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Reconstructing consequences of lifetime hypoxia exposure on metabolism of demersal fish in the northern Gulf of Mexico using otolith chemistry

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Abstract Hypoxia is a worldwide natural phenomenon that is becoming increasingly more severe in the northern Gulf of Mexico (nGoMex) where it is driven by anthropogenic nutrient loading in riverine runoff. In addition to outright mortality, hypoxia can have a range of ecophysiological effects on demersal and pelagic organisms, from altering species niche widths to stifling growth rates. While hypoxia exposure is known to have many sub-lethal effects for early life history stages of demersal fishes, quantifying exposure histories and consequences for the entire lifespan of exposed individuals is critical for understanding how hypoxia impacts ecosystem functions. Lifetime hypoxia exposure patterns and potential consequences to metabolism were reconstructed using otolith chemical analyses (Mn:Ca, Mg:Ca and Mn:Mg values) in Atlantic Croaker (*Micropogonias undulatus*) collected in the northern Gulf of Mexico. Age-0 croaker were found to have the highest Mn:Ca duration fractions, indicating that a large proportion of juveniles are exposed to hypoxia within the first year of life. However, otolith chemical proxies of metabolism (Mg:Ca) did not differ by hypoxia exposure groups until Age-1 and Age-2. These patterns were unexpected and contrary to those found for comparable assessments of putative metabolic response to hypoxia in other systems, potentially due to the relative hypoxia tolerance of Atlantic Croaker that may allow increased foraging rates in low oxygen waters. Understanding the complex interactions between hypoxia and the lifetime growth and metabolism in mobile fishes is necessary to make robust predictions about the ecosystem consequences of this pervasive and growing environmental stressor in the Gulf of Mexico.

Keywords Otolith chemistry · Hypoxia exposure · Atlantic Croaker · Metabolic proxy

Introduction

Hypoxia is a worldwide phenomenon that is increasing in magnitude and severity across freshwater and marine habitats (Diaz and Rosenberg 2008). In marine coastal and shelf systems, bottom water hypoxia is enhanced by thermohaline stratification during hot

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summer months that limits water column mixing and prevents reoxygenation of bottom waters. The northern Gulf of Mexico (nGoMex) experiences natural hypoxia that is exacerbated by anthropogenic nutrient input from the Mississippi River and the Atchafalaya watershed (Rabalais et al. 2001). Monitoring that began in 1985 indicates that hypoxic zones occur annually and often exceed 5000 km^2 (LUMCON Gulf Hypoxia Program 2021). Under severe conditions when dissolved oxygen concentrations drop to lethal concentrations for many organisms (Breitburg et al. 2018), the resulting "Dead Zone" may be spatially extensive and result in die-off events (Rabalais and Turner 2019). However, a more likely scenario is that hypoxia causes sublethal effects on demersal marine organisms (Thomas et al. 2007; Ludsin et al. 2009; Long et al. 2014; Steube et al. 2021).

A variety of sublethal ecophysiological effects in demersal fishes can result from hypoxia exposure due to individualistic, phenotypic plasticity in physiological and behavioral responses to oxygen stress (Stramma et al. 2008, 2012; Blewett et al. 2022). Altered metabolic and respiratory rates may redirect energy away from growth, leading to lower size-at-age or impaired condition factors (Mohan et al. 2014; Limburg and Casini 2018). Demersal fish such as Atlantic Croaker *Micropogonias unduatus* also experience significant gonadal dysfunction and endocrine pathway disruption due to continued exposure to hypoxia (Thomas and Rahman 2009b; Thomas et al. 2007). Collectively, growth and reproduction impairments may lead to declining populations of demersal fishes that experience hypoxia over multiple generations (Rose et al. 2018a, b).

Sublethal impacts of hypoxia may also arise due to behavioral avoidance of hypoxic waters and altered ecosystem interactions (Ludsin et al. 2009). For instance, low fish biomass can often be found in hypoxic waters, whereas increased biomass is found at the edges of hypoxic waters where laterally or vertically displaced fish aggregate (Zhang et al. 2009). Displaced fish may experience increased physiological and metabolic stress due to conspecific crowding, increased predation and decreased resource availability when forced to avoid their preferred habitats (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). Food web topologies can therefore be reorganized when hypoxia displaces consumers and forces them to consume alternate prey items, although some predators may tolerate hypoxia and remain feeding on stressed prey items (Mohan and Walther 2016). Recently, the spatial response of Atlantic Croaker to hypoxia was found to be individually variable and subsequent displacement may lead to an overall expansion of trophic niches at the population level, a phenomenon termed the 'Distributive Stress Model' (Steube et al. 2021). Quantifying these complicated impacts of hypoxia requires reliable reconstructions of lifetime hypoxia exposure patterns at the individual level.

Many benthic and demersal fishes have demonstrated individual variability in their physiological responses to hypoxic stress. In an experiment to test the hypoxia-induced metabolic stress of a member of the Sciaenidae family, *Leiostomus xanthurus*, or Spot, researchers found that while there was a significant increase in enzyme indicators for anaerobic metabolism, there was substantial interindividual variation between treatments, perhaps explained by prior exposure to hypoxia (Cooper et al. 2002). Experiments testing the effects of hypoxia exposure on other benthic and demersal species have found similar results. Pichavant et al. (2000) found that, for Turbot *Scophthalmus maximus*, feed conversion ratios were not significantly affected by oxygen concentrations, and that Turbot have some capacity to regulate their energic demands during exposure to relatively low oxygen concentrations. An experiment done to test the metabolic capacity of Atlantic Cod *Gadus morhua* exposed to acute and chronic hypoxia found that hypoxia-acclimated cod were able to consume more oxygen for a given cardiac output (Petersen and Gamperl 2009). These studies showed that while hypoxia can have negative physiological consequences, some individuals within a species may have counterintuitively positive responses to hypoxia due to complex physiological responses and acclimation histories (Gallo et al. 2019). The collective impact of complex and variable responses to hypoxia can lead to an apparent paradox where hypoxic events lead to high levels of fisheries production in some systems (Breitburg et al. 2018).

In order to quantify sublethal impacts of hypoxia on individual fish, internal records of lifetime exposure histories of individual fish are required. Otolith microchemistry is an important tool that can be used to understand how hypoxia exposure affects individual fish both physiologically and ecologically. Otoliths are fish "ear stones" composed primarily of incrementally accreted calcium carbonate layers and can be used to estimate age and growth as well as analyzed for lifetime variations in chemical compositions driven by endogenous and exogenous factors (Elsdon et al. 2008; Walther 2019). The chemical composition of water masses in which fish reside can be unique, as abiotic factors like temperature, salinity, and dissolved oxygen alter the dissolved concentrations of certain elements as well as the rates of uptake and incorporation into otoliths of fishes (Bath et al. 2000; Grønkjær 2016; Thorrold et al. 1997). Manganese (Mn:Ca) has proven to be a useful elemental tracer for hypoxia exposure, as dissolved Mn concentrations increase in the water column due to hypoxia-induced redox reactions (Limburg et al. 2011). Other elements in otoliths may be strongly impacted by endogenous factors such as growth and metabolism. Magnesium (Mg:Ca) has been found to be a promising proxy for metabolic rates and growth rates, as Mg appears to largely reflect metabolic activity (Limburg et al. 2018). Even though Mn is an established proxy for hypoxia exposure, hypothesizing its effect on growth can be complicated as Mn incorporation into otoliths may also be sensitive to growth. In order to correct for this, Mn can be ratioed with Mg (Mn:Mg), as Mg uptake is sensitive to metabolism, but is relatively independent of hypoxia and other environmental factors (Limburg and Casini 2018). This way hypoxia exposure and duration can be estimated from otolith chemical records independently from any possible changes in metabolism due to stress. However, the necessity of ratioing Mn to Mg has not yet been assessed in many species, including Atlantic Croaker.

In the nGoMex, prior otolith microchemical approaches to understanding hypoxia exposure patterns and impacts have used Atlantic Croaker as a model species (Mohan et al. 2014; Mohan and Walther 2016; Altenritter et al. 2018; Altenritter and Walther 2019). This species was chosen given the extent of knowledge about hypoxia impacts on its reproductive dynamics and subsequent population modelling efforts (e.g., Thomas and Rahman 2012; Rose et al. 2018a), as well as its abundance and widespread distribution across the typical hypoxic zone in the nGoMex region. Two prior otolith chemistry studies in particular laid the groundwork for the current study. First, Altenritter et al. (2018) used nGoMex Atlantic 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. In addition, Altenritter and Walther (2019) tracked carryover effects of hypoxia exposure during the early years of life on survival and growth. They found no evidence that traits of survivors changed from 1 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 Atlantic 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 this species. However, these prior studies only focused on the first year of life of Atlantic Croakers due to the highest spatial resolution of chemical proxies in the early portions of their otoliths.

While prior studies on Atlantic Croaker otolith chemistry provided substantial insight into exposure dynamics and carry-over consequences for critical juvenile periods, a full analysis of lifetime hypoxia exposure patterns has not yet been conducted. In addition, lifetime patterns of Mg:Ca in nGoMex Atlantic Croaker have not yet been investigated. Otolith Mg:Ca has been used to assess lifetime metabolic responses in Baltic Cod (*Gadus morhua*) exposed to hypoxia (Limburg and Casini 2018, 2019). Similarly, a new proxy for hypoxia exposure which corrects for the metabolic influence of Mn uptake in the otolith, Mn:Mg, has been shown to reflect long-term hypoxia status in Baltic Cod (Limburg and Casini 2019). Because of this, comparable evaluations in nGoMex Atlantic Croaker are needed to determine whether putative metabolic influences on stress responses and manganese uptake into the otolith are consistent across systems and species.

This study analyzed the complete lifetime otolith chemical transects of Atlantic Croaker in the nGoMex collected across multiple years. The primary goal of the study was to assess lifetime patterns of hypoxia exposure and potential metabolic responses that have not yet been quantified in this species. We did so by incorporating an established proxy for hypoxia exposure, Mn:Ca, as well as an emerging potential proxy for metabolism, Mg:Ca, to better understand the metabolically corrected responses of fish to hypoxia. Our objectives for this project were to (1) quantify lifetime trends in chemical proxies of hypoxia (Mn:Ca) and putative metabolism (Mg:Ca) for Atlantic Croaker in the nGoMex, (2) explore a new proxy for hypoxia exposure, Mn:Mg, and (3) examine relationships between hypoxia exposure and Mg:Ca, which may indicate metabolism. Collectively, we sought to better understand the sublethal effects of hypoxia over entire lifetimes of demersal fish in the northern Gulf of Mexico.

Methods

Atlantic Croaker otoliths were collected from the nGoMex over two years and otolith chemical compositions were quantified from a total of 323 individuals. Prior studies by Altenritter et al. (2018) and Altenritter and Walther (2019) only reported on Mn:Ca and Ba:Ca compositions of the first year of life from these otoliths. However, complete lifetime chemical transects for Mn:Ca and additional elements such as Mg:Ca were also quantified but have not been statistically analyzed beyond the compositions of the first years of life. Therefore, the current study revisited the complete life history transects compiled from these previously analyzed samples in order to assess lifetime patterns of Mn:Ca and Mg:Ca compositions. Additionally, these previous studies identified individuals with otolith transects that contained high concentrations of both Mn:Ca and Ba:Ca, indicating they were potentially estuarine residents simultaneous with potential hypoxia exposure. These estuarine-type fish were excluded from the present study, and the subsequent analyses here only include fish with low Ba:Ca concentrations and thus focused on individuals primarily residing in coastal and shelf habitats.

Fully detailed methods of specimen collections and otolith preparation and chemical analyses are described by Altenritter et al. (2018). Collections took place around and within the hypoxic zone of the continental shelf offshore of Louisiana and Texas. Collections occurred between October 23rd and November 5th of 2014 and 2015 to allow for possible records of summer hypoxia exposure to be recorded in otoliths. In 2014, the extent of bottom water hypoxia covered an area of 13,080 km^2 (5052 mi^2), which increased to 16,760 km^2 (6474 mi^2) in 2015. These values fell 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 Croaker were collected by the National Oceanic and Atmospheric Administration's (NOAA) Southeast Monitoring and Assessment Program's (SEAMAP) Fall Groundfish Surveys aboard the RV *Oregon II*. Sampling stations were chosen via a stratified random sampling design. Twenty-two stations were sampled in 2014 and twenty-one were sampled in 2015 (for a total of 43 stations) using a 12.8 m beam benthic shrimp trawl with 41-mm stretch mesh deployed for 30 min (GSMFC 2001). When present, between 10 and 50 Atlantic Croaker were collected at each station and stored frozen at -20 °C. A total of 463 Atlantic Croaker were collected during the 2014 cruise, and 962 in 2015. A subset of these fish were used for aging and chemical analyses, as detailed below.

Collected and frozen fish were thawed, patted dry, and measured for total length (± 0.5 mm), and weighed (± 0.5 g). The sagittal otoliths were then removed, rinsed in DI water, and dried. The left otolith was thick-sectioned (1–2 mm; 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-µm and 3-µm 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 (Barger 1985), it was assumed that one pair of opaque and translucent bands was indicative of 1 year of growth. Atlantic Croaker were assumed to have hatched during October and November of the year before their respective year of capture (Hernandez et al. 2010; Thomas et al. 2015). Therefore, an Age-0 fish was nearly 1 year old at capture but had not yet accreted a winter opaque band. The fish used for subsequent analyses were between Age-0 and Age-3, and all fish were captured in either 2014 or 2015 (Table 1). In order to analyze for distinct age-year trends, fish otolith elemental transect data were partitioned corresponding with 10 unique groups according to their age during a given calendar year (Table 2): Age-0 data from 2012, 2013, 2014, and 2015; Age-1 data from 2013, 2014, and

Table 1 Sample sizes, total lengths, and masses for Atlantic Croaker used for otolith chemical analyses categorized by age and year of capture. Values are means with standard deviations in parentheses

Collection year	Age	Sample size	Total length (mm)	Mass (g)
2014	0	70	151 (11.1)	36.2 (9.7)
2014	1	49	172 (16)	52.9 (18.4)
2014	2	1	200 (n/a)	84.4 (n/a)
2015	0	85	156 (10)	36.6 (8.3)
2015	1	79	173 (17.3)	49.8 (17.4)
2015	2	30	169 (13)	46.5 (12.6)
2015	3	9	189 (16)	68.2 (17.7)

Table 2 Sample sizes of otolith chemistry data categories for Atlantic Croaker

Age	Calendar year	Total
0	2012	13
	2013	80
	2014	152
	2015	85
1	2013	13
	2014	80
	2015	82
2	2014	13
	2015	30
3	2015	9

2015; Age-2 data from 2014 and 2015, and Age-3 data from 2015. For example, the oldest fish analyzed were Age-3 fish, all of which were caught in 2015. This means their otolith elemental transects were partitioned using annuli and analyzed distinctly as Age-0 data from 2012, Age-1 data from 2013, Age-2 data from 2014, and Age-3 data from 2015.

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, USA. 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. 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). Otoliths were ablated over two separate analytical sessions of 17- and 15-day duration, respectively. Mean laser power was 5.73 J/cm^2 (SD = 1.70) and 3.31 J/cm^2 (SD = 1.37) for the two sessions. 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. Mean relative standard deviations of repeated measurements of NIST-612 as a metric of analytical precision across all sampling days were 2.96% for Mn and 6.07% for Mg. Percent recoveries calculated based on NIST-612 measurements compared to certified reference values as a metric of analytical accuracy were 102% for Mn and 91% for Mg. Limits of detection (LOD) were on average 0.40 ppm for Mn and 2.01 ppm for Mg. Measurements for each analyte were consistently above the LODs, and no data had to be discarded due to sub-LOD values.

The ablated otoliths were inspected under microscopes and laser transect lengths were 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). Age-0 distances were thus measured from the primordium to the leading edge of the first opaque zone. Because opaque zones are assumed to form in approximately November for this species (Barger 1985), and fish were collected in October and early November, the Age-0 portion of otoliths did not include material from the first opaque band. Afterwards, annuli distances were measured from the beginning of an opaque zone and including the subsequent translucent zone. Annuli distances along the transect axes 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 partitioned 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 yearly elemental ratios, otolith microchemical transects were first partitioned by annual otolith growth zones, or ages by year (i.e., one of the 10 unique age classes mentioned above). Then, age-specific metrics of chemical compositions were calculated following prior work assessing hypoxia exposure and metabolic responses by Limburg and Casini (2018; 2019). The metrics used for each elemental ratio (Mn:Ca, Mg:Ca, Mn:Mg) are as follows, using Mg:Ca as an example: mean Mg:Ca (average Mg:Ca in an annulus) and Mg:Ca duration fraction (the number of points within an annulus that exceed the median Mg:Ca for that specific age and cohort year divided by annulus width). These metrics were then calculated separately for Mn:Ca and Mn:Mg ratios. All Mg:Ca and Mn:Ca values were reported in units of μmol/mmol, and Mn:Mg values were reported in units of mol/mol.

To calculate hypoxia exposure groups (HEGs) 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). Median Mn:Ca for each specific age class per year were calculated and used to define Mn:Ca duration "thresholds" for each age and cohort group. These thresholds were used to calculate the duration fractions for each year of life as defined above. Then, Mn:Ca duration fractions were used to group fish into quartiles (Mn:Ca duration fraction values in categories of less than 25%, 25–49.9%, 50–74.9%, greater than 75%). These groups were designated as hypoxia exposure groups, where Low represents the group that was the least exposed, Low-Medium represents the next highest exposed group, Medium–High represents the second highest exposed group, and High represents the group that was the most exposed.

The residuals of the data were inspected for normality and tested as appropriate with methods fit for continuous data. One-way and two-way ANOVAs were used to test for significant effects of age and group on the multiple proxy values. Post-hoc pairwise comparisons between groups followed, using a Shaffer correction given that there were always fewer than six groups and the sample sizes were unbalanced among groups. Full ANOVA tables are provided in the Supplementary File 1.

Results

A total of 10 unique categories of otolith chemistry data were identified depending on the age of the fish during each calendar year of life: Age-0 data from 2012, 2013, 2014, and 2015; Age-1 data from 2013, 2014, and 2015; Age-2 data from 2014 and 2015; and Age-3 data from 2015. Sample sizes for these categories as well as their lengths and masses are reported in Table 2.

Duration fractions based on either Mn:Ca or Mn:Mg were compared among years to determine if interannual variation in either metric was significant. There were no significant differences among calendar years for either duration fraction (Mn:Ca $p = 0.82$, Mn:Mg $p = 0.99$; Fig. 1). Because the patterns of Mn:Ca and Mn:Mg variation were similar across time, we decided to continue our analyses of tracking lifetime hypoxia exposure trends with the uncorrected hypoxia exposure proxy Mn:Ca, as normalizing to Mg did not appear to significantly influence the Mn-based hypoxia proxy in this dataset.

There were significant differences between mean Mn:Ca values across ages ($p < 0.001$). Age-0 was significantly greater than Age-1, Age-2, and Age-3 ($p < 0.001$ for each pairwise comparison). Values of Mn:Ca were not significantly different between other age groups ($p > 0.21$ for each pairwise comparison; Fig. 2). Values of Mg:Ca were significantly different between age groups ($p < 0.001$), and pairwise comparisons found that Age-0 fish had a significantly higher mean Mg:Ca than Age-1, Age-2, and Age-3 ($p < 0.001$ for each comparison; Fig. 2). Similarly, values of Mn:Mg were significantly different between age groups ($p < 0.001$), and pairwise comparisons found that Age-0 fish had a significantly higher mean Mn:Mg than Age-1, Age-2, and Age-3 ($p < 0.001$ for each comparison). Thus, Mn:Ca, Mg:Ca, and Mn:Mg absolute concentrations declined asymptotically with age, as expected given nonlinear declines in growth, metabolic, and otolith accretion rates with age.

A two-way ANOVA was used to test for the relationship between mean Mg:Ca and age among hypoxia exposure groups (Fig. 3). The main effect of age, the main effect of hypoxia exposure grouping, 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 hypoxia exposure groups for each

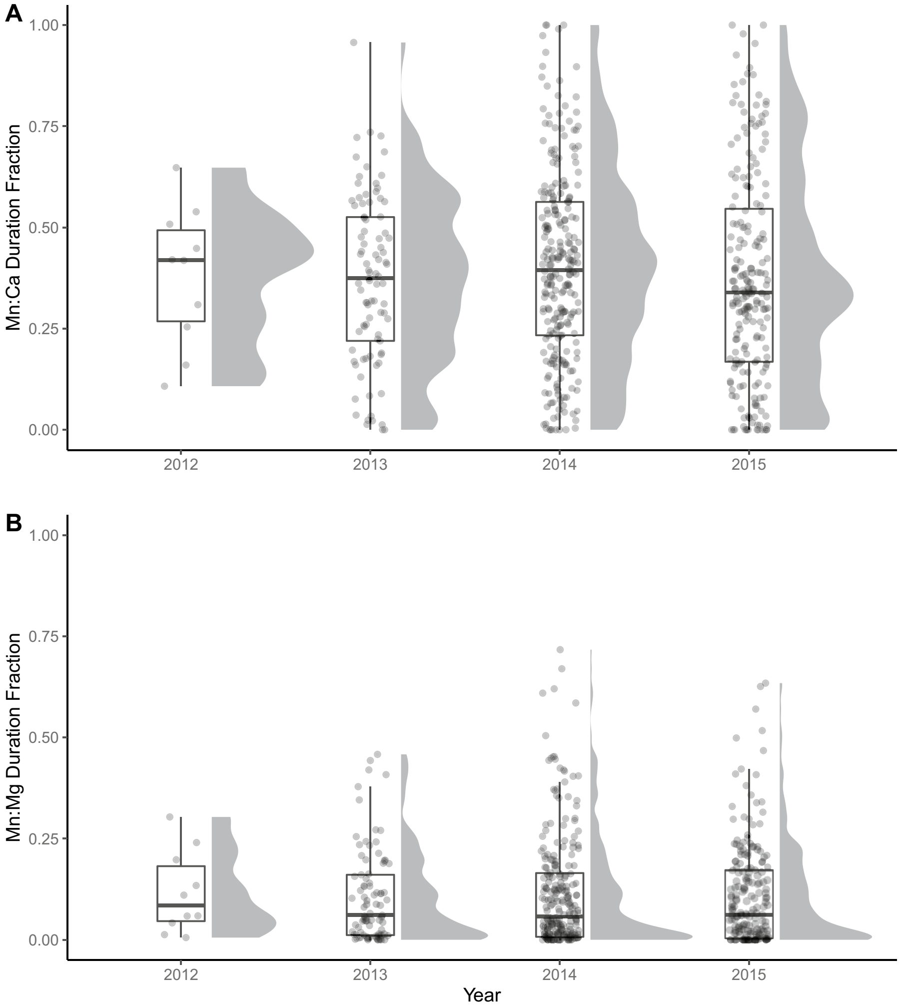

Fig. 1 Duration fractions of otolith **A** Mn:Ca and **B** Mn:Mg ratios for each calendar year of life for individual Atlantic Croaker. Boxplots depicting minimums, maximums, medians, and interquartile ranges are overlain for each age group. Shaded areas adjacent to each calendar year grouping depict density distributions of duration fraction values within calendar year groups

age separately using post-hoc pairwise comparisons between groups. The Shaffer correction was used, as there were less than six groups and the sample sizes within groups were unbalanced. For Age-0, there was no significant difference in mean Mg:Ca between hypoxia exposure groups ($p = 0.0664$). For Age-1, mean Mg:Ca values were significantly different among HEGs ($p = 0.027$). The highest mean Mg:Ca value was observed in the High hypoxia exposure group, while decreasing mean values of Mg:Ca were observed in Medium–High, then Low-Medium, and finally Low, the group with the lowest hypoxia exposure, which contained the lowest mean Mg:Ca value. For Age-2 fish, the Low hypoxia exposure group had significantly lower mean Mg:Ca values than the Low-Medium ($p = 0.042$), the Medium–High ($p = 0.011$), and the High ($p < 0.001$) hypoxia exposure groups. Values of Mg:Ca in Age-2 Low-Medium and Medium–High hypoxia exposure groups were not significantly different from one another ($p = 0.16$). Lastly, Age-2 High hypoxia exposure groups had the highest mean Mg:Ca values compared to Age-2 Low ($p < 0.001$), Low-Medium ($p < 0.001$), and Medium–High ($p = 0.0019$) hypoxia exposure groups. For Age-3, there was no significant difference in Mg:Ca values among hypoxia exposure groups ($p = 0.47$).

Discussion

Otolith chemical proxies of exogenous and endogenous dynamics have the potential to reveal the complex impacts of a temporally variable and spatially heterogeneous environmental stressor such as

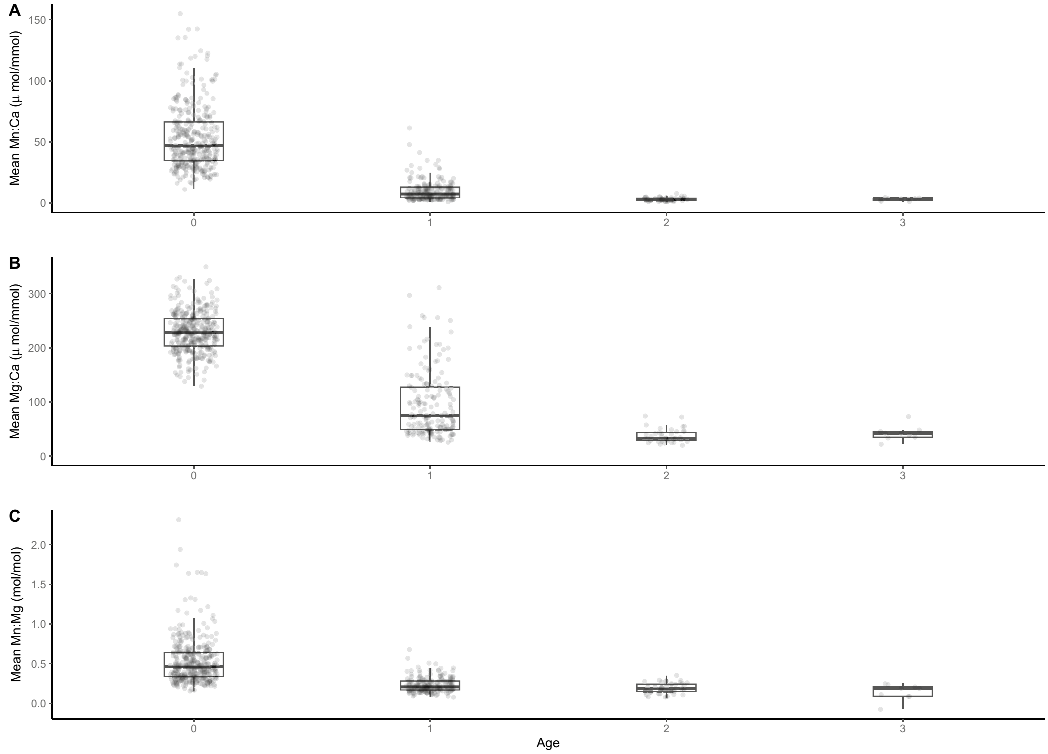

Fig. 2 Mean values of otolith **A** Mn:Ca in µmol/mmol, **B** Mg:Ca in µmol/mmol, and **C** Mn:Mg in mol/mol, by age for individual Atlantic Croaker. Boxplots depicting minimums, maximums, medians, and interquartile ranges are overlain for each age group

hypoxia. Prior assessments of hypoxia exposure and performance consequences of wild-caught Atlantic Croaker in the nGoMex have been restricted to either the first year of life (Altenritter et al. 2018; Altenritter and Walther 2019) or a few months just prior to capture (Mohan and Walther 2016; Steube et al. 2021). However, lifetime patterns of otolith Mn:Ca and estimated hypoxia exposure have not yet been reported for this species, although comparable analyses have revealed the importance of interannual exposure and performance consequences in other species such as Baltic Cod (Limburg and Casini 2018). In addition, otolith Mg:Ca has recently been identified as a potential proxy for metabolic rates (Limburg et al. 2018), and otolith Mn:Mg has also recently been identified as a useful proxy for hypoxia exposure in some species (Limburg and Casini 2019). In the current study, the first lifetime analyses of otolith Mn:Ca, Mn:Mg, and Mg:Ca in Atlantic Croaker from the nGoMex revealed complex variation in these proxies of hypoxia exposure and metabolism across multiple calendar years and age classes.

There was no clear pattern in otolith Mn:Ca duration fractions by calendar year. For the four calendar years 2012 to 2015 covered by the otolith analyses reported here, hypoxia volume ranged from 7480 km^2 in 2012, to 16,760 km^2 in 2015 (LUMCON Gulf Hypoxia Program 2021). If the overall magnitude of summer hypoxia extent in the nGoMex was the primary factor determining fish hypoxia exposure in a given year, otolith Mn:Ca duration fractions would be expected to be highest in 2015 and lowest in 2012. It should be noted that limited data was available for 2012, reducing the ability to fully assess relationships between hypoxic extent and hypoxia exposure proxies in otoliths in all years. Nonetheless, these duration fractions were consistent across each calendar year, suggesting the total spatial extent of hypoxia and the likelihood of hypoxia exposure for individual fish were decoupled. Two likely factors could explain this observation. First, the distribution of hypoxia in the nGoMex is spatially and temporally patchy, meaning overall hypoxic volume recorded during the height of the summer season does not guarantee uniform

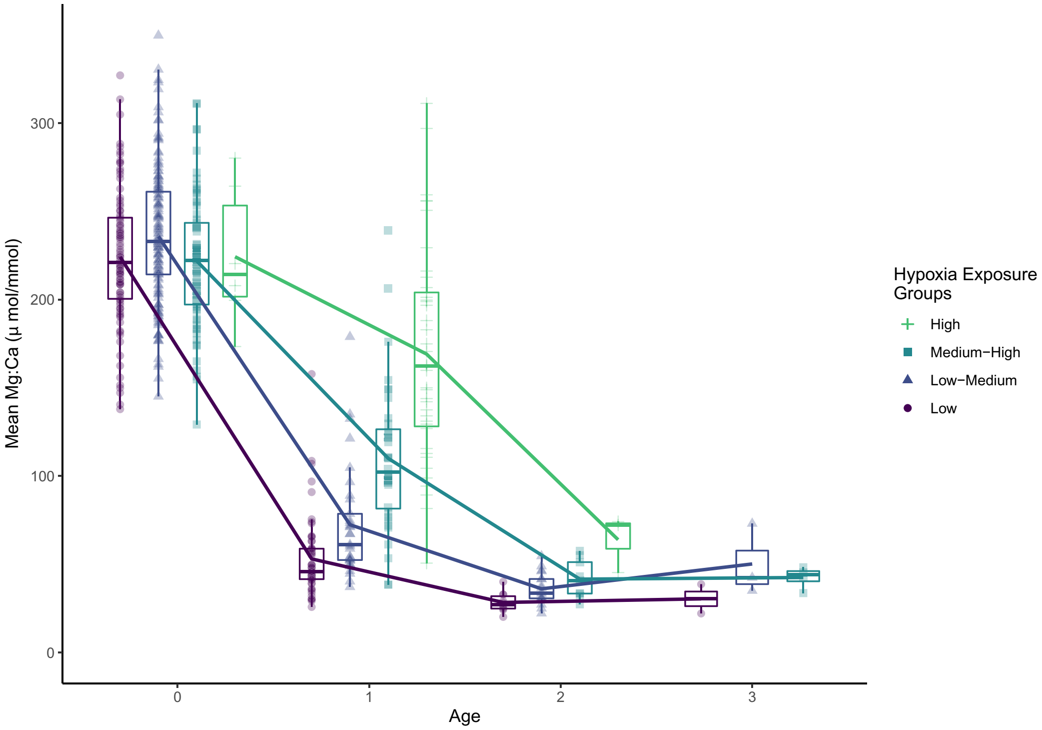

Fig. 3 Values of mean otolith Mg:Ca (μmol/mmol) by age class separated by hypoxia exposure group (HEG) as defined by quartiles of Mn:Ca values. HEG-Low has the lowest Mn:Ca values and therefore lowest estimated hypoxia exposure, while HEG-High has the highest Mn:Ca and highest estimated hypoxia exposure. Boxplots depicting means, 25th and 75th percentiles are overlain for each age group. Lines connecting groups are to assist visual interpretation of group trajectories only

hypoxic coverage nor uniform exposure likelihood at the scale of individual fish (Rabalais and Turner 2019). In addition, fish including Atlantic Croaker are known to engage in hypoxia avoidance behavior both laterally and vertically, meaning individuals may escape hypoxic waters and reduce exposure durations even during periods of high hypoxia extent (Craig and Bosman 2013; Eby et al. 2005). Second, individual Atlantic Croaker may make foraging forays into benthic hypoxic waters to continue foraging on hypoxic stressed prey, thereby elevating exposure magnitudes even in years with smaller hypoxia coverage (Mohan and Walther 2016). As a result, hypoxia exposure at the individual scale appears to be proportionally consistent across calendar years despite substantial fluctuations in the volume and areal extent of hypoxic waters at the shelf-wide scale.

Because hypoxia can influence growth and metabolism, other otolith chemistry investigations have used Mn:Mg ratios (e.g., Limburg and Casini 2018) to normalize the hypoxia exposure proxy (Mn) by a metric of estimated metabolism (Mg). We found that using Mn:Mg ratios in Atlantic Croaker did not significantly alter interpretations of hypoxia exposure inferred from Mn:Ca alone. Both Mn:Ca and Mg:Ca declined exponentially with age, meaning the ratio of the two would not provide substantial insight into differential trends across age classes. In addition, Mn:Mg duration fractions were consistent across calendar years much the same as Mn:Ca duration fractions, further suggesting that the Mn:Mg ratio was not substantially more informative than Mn:Ca alone. A prior experiment that evaluated the impacts of hypoxia stress on Atlantic Croaker otolith chemistry found that fish exposed to 4 weeks of constant hypoxia had no significant differences in their otolith Mg:Ca values (Mohan et al. 2014). Although fish exposed to 10 weeks of fluctuating hypoxia experienced a statistically significant decline in Mg:Ca values, the absolute magnitude of the decrease was minimal (Mohan et al. 2014). Notably, this experiment was conducted without manipulating water chemistry itself, so those experiments assessed endogenous stress response of otolith chemical proxies to hypoxia exposure alone. The lack of substantial responses of otolith Mg:Ca to hypoxia stress in those experiments in addition to the results presented here collectively suggest that the Mn:Mg ratio may not be necessary to assess hypoxia exposure in all species, particularly for Atlantic Croaker in the nGoMex. As a result, hypoxia exposure in this study was assessed using Mn:Ca values and duration fractions.

A putative metabolic proxy based on Mg:Ca in otoliths for inferring metabolic rates in fish was compared across lifetimes against hypoxia exposure groups (HEGs) to understand how repeated hypoxia exposure affects metabolism. When Mg:Ca was compared against the four HEGs, the groups by age showed an 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 Atlantic Croaker in the nGoMex, particularly for Age-1 and Age-2 individuals where the highest hypoxia exposure group had significantly greater mean Mg:Ca than the other groups, and the lowest hypoxia exposure group had the lowest mean Mg:Ca values overall. Thus, the divergence in Mg:Ca values is opposite of that observed for other hypoxia-stressed fishes.

The unexpected pattern of otolith Mg:Ca among hypoxia exposure results may be due to a variety of non-mutually exclusive mechanistic explanations. First, Atlantic Croaker are known to be relatively tolerant to low dissolved oxygen levels that other, more sensitive organisms avoid (Bell and Eggleston 2005; Long and Seitz 2008; Thomas and Rahman 2009a). Accordingly, Atlantic Croaker are known to aggregate on the edges of hypoxic zones, both laterally and vertically (Craig and Crowder 2005; Eby et al. 2005). Hypoxia avoidance may also influence metabolic rates. In a recent assessment of avoidance behavior of closely related Spot *Leiostomus xanthurus*, Craig et al. (2023) found that fish exposed to the highest magnitudes of hypoxia exhibited significantly increased swimming speeds in order to avoid hypoxic stress. This increased activity rate could elevate metabolism and potentially explain the counterintuitive result of increased Mg:Ca ratios, as a metabolic proxy, in the most hypoxic exposed Atlantic Croaker found in this study. Atlantic Croaker may also make occasional or frequent foraging forays into hypoxic zones to access preferred benthic prey (Mohan and Walther 2016). Despite the hypoxic stress endured during these forays, these fish may in fact be more successful at obtaining prey that are moribund and readily accessible at the sediment interface, and subsequently experience an increase in metabolic rates as a consequence (Pihl et al. 1992; Long et al. 2014). Importantly, the effects of hypoxia on organismal metabolism are a complex function of resting and maximum metabolic rates as well as oxygen supply capacity, and the tolerances and responses are likely highly variable among taxa (Esbaugh et al. 2021). In some cases, fish may increase their respiration rates to compensate for low dissolved oxygen (Pörtner 2001). An additional critical factor is temperature, which directly affects metabolism as well as modifies oxygen uptake rates that define aerobic scope, as well as potentially impacting Mg:Ca uptake into otoliths. The current study focused on classifying fish according to their Mn:Ca values as a proxy for hypoxia exposure, but if temperature co-varied with the distribution of hypoxia in the system it could be an additional explanatory factor leading to divergent metabolic rates among hypoxia exposure groups. Future experimental work that exposes fish such as Atlantic Croaker to individual and combinatorial environmental stressor variables including dissolved oxygen, temperature, and food ration will be needed to fully disentangle the driving factors behind the patterns reported here for wild-captured individuals.

Some prior investigations into otolith Mn:Ca have encountered spikes in this analyte around the larval core that are likely associated with maternal transfer or physiological and metabolic processes inherent to embryonic and larval development (Ben-Tzvi et al. 2007; Ruttenberg et al. 2005; Limburg et al. 2015). It is unlikely, however, that any spikes in Mn:Ca have systematically biased the interpretation of hypoxia exposure during the first year of life in Atlantic Croaker for this study. First, otolith core spikes in Mn:Ca were rarely observed in Atlantic Croaker transects (see examples in Altenritter et al. 2018). In addition, prior analyses of larval Atlantic Croaker otolith composition found that core Mn:Ca compositions did not exceed 1 µmol/mol (Schaffler et al. 2009), which is one to two orders of magnitude below the concentrations of average Mn:Ca values in Age-0 otolith portions in this study. Second, the larval core diameter is a very small proportion of the Age-0 transect lengths analyzed in this study. Atlantic Croaker larval cores are approximately 50 µm in diameter (Schaffler et al. 2009). Age-0 transect lengths in this study were primarily between 2500 and 3000 µm (Valenza 2021), meaning core material represented approximately 2% of the total transect length. Thus, it is unlikely that larval otolith spikes in Mn:Ca are a major source of bias for this particular species. The interpretation that elevated Mn:Ca in Atlantic Croaker otoliths in the first year of life reflect elevated water Mn:Ca concentrations due to altered redox conditions from hypoxic conditions is robust, at least for this study. Future analyses that specifically analyze the larval portion of Atlantic Croaker or other species must take care to distinguish between external water-derived processes versus maternally derived or developmental processes that may influence the core region of the otolith in particular.

For Atlantic 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 Mn:Ca duration fractions. But Age-0 fish also recorded the highest Mn:Ca duration fractions in their otoliths. Altenritter and 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, in order to survive hypoxia exposure, these Age-0 fish 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). Given that these otolith chemistry patterns in Mg:Ca were obtained from wild individuals, a full evaluation of the links between foraging dynamics, differential survival, or other explanatory mechanisms will require careful long-term experimentation that ideally occurs over multiple life history stages to disentangle age-specific responses to hypoxia exposure.

Investigations into overall lifetime trends of growth, metabolism, and hypoxia exposure are key to understanding the population dynamics of Atlantic Croaker and other demersal fish species in habitats that experience anthropogenically exacerbated hypoxic episodes. Hypoxia is arguably one of the largest stressors to the Atlantic Croaker population in the nGoMex, causing direct mortality and a slew of sublethal effects. Our results found complicated evidence that (1) Age-1 and Age-2 fish with high values of Mn:Ca, a proxy for hypoxia exposure, also had high values of Mg:Ca, a potential proxy for metabolism, and (2) Age-0 fish showed high Mg:Ca and Mn:Ca duration fractions. These findings may be due to increased foraging efficiency within the hypoxic zone or from differential mortality of slow-growing and lower-metabolic fish. Additionally, the complexity and variability of the results of this study signify the individual variability of Atlantic Croaker exposure and movement in the hypoxic zone. In order to directly quantify trends, further research should explore metabolic and age-specific responses to hypoxia exposure through long-term experimentation. Therefore, measuring the responses of Atlantic Croaker metabolism or growth across years will provide a more straightforward understanding of the consequences of hypoxia exposure for fishes.

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Data availability The data that support these findings and conclusions are available upon reasonable request from the corresponding author.

Declarations

Ethical approval Fish carcasses were obtained already deceased from standard government groundfish surveys performed by the National Marine Fisheries Service. Because only deceased specimens were used and no additional mortality was incurred by project personnel for this research project, further IACUC approval was not deemed necessary.

Competing interests The authors declare no competing interests.

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