a biological phenomenon or mathematical artefact.

Additionally, there were significantly fewer Age-3 individuals analyzed than any other age. When assessing Mn:Ca-defined hypoxia exposure groups for age relationships with mean Mg:Ca, Age-0 has the strongest correlation, followed by Age-1 and Age-2. More data is needed for individuals of Age-3 to determine if this trend holds. However, that is easier said than done due to the life history of croaker in the nGoMex. While croaker can reach ages older than 3-years-old, it is rarely seen because many die due to hypoxia exposure as well as shortened life histories that may have resulted from decades of bycatch and size-selective mortality (Barbieri et al. 1994; Diamond et al. 1999).

An important goal of this study was to analyze growth and hypoxia exposure histories over entire lifetimes of individual Atlantic Croaker, given that prior work only focused on the

first year of life for this species. By using known and accepted proxies for hypoxia exposure and metabolic growth, established through previous research, this study aimed at elucidating trends over entire lifetimes that could have serious implications for Atlantic Croaker population dynamics. However, the trends analyzed were not as strong as those seen in other studies using the same proxies (Limburg and Casini 2018; Limburg and Casini 2019). In those studies, the study species was Baltic cod, an economically and ecologically important demersal fish species in the North Atlantic that experiences severe, and continually increasing, hypoxia in the Baltic Sea over the past century (Limburg and Casini 2018). Besides being two separate species that reside in different water masses, there are two critically important differences between the Atlantic Croaker and Baltic Cod. First is that Baltic Cod have a much longer reported lifespan than croaker, with individuals reported to be 4, 5, or even 5+ years old. Because croaker normally caught and studied do not live this long, the dynamic range in growth and age for croaker is significantly less than for cod. As a result, the overall signal of hypoxia effects on the long-term growth responses to stress may be more difficult to identify. This is reflected in the fact that most of the significant patterns identified in this study were found within the Age-0 and Age-1 years of croaker, the first few years of life where growth rates are highest and have greater dynamic scope. The second difference is that the hypoxic areas are very different for each species, which poses unique challenges to each. Baltic cod require a specific salinity of $\geq$11 at depths of >80m for successful spawning (Limburg and Casini 2018). While there are many other factors affecting cod in the Baltic Sea, increasing hypoxia severity and area is one that inflicts a serious and direct impact to the population. While the Atlantic Croaker spawns offshore on the continental shelf, they spawn during the fall, in the months of October/November. In the nGoMex, the dead zone typically dissipates by this time, and hypoxia is no longer a significant

threat for the year. It is only during the summer months of May/June – September that hypoxia threatens croaker directly. In this case, for croaker, it is primarily a reduction of foraging habitat, while for cod, hypoxia is a threat to spawning grounds and therefore reproductive success.

Atlantic Croaker are tolerant of low levels of DO, as they are reported to survive 1.6 – 3.7 mg of DO per liter in lab and field observations (Bell and Eggleston 2005; Thomas and Rahman 2009a; Thomas and Rahman 2009b; Wannamaker and Rice 2000). This hypoxia tolerance allows croaker to survive in hypoxic waters for a period of time and take advantage of benthic invertebrates migrating to the sediment-water interface. Counterintuitively, this may result in hypoxia-exposed croaker having elevated metabolic and/or growth rates if they are allowed greater access to easily obtained benthic prey.

Another reason why croaker may show fewer signs of decreased growth in otolith microchemical signatures is that Mg:Ca may not be the best indicator of growth in Atlantic Croaker, especially if they have a smaller range of growth than other species. Mg:Ca is still a relatively new proxy, and the proposed ideas of how it becomes incorporated into the otolith lean more towards the fact that is a proxy for metabolism, rather than growth alone. While metabolism and somatic growth are linked, and on the simplest level, increased metabolism shows increased growth, the reality is that the relationship between metabolic rate and growth is much more complicated. Metabolism controls both somatic and gonadal growth, as well as digestion and homeostasis. For fish that experience hypoxia, their metabolic rate may increase to offset any detrimental effects, leaving an observable "net effect" that looks no different than a fish that was not exposed to hypoxia. One way this could work is by re-allocating energy from gonadal growth to homeostasis when exposed to hypoxia. In this way, an increased metabolic rate would be observed, not exactly increased growth. However, there is no other metabolism

metric that could be used to gather information about the metabolic rates of these fish when exposed to hypoxia, other than otolith widths.

This study found that there was no significant difference in mean Mg:Ca by age when grouped according to metabolism-normalized hypoxia exposure groups (nHEGs). This may be because croaker are resilient to hypoxia and use it to exploit their prey and gain a growth advantage. However, this may also be due to the fact that the proxy Mg is used twice in this comparison (as Mg:Ca, the growth proxy, and as Mn:Mg, correcting for growth-related effects of Mn uptake). In this case, Mg:Ca might not be the best proxy to define growth-related effects of hypoxia on Atlantic Croaker. As the field of otolith chemistry develops and identifies additional validated growth and physiological status proxies (such as Na:Ca), these alternative explanations may be able to be distinguished (Grammer et al. 2017).

Similarly, growth itself may not be the ideal response variable to quantify stress and sublethal effects of hypoxia in Atlantic Croaker. Other individual, population and ecosystem level metrics may more clearly respond to sublethal hypoxic stress. Sublethal effects that have been observed for Atlantic Croaker are habitat displacement, endocrine disruption and reproductive impairments (Craig and Crowder 2005; Thomas and Rahman 2009b; Thomas and Rahman 2012). Habitat displacement can result in density-dependent effects and rapid declines in available prey (Craig and Crowder 2005). This could lead to selective mortality and altered population sizes without necessarily affecting growth rates directly. In addition, hypoxia can cause severe disruption to reproductive pathways in fishes and croaker specifically. Not only does production of oocytes in female croaker significantly decrease during hypoxia exposure, but roughly 20% of female croaker in the nGoMex experienced gonadal masculinization after being exposed to high levels of hypoxia (Thomas and Rahman 2009a; Thomas and Rahman 2012). Yet

when sublethal carryover effects of hypoxia were examined for growth impairments of juvenile croaker, Altenritter & Walther (Altenritter and Walther 2019) found that YOY fish that were exposed to hypoxia showed no decreased somatic growth in later years compared to fish that have never experienced hypoxia. Thus, all relevant physiological processes and vital rates may not respond uniformly to sublethal hypoxia exposure, and some such as fecundity and reproductive potential may be more sensitive. Understanding the complexities of hypoxia effects on individuals, populations and communities is critical to predict future responses to this growing stressor.

Management Implications

Because Atlantic Croaker are highly abundant the Atlantic Croaker fishery is currently unmanaged. The most critical impact on croaker from the commercial fishing industry is that they are still caught as bycatch in shrimp trawls. From 2007 – 2010, roughly 342 metric tons of croaker were caught as bycatch in coastal waters around Texas and Louisiana (Scott-Denton et al. 2012). Despite populations and stocks being unmanaged, bycatch data has been analyzed, and croaker biomass is and has been predicted to fall below maximum sustainable yield (Porch 2009).

In order to remediate this situation, a Fisheries Management Plan needs to be established that protects croaker from being caught as bycatch, and effectively acts to conserve the population from its primary environmental stressors. This study finds that croaker are exposed to detrimental levels of hypoxia but that the effects on growth performance are complex and variable across individuals and ages. Additionally, previous research has established that low levels of DO can have significant detrimental effects on the reproductive viability of mature

croaker (Thomas and Rahman 2009a). Predicting future trends in stock biomass is never easy, let alone with an added environmental stressor like hypoxia. However, it is critical that conservation incorporates a nuanced understanding of the complex responses of species and systems to a stressor such as hypoxia. Models developed by Rose et al. (2018b) predict that repeated severe hypoxia events over a 100-year period will result in a 19% reduction in long-term croaker population abundance. Because there are so many environmental factors that contribute to the cause and severity of hypoxia, each individual factor, like eutrophication and displacement, have their own detrimental effect on croaker populations. Eutrophication may shift the energy flow within the food web amongst functional groups, changing the phytoplankton and fish community entirely. Displacement into inferior habitats may results in changes to typical predator-prey interactions, resulting in slower growth and higher mortality rates (Rose et al. 2018b). All of these indirect factors cause change, stress and sublethal effects to the nGoMex ecosystem, in addition to the direct effect of mortality from hypoxia. It is these indirect effects that will eventually cause the most damage. From a more realistic model by Rose et al. (2018a), the predicted long-term effect of hypoxia on Atlantic Croaker is population reduction of about 25%.

With the effects of climate change as well as increased freshwater nutrient runoff, seasonal hypoxia is predicted to worsen in magnitude and area in the years to come (Rabalais and Turner 2019). However, with climate change threatening to affect ocean chemistry in multiple ways, low oxygen conditions are expected to occur more frequently. Rising temperatures decrease oxygen solubility, increase thermal stratification of the water column, and create stronger winds which, in turn, create more upwelling, bringing more CO_2 to surface waters (Breitburg et al. 2018). Not only does oxygen have a physiological effect on fish by defining their metabolic scope, but it also plays a key role in the biogeochemical cycle as it

affects remineralization processes. With low DO, ocean waters are expected to have larger concentrations of H_2S, methane, arsenic, cadmium, zinc, copper, and many other dangerous heavy metals (Breitburg et al. 2018). All these factors will affect fish populations in the nGoMex and across the oceans. Breitburg et al. (2018) suggest that the first step in reducing hypoxic and anoxic areas is to reduce nutrient loads, as the failure to do so in past years is the primary reason that oxygen levels have not improved in coastal areas.

Analyzing how Atlantic Croaker are affected across their lifetime is the first step in establishing an effective plan to monitor and protect this ecologically important species (Diamond et al. 2013). The use of otolith microchemistry proxies provides an abundance of knowledge for exploring life history patterns, metabolic effects, and exposure histories across an individual's lifetime. Continuing investigations into how croaker are affected by hypoxia will contribute greatly to how this species is managed.

Future Directions

One important aspect of any chemical proxy investigation that is usually over-looked or forgotten is the establishment of an environmental baseline. In this study, because croaker were split by age and calendar year, each individual's analysis was done with respect to their specific age or calendar year to identify mean or median values of the chemical metrics. This was done, in part, to correct for any temporal changes in ambient elemental concentration. However, the best way to approach normalizing for shifting baselines is to collect water or sediment samples when collecting fish. That way, the same analysis can be done on the water as well and correct for any differences between years. Where historical water and sediment chemistry data are unavailable, it may be possible to reconstruct ambient elemental compositions with long-lived

and stationary organisms such as bivalves or corals when they are present. Cross-taxonomic investigations may also help evaluate community-level responses to hypoxia exposure, although careful experimentation and validation of elemental incorporation processes into the targeted taxa will be needed.

Additionally, this study compared the Mg:Ca metabolic rate proxy among individuals after they were grouped according to hypoxia exposure, measured with both uncorrected (Mn:Ca) or metabolism-normalized (Mn:Mg) metrics. For the uncorrected hypoxia exposure groupings, there were significant differences in Mg:Ca between the groups, showing that hypoxia exposure has a significant effect on metabolic rate. But there were no significant differences in Mg:Ca between the groups for the metabolism-normalized hypoxia exposure groups. This pattern may be due to the fact that croaker truly do not show significant growth impairments due to hypoxia exposure, or it may be because the use of Mg twice in the comparison (as mean Mg:Ca and as part of the nHEG, Mn:Mg), cancelled out the effect. Therefore, in future studies when using Mn:Mg as a proxy for hypoxia exposure, it may be best to use a different proxy for metabolism, like P:Ca or Na:Ca, as other elements may be good indicators of metabolic activity (Hüssy et al. 2020; Thomas et al. 2017). The use of these two new robust proxies may provide more, clearer answers about hypoxia-related growth effects on croaker in the nGoMex.

Finally, this study used otolith microchemistry metrics to compare age class and lifetime hypoxia exposure and metabolic trends. The metrics used were the same ones as those used in previous studies done by Dr. Karin Limburg and her research team on Baltic Cod. The work done in this study is part of a broader collab with these researchers in an effort to compare how Atlantic Croaker respond to hypoxia to other species in other systems around the world. In this

way, an effort was made to similar metrics in order to stay consistent for future comparison and analysis.

Summary

Investigations into overall lifetime trends of growth and hypoxia exposure is key to understanding the population dynamics of Atlantic Croaker, which in turn, provides valuable information for fisheries management. Hypoxia is arguably one of the largest stressors to the croaker population in the nGoMex, causing direct mortality and a slew of sublethal effects. Young of the year croaker showed the highest values of Mg:Ca duration fractions, as well as Mn:Ca duration fractions, indicating that a large proportion of juvenile croaker are exposed to hypoxia within the first year of life, but their growth is not directly affected by it. In later years of life, ages 1 and 2, croaker that experienced high levels of hypoxia were found to also have high levels of mean Mg:Ca, indicating that hypoxia may give croaker an advantage in foraging for benthic prey. While Mn:Mg did not show as distinct trends as Mn:Ca with Mg:Ca, it may be because of repetitive use of Mg in the chemical metrics. Understanding the interaction between hypoxia and lifetime growth trends in Atlantic Croaker will provide invaluable information for a sustainable fishery and will prepare for the anticipated environmental change and stress affecting the Gulf of Mexico in future years.

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