TRACKING HYPOXIA INDUCED TROPHIC SHIFTS OF ATLANTIC CROAKER (*MICROPOGONIAS UNDULATUS*) IN THE GULF OF MEXICO USING STABLE ISOTOPES

A Thesis

by

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

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ABSTRACT

Seasonal hypoxia in the northern Gulf of Mexico (nGoMex) occurring during the summer months can have sub-lethal effects on fishes by impairing reproductive capabilities, reducing growth rates, displacement to sub-optimal habitat, and altering trophic interactions. Atlantic Croaker, (*Micropogonias undulatus)* hereafter referred to as croaker, are demersal omnivorous fish found throughout the nGoMex including the area affected by the hypoxic zone. Bottom-water hypoxia may displace croaker from preferred benthic prey to pelagic alternatives. Pelagic shifts will not occur if resilient consumers can withstand hypoxia enough to continue foraging on stressed benthic prey. Stable isotopes can be used to resolve benthic to pelagic food web shifts given known differences in primary producers, known as isotopic endmembers. To identify recent hypoxia and estuarine residence, I used microchemical otolith markers for hypoxia (manganese) and salinity (barium), thereby clustering fish by exposure histories. Time periods of recent otolith exposure histories were matched to experimentally-validated turnover rates of $\delta^{13}C$ and $\delta^{15}N$ in croaker muscle allowing direct comparisons between exposure type and food web dynamics. Isotope niche widths revealed variable trophic shifts among individuals across two years using standard ellipse area (SEA). On average, hypoxia exposed fish had depleted $\delta^{13}C$ values relative to normoxic fish indicating shifts to pelagic food webs, although the magnitude of displacement differed among individuals. SEAs for hypoxic individuals showed expanded isotopic niche widths of $\delta^{13}C$ and $\delta^{15}N$ indicating variable trophic shifts following benthic food web displacement. Greater magnitudes of hypoxia exposure index values in the otolith were correlated with pelagic $\delta^{13}C$ values in muscle tissue, suggesting individual differences in benthic displacement may have been driven by variability in hypoxia exposure. Combining otolith

microchemistry with stable isotopes enhances our understanding of sub-lethal hypoxia, trophic webs, and feeding ecology and will inform management of key demersal fish species in the northern Gulf of Mexico

DEDICATION

To my parents, Jennifer and Philip, for showing me the sea

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INTRODUCTION

Elevated anthropogenic nutrient loading and artificial flow regimes have increased the frequency of hypoxia events worldwide (Rabalais et al. 2010). Documented in over 500 coastal locations (Breitburg et al. 2018) as well as large inland freshwater systems (Scavia et al. 2014) hypoxia has clear global relevance. Hypoxia is operationally defined by dissolved oxygen levels at or below 2.0 mg L^{-1} (Diaz and Rosenberg 2008) and can cause mortality as well as sub-lethal effects in many aquatic organisms (Bell and Eggleston 2005). Hypoxia occurs naturally through fluctuations in primary production, oceanography, and coastal meteorology (Rabalais et al. 2001). Increased nutrient input from agricultural fertilizer, treated wastewater effluent and other nitrogen sources can lead to phytoplankton blooms, which result in elevated primary biomass (Rabalais et al. 2007). This biological material is processed through benthic bacterial respiration (Turner and Rabalais 1994) which consumes substantial amounts of dissolved oxygen in the process. Thermohaline stratification, particularly during warmer summer months, prevents mixing and therefore limits replenishment of oxygen to the benthic layer. Prolonged seasonal stratification intensifies the effects of hypoxia on aquatic organisms, including populations of fishes (Breitburg et al. 2009).

The northern Gulf of Mexico (nGoMex) is the largest anthropogenic hypoxic system in the Western Hemisphere (Rabalais et al. 2001). The extent of hypoxia in the Gulf varies spatially and temporally on an annual basis, typically developing and persisting throughout the summer months (Rabalais et al. 2010). In recent years in the nGoMex has experienced greater magnitude and duration of seasonal hypoxia. Aptly named “The Dead Zone”, greater nutrient input over the last three decades has augmented eutrophication and expanded this suboxic area.

Research from other hypoxic zones have reported vertical and lateral displacement of fish populations (Craig and Crowder 2005, Hazen et al. 2009, Ludsin et al. 2009, Zhang et al. 2009). To date, research on how coastal and pelagic fisheries are affected by increased hypoxia in the nGoMex have been limited (Rose et al. 2009). Hypoxic conditions are hypothesized to alter fish feeding ecology by stressing metabolic function in benthic predator and prey species. Predators who have higher relative tolerance to hypoxia benefit when oxygen stressed prey become more easy to consume (Long and Seitz 2008). For instance, benthic prey displaced to the margins of hypoxic zones have shown increased predation by hypoxia tolerant predators (Craig and Crowder 2005, Craig 2012). This behavior results in a net benefit to predators during hypoxic episodes. Conversely, predators may pursue pelagic prey items to avoid hypoxic conditions creating predation refuges for benthic prey (Sagasti et al. 2001). This behavior indicates vertical shifts in the water column allow predators to escape costs of bottom water hypoxia and switch to pelagic food web resources (Essington and Paulsen 2010) and hypoxia induced trophic shifts have been documented in nGoMex demersal fishes.

Atlantic Croaker

Atlantic Croaker, *Micropogonias undulatus*, are widespread demersal omnivorous fish found in the Gulf of Mexico and along the Atlantic coast of North America (Sheridan and Trimm 1983, Sheridan et al. 1984). In the Gulf of Mexico, annual commercial landings were between 47-64 metric tons from 2000 and 2013 (NMFS 2018a) and annual recreational landings were between 373-467 metric tons between 2000 and 2014 (NMFS 2018b). Atlantic Croaker in the Gulf of Mexico also experience elevated mortality due to commercial penaeid shrimp trawl bycatch. From 1972 to 1995 bycatch of this species exceeded direct landings by an order of magnitude (Diamond et al. 1999). Between 2007 and 2010, Scott-Denton et al. (2012) reported

Atlantic Croaker remained the largest finfish bycatch in Gulf penaeid trawls, with 342 metric tons harvested totaling 16% of landings by mass. Coastal Louisiana and Texas account for the most intense bycatch regions, which overlap the geographic extent of nGoMex hypoxia.

Atlantic Croaker are an ideal research species to investigate hypoxia effects on food webs. They are widely distributed and abundant throughout the northern Gulf of Mexico's hypoxic zone and have been previously investigated for sub-lethal hypoxic effects in growth, reproduction and habitat displacement (Rahman and Thomas 2007, Diamond et al. 2013, Craig and Crowder 2005). Hypoxia often leads to habitat displacement of consumers the edges of suboxic waters where predators may experience energetic cost dependent on several metabolic factors (Craig and Crowder 2005, Craig 2012). Further, croaker exposed to elevated of hypoxia show impaired ovarian and testicular growth resulting in lower fecundity (Thomas et al. 2007, Thomas and Rahman 2009b). Significant proportions of female NGoMex Croaker exhibited germ line masculinization resulting from hypoxia exposure (Thomas and Rahman 2012). One sub-lethal effect yet to be investigated is displacement from benthic to pelagic food webs.

Trophic Dynamics

Atlantic Croaker are omnivorous generalists, with a preference for benthic prey but also demonstrating significant pelagic piscivory (Overstreet and Heard 1978, Sheridan et al. 1984, Nye et al. 2011). From populations in the Chesapeake Bay, Nye et al. (2011) described eleven categories of prey found by stomach content analysis: polychaetes, mysids, amphipods, bivalves, other benthic prey (barnacles, hydroids, gastropods, isopods), crabs, shrimp, detritus, other fish species, anchovies (*A. mitchilli, & A. hepsetus),* and other pelagic invertebrates (squid, nettles, terrestrial insects). Polychaetes were the dominant prey by mass (62%) and occurrence (84%), followed in occurrence by mysids (37%) amphipods (21%) other benthic items (20%) and

anchovies (13%). Thus, although Atlantic Croaker have a clear preference for benthic items, they have diet flexibility to opportunistically pursue pelagic prey items.

Consequences of hypoxia on food web dynamics have been investigated in the Mid-Atlantic Bight and Northern Gulf of Mexico. Atlantic Croaker are relatively tolerant to hypoxia, able to survive 1.6 – 3.7 mg of dissolved oxygen (DO) per liter in lab and field observation (Wannamaker and Rice 2000, Bell and Eggleston 2005, Thomas and Rahman 2009a, b). Diet contents reveal croaker not only survive suboxic conditions, but continue to feed on benthic prey items during hypoxia in the Neuse River, North Carolina (Powers et al. 2005). Despite this, croaker are known to avoid habitats lower than 2 mg/L DO, and have been found to consume higher quantities of pelagic prey during hypoxia in the Chesapeake Bay (Nye et al. 2011). Variability in hypoxia magnitude may account for observed differences in benthic food web displacement. If individuals are not uniformly displaced and exhibit punctuated foraging dives into hypoxic benthic habitat fish would contain benthic prey items in their stomachs while still primarily residing in pelagic habitat above hypoxic waters. The ability to reconstruct individual hypoxia exposure alongside dietary history is needed to distinguish trophic response. Individual hypoxia exposure markers are required to distinguish and measure the variable magnitude of hypoxia induced sublethal trophic shifts.

Hypoxia Markers

Developing a reliable marker for detecting hypoxia exposure in fish is crucial for subsequent ecological comparisons. Research in the Gulf of Mexico (Thomas and Rahman 2009a, Thomas and Rahman 2012) has shown croaker will upregulate the transcription factor Hypoxia Inducible Factor 1α (HIF-1α) when activating molecular and physiological stress responses to dissolved oxygen concentrations below 2.0 mg L^{-1}. While HIF-1α can be a useful

molecular indicator of whether a given fish has been exposed to hypoxia, HIF-1α expression levels fall to background levels within 24 hours after the fish is returned to normoxic water. Therefore, this molecular marker cannot be used to investigate exposure histories on the order of months to years, and markers better suited for detecting long term hypoxia exposure in Atlantic Croaker are required.

Lifetime environmental exposure is recorded on calcium carbonate structures called otoliths found in the inner ear of bony fishes. Otoliths have been used extensively in fisheries science and are renowned for serving as reliable aging structures as well as using natural trace elements as tags for reconstructing environmental conditions (Campana 1999, Elsdon et al. 2008). The latter, known as otolith microchemistry, has seen recent advances in detection of hypoxia exposure in fish by using dissolved manganese (Mn) found naturally elevated in low oxygen conditions (Limburg et al. 2011, Limburg et al. 2015). Across multiple habitats and species (including nGoMex Atlantic Croaker) Mn was found incorporated into the otoliths of fish taken from hypoxic waters. Hypoxic conditions facilitate Mn flux from benthic sediment (Limburg et al. 2015), allowing diffused Mn to become available in bottom water and integrate into the otolith via transport across gill membranes. Mn levels in waters above sediments are not elevated during normoxic conditions. Therefore, stage specific hypoxia exposure can be determined using the chemical composition across growth increments found within otoliths. Using the magnitude of the Mn:Ca ratio and the locations of peaks in Mn:Ca across the otolith, hypoxia timing, severity and duration can be calculated. Otoliths from normoxic individuals will not contain elevated Mn, allowing effective separation of hypoxia exposure among Croaker. One confounding variable for hypoxia detection arises from alternative sources of Mn in estuarine habitats (Walther and Nims 2015). Croaker are also known to utilize estuarine habitat (Thorrold

et al. 1997, Rooker et al. 1998), necessitating separation of estuarine-derived Mn from coastal hypoxia derived Mn which is the focus of this study. Barium (Ba) is another reliable environmental marker for movement across salinity gradients (Walther and Nims 2015). Elevated levels of Ba: Ca in the otolith identify individuals with estuarine (estimated <20 salinity) residence. Therefore, simultaneous measurement of Mn and Ba across individual otoliths can be used to distinguish periods of residence in estuaries and coastal shelf habitats (distinguished by high or low Ba, respectively) and hypoxia or normoxia (distinguished by high or low Mn, respectively). These elemental markers derived otolith values are used to cluster individuals into "normoxic", "hypoxic", and "estuarine" groups and therefore enable comparisons of trophic shifts between benthic and pelagic food webs using tissue isotopes from these identified groups.

Stable Isotopes

Stable isotopes provide a unique analytical tool for tracing movements between habitats in addition to trophic interactions (Fry 1983). Key biogeochemical interactions influence the levels of heavy isotopes relative to lighter isotopes. Stable isotopes are measured in δ notation in ‰ ("per mil") units and defined by the equation:

$$\delta = [(R_{SAMPLE} / R_{STANDARD} - 1)] * 1000$$

where *R* represents the ratio of heavy to light isotopes in the sample or standard (Fry 2006). Variation in photosynthetic pathways among primary producers can lead to distinct values of $\delta^{13}C$ in organic material at the base of food webs in different habitats. This primary biomass is referred to as the isotopic endmember (Fry 2006). Endmember $\delta^{13}C$ values can propagate upwards through food webs with some modification due to trophic fractionation in isotope ratios.

Stable isotopes are a useful tool when identifiable and distinct endmembers occur, meaning isotope values do not overlap between sources.

Terrestrial derived bulk $\delta^{13}C$ has a distinct isotopic signature when compared to pelagic derived production (Post 2002). Inshore terrestrial derived food webs may have depleted (lower $\delta^{13}C$ values) for C3 plants (mean -27‰) or enriched (higher $\delta^{13}C$ values) for C4 plants (mean -13‰) compared to offshore marine food webs which have a mean $\delta^{13}C$ of -21.4 ‰ (Boutton 1991, Peterson 1999, Rosenheim et al. 2016). Within marine systems, benthic carbon isotopes have a different signature than pelagic derived carbon (Radabaugh et al. 2013) due to photosynthetic fractionation between producers. In the northern Gulf of Mexico, benthic algae have a mean $\delta^{13}C$ of -19.3‰ and particulate organic matter (POM) has a mean $\delta^{13}C$ of -23.3‰. Due to fractionation, benthic-associated fish collected from the nGoMex exhibited mean $\delta^{13}C$ values of −16‰, while pelagic-associated fish displayed mean $\delta^{13}C$ values of −20‰ (Radabaugh et al. 2013). Fish which exhibit a significant shift in feeding on benthic to pelagic food webs will increase the proportion of pelagic derived $\delta^{13}C$ in their tissues. Over time, croaker feeding at higher rates on pelagic prey lower their tissue isotopic carbon values. Proportions of prey consumed from pelagic food webs impacts the magnitude of the $\delta^{13}C$ shift. Fish will only display mean isotope values of -20‰ when the majority of their diet originates from pelagic sources. Fish which continue to feed on benthic sources will not show shifts in $\delta^{13}C$. In this manner a 4.0 ‰ shift in marine carbon isotopes between heavier benthic to lighter pelagic food webs can identify food web origin in consumer tissue.

Variation in $\delta^{13}C$ baselines exists between marine systems (Post 2002). However, differences in mean benthic to pelagic $\delta^{13}C$ values are similar across other marine systems: Southeast Australia, South Pacific (Davenport and Bax 2002), in the Barents Sea, Arctic

(Tamelander et al. 2006), in the Southern Ocean, Antarctica (Cherel and Hobson 2007), in Northern California, Eastern Pacific (Miller et al. 2008), in Chesapeake Bay, Western Atlantic (Woodland and Secor 2013), the English Channel, Eastern Atlantic (Kopp et al. 2015) and can be used as reliable indicators of benthic and pelagic food webs.

Values of a second stable isotope, $\delta^{15}N$, can be used as a trophic position indicator. Values of $\delta^{15}N$ increase 3.4‰ on average between trophic levels (Post 2002). Organisms exhibit changes in bulk $\delta^{15}N$ with ontogeny and changes in the trophic positioning of their prey. For example, Atlantic Croaker in the nGoMex at age 0+ would be expected to show lower $\delta^{15}N$ values than an individual at 3 years due to limitations on gape size, and greater trophic positioning of larger prey (Akin and Winemiller 2008). $\delta^{15}N$ is also a useful marker to tease out individual variation in prey selection by omnivorous consumers with diverse diets among fish of the same age. Croaker consume a range of prey types from many trophic levels (Nye et al. 2011). Combining $\delta^{13}C$ and $\delta^{15}N$ into a bivariate plot measures total isotope niche width, which should reflect food web dynamics of consumers.

Isotope values change within an organism's tissue due to feeding, metabolism, and somatic growth. When a consumer switches to a new diet with a distinct isotope signature, that tissue will eventually converge, or equilibrate, on the new dietary signature. This change occurs because of dilution due to growth and addition of new biomass synthesized from the new diet as well as metabolism and replacement of existing biomass. Tissues such as white muscle are therefore time-integrated indicators of dietary histories reflecting a specific time period prior to capture. Determining the isotopic turnover rate, or the time needed to replace $\delta^{13}C$ and $\delta^{15}N$ in muscle tissue varies with age, physiological status, and tissue types. These rates are best determined through controlled diet switch experiments that establish rates of equilibration for the

tissue of interest. Fortunately, prior experiments on Age-0 nGoMex Croaker muscle tissue were performed by Mohan et al. (2016). In these experiments, 95% equilibration took an average of 129 days for $\delta^{13}C$ and 115 days for $\delta^{15}N$. Thus, isotope signatures of muscle tissue from Croaker in their first year of life reflects diet histories from the prior three to four months before capture. This information can then be coupled with the same time period in the exterior of otoliths to compare time-matched signatures of hypoxia exposure (from otolith chemistry) and diet histories (from muscle tissue isotopes).

Research Questions

This study looks to resolve the trophic shifts of Atlantic Croaker following hypoxia exposure in the Northern Gulf of Mexico. Croaker have been observed to congregate at the vertical margins of the hypoxic zone in the nGoMex (Craig and Crowder 2005) and acoustic scans and trawls reveal croaker among demersal fishes in the seven meters of water above hypoxic zones in the Chesapeake Bay (Hazen et al. 2009). These results support the physical displacement of fish from the benthic food web. However, if individuals are not uniformly displaced, or exhibit punctuated foraging dives into benthic habitat, fish will continue to display benthic $\delta^{13}C$ while still primarily residing in pelagic habitat above hypoxic waters. Mohan and Walther (2016) found that coastal hypoxic fish had limited benthic displacement compared to normoxic fish as indicated by muscle tissue $\delta^{13}C$ isotopes and concluded hypoxic resilience for this species. However, small sample sizes used in this research and substantial temporal factors across multiple years prompts this current study.

Our first hypothesis looks to determine whether hypoxia exposed Atlantic Croaker are displaced from benthic to pelagic food webs. Otolith microchemistry will separate hypoxic from normoxic fish and stable isotopes $\delta^{13}C$ and $\delta^{15}N$ will reveal trophic shifts. No shift in trophic

ecology would occur if hypoxic and normoxic muscle isotope mean and deviation directly overlap benthic endmember $\delta^{13}C$ and $\delta^{15}N$ values (no response, left panel Fig. 1.). This outcome indicates hypoxic resilience and continued benthic consumption. Our second hypothesis examines whether hypoxia exposed fish have uniform or variable trophic shifts. If all hypoxic exposed individuals are entirely displaced from benthic to pelagic habitats, then the bivariate mean of $\delta^{13}C$ and $\delta^{15}N$ isotopic signatures of hypoxic fish would shift towards the pelagic endmember, but the group variance (total isotopic niche area occupied by this group) would not change (uniform response, center panel Fig. 1). Alternatively, the magnitude of hypoxia induced trophic shifts could vary among individuals, with some continuing to forage on benthic prey and others being displaced to pelagic food webs. This individually variable scenario would lead to shifts in both mean and variance of $\delta^{13}C$ and $\delta^{15}N$ isotope values to form a much larger isotopic niche space for hypoxic individuals, (variable response, right panel, Fig. 1). I predict exposure to seasonal bottom water hypoxia will displace Atlantic Croaker (*Micropogonias undulatus*) from benthic food webs to pelagic food webs, and the extent of trophic shift will be determined by individual hypoxia exposure variation within the hypoxic group (variable response, right panel, Fig. 1).

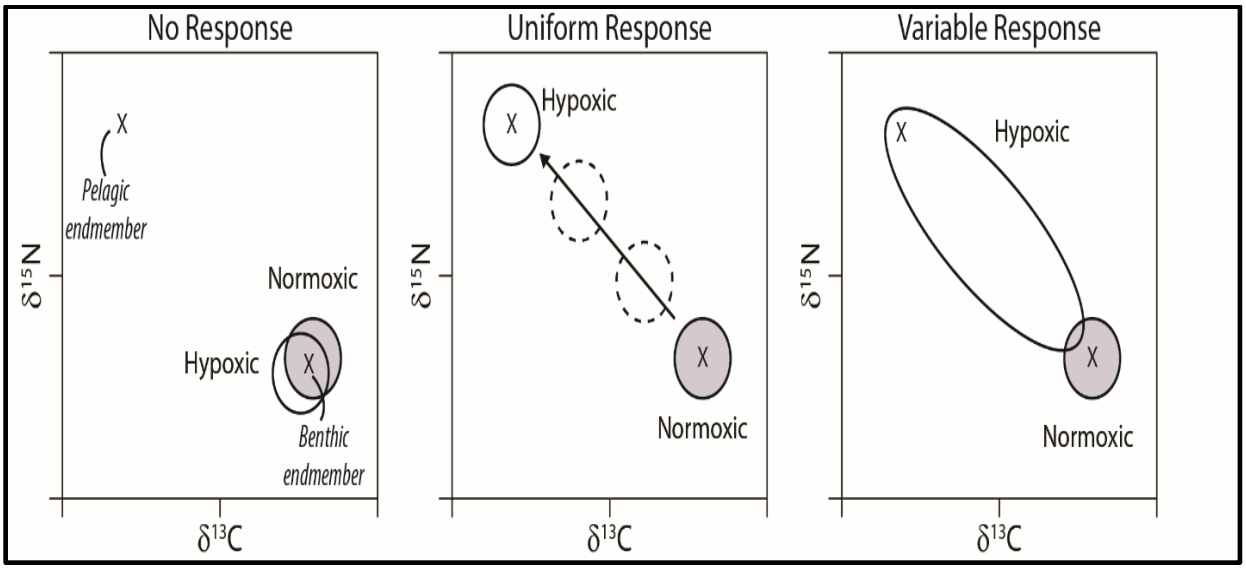

Fig. 1. Visual representation of expected trophic shifts in hypoxic Atlantic Croaker. Redrawn with permission from Mohan and Walther (2016).

STUDY AREA

To effectively characterize offshore seasonal hypoxia induced trophic shifts, collection locations were selected around and within the coastal hypoxic zone of Louisiana and Texas (Fig. 2). Since records began in 1985 (Turner and Rabalais 1994) this region has experienced expanding bottom water hypoxia which reaches peak severity in late summer and dissipates in September, October and November. Collections took place October 23rd until November 5th of 2014 and 2015 to allow any impacts of summer hypoxia exposure to be reflected by otolith chemical composition and changes in soft tissue isotopes. 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 spatial extent of the previous 10-year footprint average for the nGoMex (LUMCON Gulf Hypoxia Program 2018), and therefore reflect offshore hypoxic conditions in the nGoMex.

MATERIALS AND METHODS

Field Sampling

Atlantic Croaker were collected from specific trawl stations inside and outside the coastal hypoxic zone of Louisiana and Texas in 2014 and 2015 (Fig. 2) during the NOAA Southeast Monitoring and Assessment Program (SEAMAP) Fall Groundfish Survey aboard the RV *Oregon II*. Sampling stations were chosen by NOAA stratified random sampling based on National Marine Fisheries Service (NMFS) Gulf of Mexico coastal shrimp fishing zones. Fish were captured using a 12.8 m beam shrimp trawl with 41 mm stretch mesh deployed for 30 minutes (GSMFC 2001). Where present, between 10 - 50 individual Croaker were collected and stored frozen (-20°C) onboard until laboratory processing. A total of 463 Croaker were collected during

the 2014 cruise, and 962 in 2015. At each sampling location bottom water temperature, salinity and oxygen concentrations were recorded.

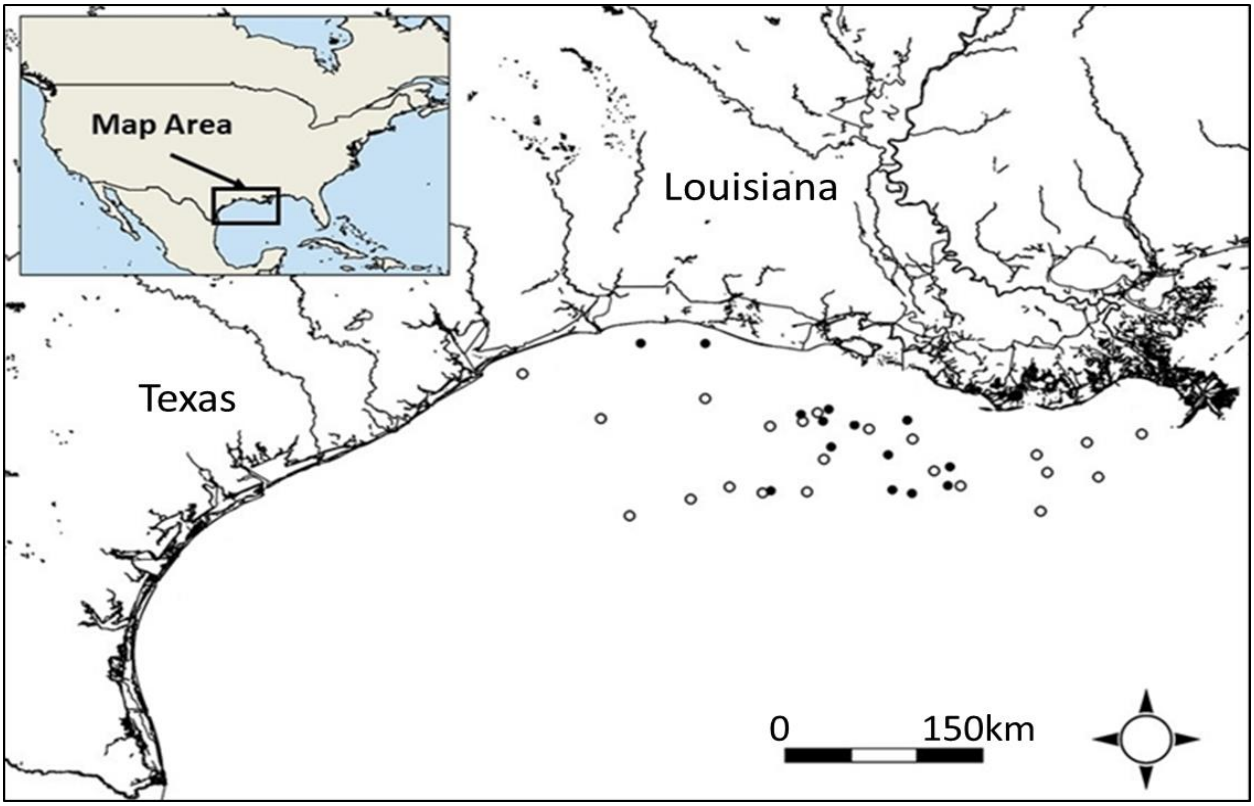

Fig. 2. Map showing sampling NOAA SEAMAP Groundfish Fall Survey sampling stations (2014=open circles, 2015=closed circles) where Atlantic Croaker were collected in October – November.

Laboratory Processing

Laboratory processing began by thawing one frozen bag of 10-50 Croaker, representing one NOAA station. Individual fish were measured for total length (TL), standard length (SL) to nearest mm, and total mass to nearest 0.1 g. Sagittal otoliths were removed, rinsed in DI water and dried in labeled 1.5 mL vials. Dorsal white muscle fillets were removed, scaled, and separated from any residual non-muscle tissue. Fillets then were rinsed thoroughly in DI water and placed in labeled 2.0 mL vials. Tissue vials were stored frozen (-20°C) before drying and grinding. Between individuals, dissection surface and utensils were cleaned with 95% ethanol to eliminate cross contamination.

Mn is a reliable indicator of hypoxic exposure when found in the otoliths of fish (Limburg et al. 2011, Limburg et al. 2015). Using the procedures described in full by Altenritter et al. 2018 otolith microchemical signatures were used to assign individuals as “hypoxic”, “normoxic”, or “estuarine”. These otolith chemistry analyses were completed prior to the tissue isotope analyses for this study. Briefly, after both sagittal otoliths were dried, the left otolith was embedded in epoxy-resin. Once hardened, otoliths were then sectioned (Buehler Isomet saw) polished and aged by two readers. Trace elements were measured using an Agilent 7500ce ICP-Q-MS coupled to a New Wave UP 193-FX laser at the University of Texas Austin Jackson School of Geosciences. A total of 674 otoliths were transected core to edge, reading the entire life history exposure of each individual. Mn:Ca was used to define hypoxia exposure and Ba:Ca to define estuarine residence based on known threshold values for each signal (Ba:Ca < 20 µmol/mol indicates estuarine fish, and Mn:Ca > 100 µmol/mol indicates hypoxic offshore fish; Mohan et al. 2014). As estimates of precision, mean relative standard deviations for Mn and Ba were 3.52% and 4.39% respectively.

For this project, only Age-0 (young-of-the-year) Atlantic Croaker were used. This age class was chosen because previous laboratory experiments determined tissue turnover rates as well as otolith accretion rates for Age-0 Atlantic Croaker (Mohan et al. 2014, Mohan et al. 2016). From these experiments, it was established that the outer 1000μm of a transect across an Age-0 otolith represents the last 3-4 months of life, which is the same period reflected in muscle tissue isotope signatures for this age class.

Collections of Atlantic Croaker yielded 101 Age-0 individuals from 2014 and 99 total Age-0 individuals from 2015. Using the Mn:Ca and Ba:Ca values from the external 1000μm of transects for each otolith, fish were clustered according to their hypoxia and estuarine exposure histories. Thresholds for either hypoxia exposure or estuarine residence were established, and each data point transects within the exterior 1000μm of the otolith was measured and assigned an index value of 1, 2 or 3 if the corresponding data point exceeded the threshold by 1x, 2x, or 3x respectively. This method preserved information about intensity and duration of chemical signatures within otoliths while remaining conservative about assigning specific elemental chemistry values to specific salinity or dissolved oxygen concentrations (information that is not currently validated). The estuarine and hypoxia index values for the exterior 1000 μm were then summed for each fish, yielding two total index values. A third index value for each individual was calculated for data points where both Mn:Ca and Ba:Ca exceeded their thresholds simultaneously, which indicated estuarine hypoxia. These three indices were used in Ward's clustering algorithm to group individuals with similar exposure histories. Once clustered, group types were determined by examining index values for each group. Groups with high hypoxia indices and low estuarine indices were considered "offshore hypoxic", while groups with low hypoxia and low estuarine indices considered "offshore normoxic". These two groups were

retained for isotope comparison in this study. Fish with high estuarine or high simultaneity indices were excluded from these analyses, as these indicated estuarine residence and thus outside the scope of this research project.

Muscle tissue samples were dried in the Isotope Core Lab (ICL) in the Natural Resource Center (NRC) of TAMU-CC for 48-72 hours at 60°C, and subsequently ground with a mortar and pestle. Once ground, samples were examined and any non-homogenous material (scales, bone, fat, or connective tissue) was removed, after which the sample was weighed on a microbalance to (+/-0.2mg of 1.0mg) and packaged into 3.5mm Costech© tin capsules. Capsules were placed into 96-well Costech© trays. Elemental Analysis (Carlo Erba© NC2500) and stable Isotope analysis (Thermo© Delta V Plus) performed at University of Texas- Marine Science Institute in Port Aransas. Daily standard calibration using USGS 40, USGS 41, and peach leaves was performed before, during and after stable isotope analysis runs. (Raw $\delta^{13}C$ and $\delta^{15}N$ isotope data are available in the Appendix).

Instrumental precision for measurements of $\delta^{13}C$ was estimated to be 0.06 ‰ based on repeated measurements of certified reference USGS-40 and USGS-41a and 0.28‰ based on repeated measurements of internal laboratory peach leaf standards. Precisions of $\delta^{15}N$ were 0.06‰ based on measurements of USGS-40, 0.07‰ for USGS-41a, and 0.13‰ for peach leaf standards. Duplicates for every 20th fish muscle sample were run to assess methodological precision. Average precision for muscle duplicates were 0.05‰ for $\delta^{13}C$ and 0.03‰ for $\delta^{15}N$. Given the small differences between duplicate muscle analyses, only one randomly selected duplicate was retained to represent the isotope composition for that individual.

Isotopic tissue values can be affected by several biometric factors including ontogeny, metabolism and diet quality (Peterson 1999, Layman et al. 2007, Mumby et al.2017). All

individuals selected for this research were Age-0, yet variable growth rates within this age class could lead to size or condition driven bias in isotope values. First, linear regressions were calculated for $\delta^{13}C$ and $\delta^{15}N$ values against fish standard length (mm) and mass (g) to check for any size related trends in isotope values. In addition, C:N ratios are used as a proxy for tissue lipid content and mean values above 4.0 indicate lipid correction steps are needed for stable isotope analysis. C:N values were calculated to determine if values exceeded established thresholds requiring lipid corrections.

Two metrics were calculated to compare isotope niche areas between hypoxic and normoxic groups in each year. First, the total area (TA) was calculated from the outermost data points for each group and calculating the area contained within the polygon. Although this method has been used frequently in the literature, TA is highly sensitive to outliers and insensitive to dispersion within groups. Thus, a second method for calculating niche area was used following Jackson et al. 2011). Here, the Stable Isotope Bayesian Ellipses package in R (SIBER; https://cran.r-project.org/web/packages/SIBER/) was used to calculate standard ellipse areas (SEA). Standard Ellipse Areas approximates total isotopic ellipse area with concessions for standard deviation. This Bayesian approach to calculate SEAs yields ellipses that contain 40% of the observed data, and were used because they are less sensitive to unequal sample sizes and outliers compared to TA. In addition, confidence intervals around estimates of the ellipse areas for each group were calculated and used to assess group overlap significance. Groups were considered to be significantly different if there was no overlap between the 95% credible intervals for the estimated SEAs for hypoxic and normoxic groups.

RESULTS

Hypoxia Exposure Clustering

Wards clustering revealed four groups for 2014 (Fig. 3); the largest was normoxic representing 67% of fish followed by two hypoxic clusters totaling 28% and one small estuarine group at 4% (n= 88 fish) (Table 1). Otolith data from 2015 yielded 5 total groups, the largest again representing normoxic fish at 63%, two estuarine groups combined to 19% and two groups of hypoxic fish totaling 18% (n= 96 fish) (Fig. 4 & Table 1). Estuarine fish were excluded from further analysis and subsequent comparisons were made between offshore normoxic and hypoxic fish. The two remaining clusters of interest, offshore normoxia, (herein referred to as normoxia) and offshore hypoxia (referred to as hypoxia) showed similar trends in their respective otolith chemistry index values. In both years normoxia was characterized by low inshore (Ba:Ca values), hypoxic (Mn:Ca values) and simultaneity index values. Hypoxia was characterized by low inshore (Ba:Ca values) and high hypoxic (Mn:Ca values) and low simultaneity index values.

Stable Isotopes

Stable isotopes $\delta^{13}C$ and $\delta^{15}N$ from croaker muscle tissue were examined over two years. 2014 $\delta^{13}C$ muscle revealed no correlation between SL (R^2= 0.056) and mass (R^2= 0.060). 2015 $\delta^{13}C$ values showed slightly higher correlation between SL (R^2= 0.29) and mass (R^2= 0.20) however these relationships are affected by several outliers with depleted $\delta^{13}C$. 2014 $\delta^{15}N$ showed very low correlation with standard length and mass (both R^2<0.01. Similar trends were observed in 2015 with $\delta^{15}N$ muscle values and SL weakly correlated (R^2=0.05) and mass (R^2=0.04). Elevated lipid content may bias values of $\delta^{13}C$, and C:N ratios above 4.00 indicate high lipid concentration (Post et al. 2007). The mean measured C:N values of muscle tissue of 3.70± 0.04 indicated lipid content was below threshold and did not warrant lipid corrections for isotope values.

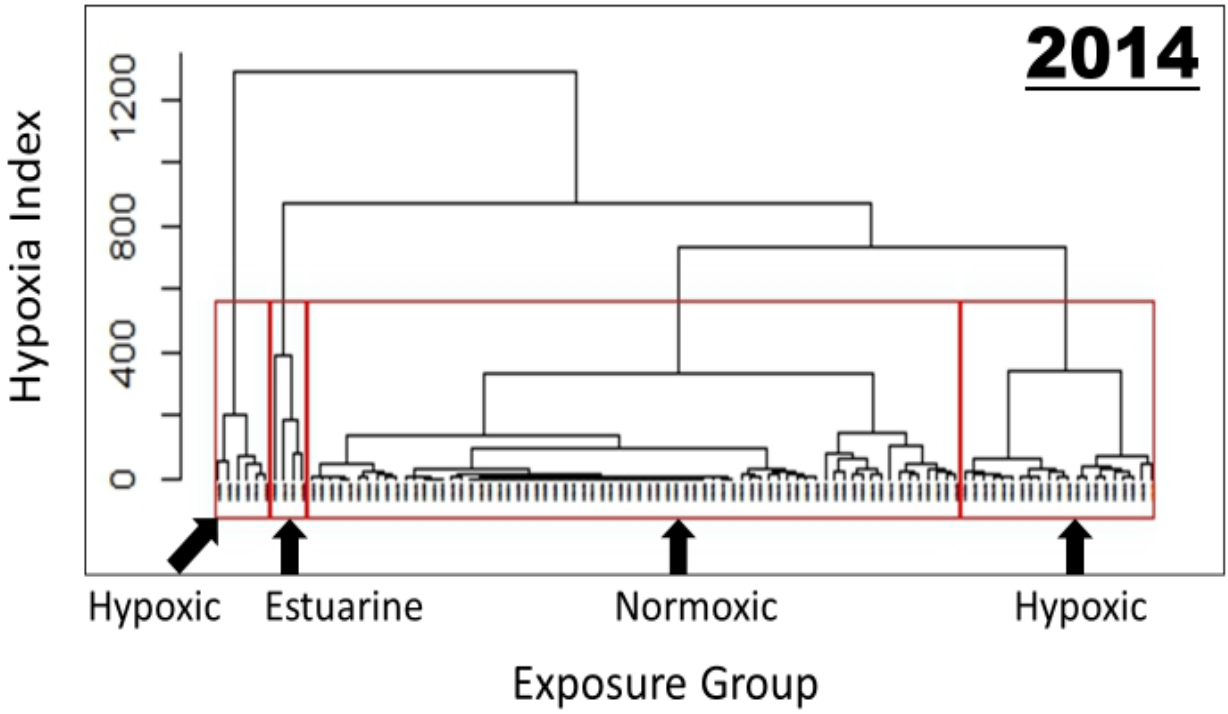

Fig. 3. Dendrogram for 2014 Age-0 Atlantic Croaker. Four clusters revealed two small Hypoxic groups, one small Estuarine group and one large Normoxic group

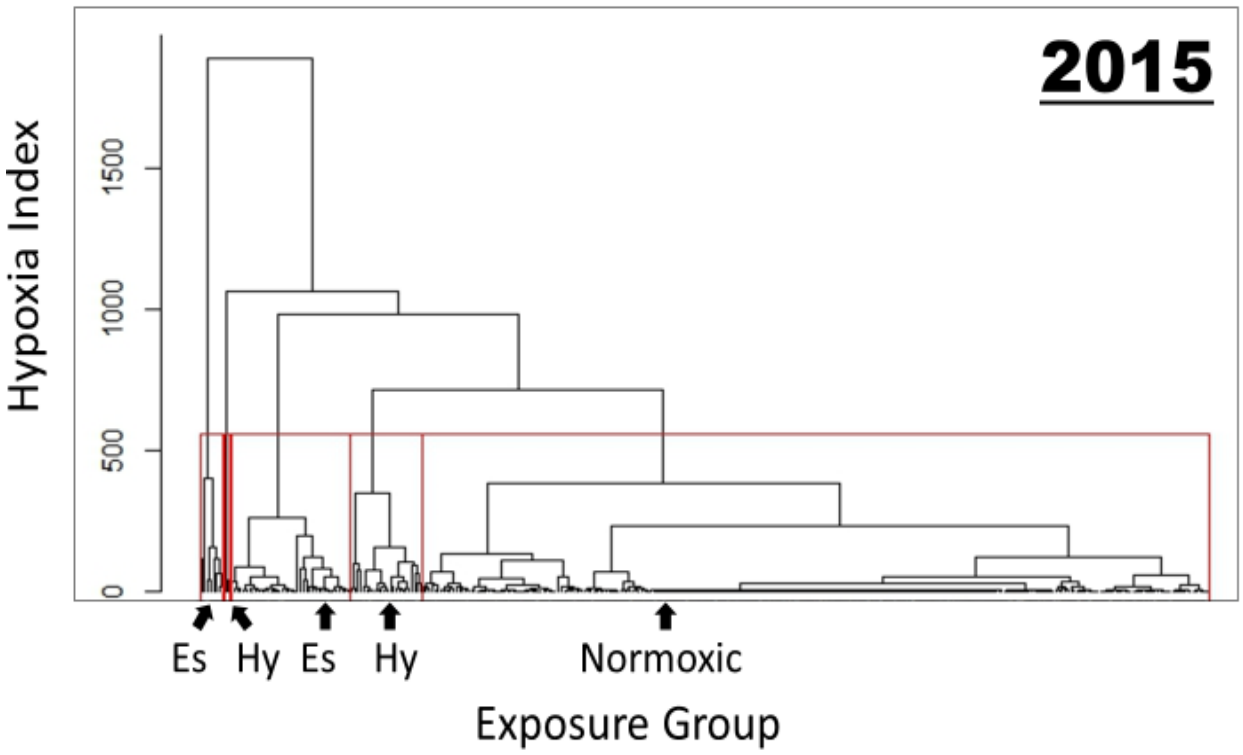

Fig. 4. Dendrogram for 2015 Age-0 Atlantic Croaker. Five clusters revealed two small Hypoxic (Hy) groups, two small Estuarine (Es) groups and one large Normoxic group.

Table. 1. Total individuals sampled with exposure groups for this study.

Year	Hypoxic	Normoxic	Estuarine*	Total
2014	25 (28%)	59 (67%)	4 (5%)	88
2015	17 (18%)	61 (64%)	18 (19%)	96
Total	**42 (23%)**	**120 (65%)**	**22 (12%)**	**184**

*Excluding Estuarine fish, in 2014 Hypoxic fish composed 30% of the remaining sample. Excluding Estuarine fish in 2015, Hypoxic fish composed 22% of the remaining sample.

Stable isotope values for $\delta^{13}C$ and $\delta^{15}N$ for all fish are pooled for each of the two sampled years shown in Table 2. In 2014 $\delta^{13}C$ values ranged between -19.79‰ and -16.82‰ with a mean value of -17.80‰. In 2015 $\delta^{13}C$ values ranged -21.24‰ to -16.97‰ with a mean of -17.80‰. Across years, $\delta^{13}C$ values were similar and not significantly different (p-value =0.966) (summarized in Table 3). $\delta^{15}N$ values of both groups averaged 15.82‰ and ranged from 12.73‰ to 19.71‰ in 2014. 2015 values of $\delta^{15}N$ averaged 15.66‰ and ranged from 14.26‰ to 18.02‰. Values of $\delta^{15}N$ were not significantly different between years of collection (p-value=0.258). Also, individuals clustered normoxic in 2014 were not significantly different from normoxic fish in 2015 using $\delta^{13}C$ (p-value=0.971) or $\delta^{15}N$ (p-value= 0.702). Likewise, fish clustered hypoxic using $\delta^{13}C$ (p-value=0.715) or $\delta^{15}N$ (p-value= 0.325) were not significantly different in either year sampled, as shown in Table 3. In contrast, the $\delta^{13}C$ values recorded for hypoxic individuals were significantly different from normoxic individuals for each year (2014 p-value= 0.007, 2015 p-value=0.005) (Table 4). $\delta^{15}N$ values between hypoxic and normoxic individuals in 2014 and 2015 were significantly different (p-value=0.04, p-value=0.05 respectfully), shown in Table 4.

Standard length (mm) and mass (g) were significantly different between individuals grouped hypoxic compared to individuals grouped normoxic. In 2014, hypoxic fish had a mean standard length of 103.5 mm compared to 110.0 mm for normoxic fish, which was significantly different (ANOVA p-value= 0.0011). In 2015, hypoxic fish were also smaller compared to normoxic individuals (110.5 mm vs 116.0 mm) and this difference was statistically significant (p-value=0.009). The mass of 2014 fish averaged 29.7 g for hypoxic individuals and 35.3 g for normoxic individuals, and the difference was statistically significant (p-value = 0.003). In 2015, hypoxic fish had a mean mass of 32.2 g and were significantly smaller compared to an average mass of 37.4 g for normoxic individuals (p-value =0.009).

Table 2. Stable Isotope Analysis statistics for Atlantic Croaker

Year	Isotope	Min (‰)	Max (‰)	Mean (‰)	St. Dev (‰)
2014	$\delta^{13}C$	-19.79	-16.82	-17.80	0.55
2014	$\delta^{15}N$	12.73	19.71	15.82	1.16
2015	$\delta^{13}C$	-21.24	-16.97	-17.79	0.59
2015	$\delta^{15}N$	14.26	18.02	15.66	0.58

Table 3. P-values for two-tailed t-tests comparing isotope values between hypoxic and normoxic groups across years. Combined year p-values indicate pooled data from both years

Between Years	**$\delta^{13}C$**	**$\delta^{15}N$**
Hypoxic	0.715	0.325
Normoxic	0.971	0.702
Combined groups	0.956	0.258

Table 4. P-values for two-tailed t-tests comparing isotope values between hypoxic and normoxic groups. Combined year p-values indicate pooled data from both years.

Exposure Between Groups	**$\delta^{13}C$**	**$\delta^{15}N$**
2014	0.007	0.04
2015	0.005	0.05
Combined years	0.001	<0.01

Standard Ellipse Area

Standard Ellipse Area (SEAs) from the R package SIBER are shown in Table 5 and Fig. 5 & 6. Temporal variation occurs in the SEA, with 2014 having a greater overlap between hypoxic and normoxic ellipses than was observed in 2015. Both years show the hypoxic cluster with depleted $\delta^{13}C$ relative to the normoxic cluster, and marginal increase in $\delta^{15}N$ of hypoxic fish compared to normoxic. Total Area (TA) measures of the largest possible niche space defined by $\delta^{13}C$ and $\delta^{15}N$. In 2014, normoxic fish TA was 11.55 $‰^2$ compared to 5.64 $‰^2$ for hypoxic fish. In 2015, normoxic fish TA was 3.50 $‰^2$ compared to 6.28 $‰^2$ for the hypoxic cluster. Standard Ellipse Areas (SEAs) for hypoxic groups were 19% larger than normoxic groups in 2014 and 268% larger than normoxic groups in 2015.

SIBER analysis comparing SEA between clusters revealed overlapping SEA for 2014 (Fig. 7.) and significantly different SEA for 2015 (Fig. 8.) Shaded boxplots separated by cluster indicate 95% confidence intervals. Scatterplots displaying $\delta^{13}C$ as explained by Hypoxia Exposure Index show a negative linear correlation for both years (Fig. 9 & 10) with fish exposed to the largest magnitudes of hypoxia have the lowest $\delta^{13}C$ values.

Table 5. Niche areas for Total Area (TA) and Standard Ellipse Area (SEA) for hypoxic and normoxic groups across years.

Year	Area	Normoxic (‰)^2	Hypoxic (‰)^2
2014	TA	11.545	5.640
2014	SEA	1.164	1.951
2015	TA	3.500	6.275
2015	SEA	0.561	2.2068

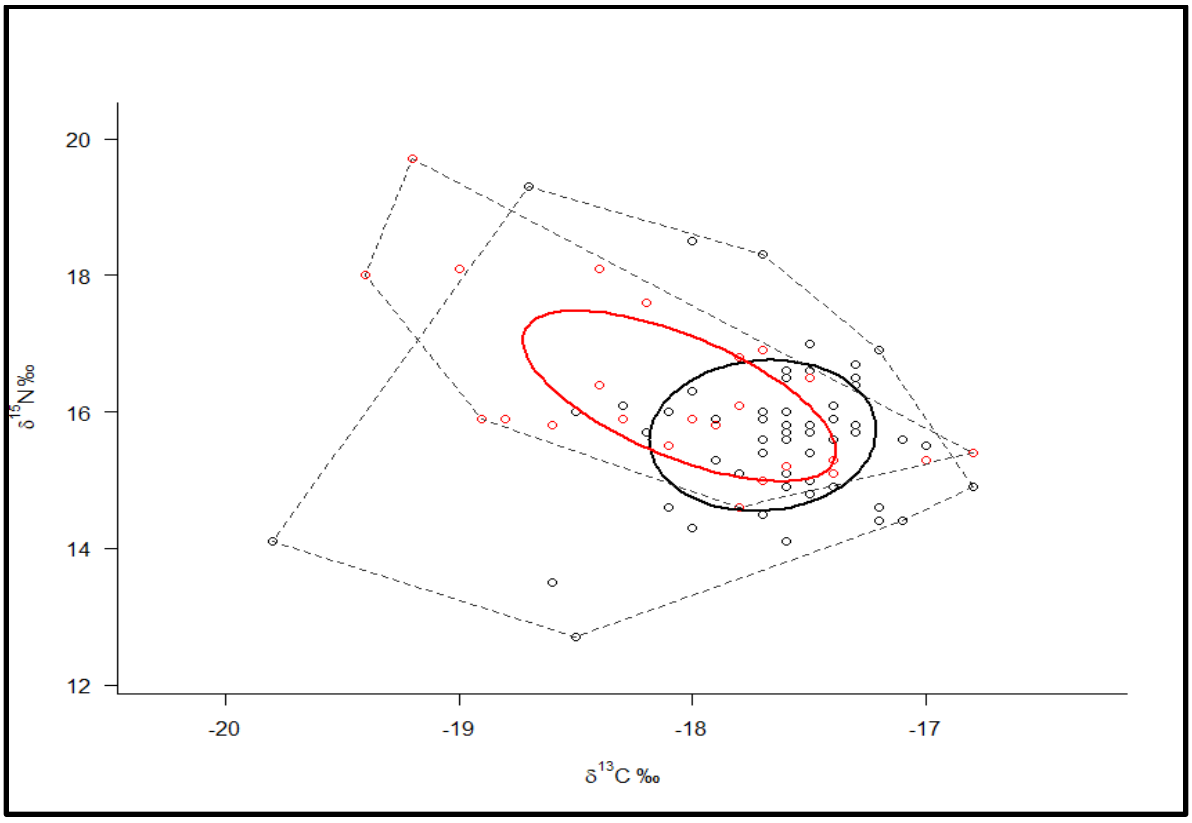

Fig. 5. Standard Ellipse Area (solid lines) and convex hull (dashed lines) for 2014 Age-0 Atlantic Croaker for isotopes $\delta^{13}C$ and $\delta^{15}N$. Black SEA= normoxic, red SEA= hypoxic

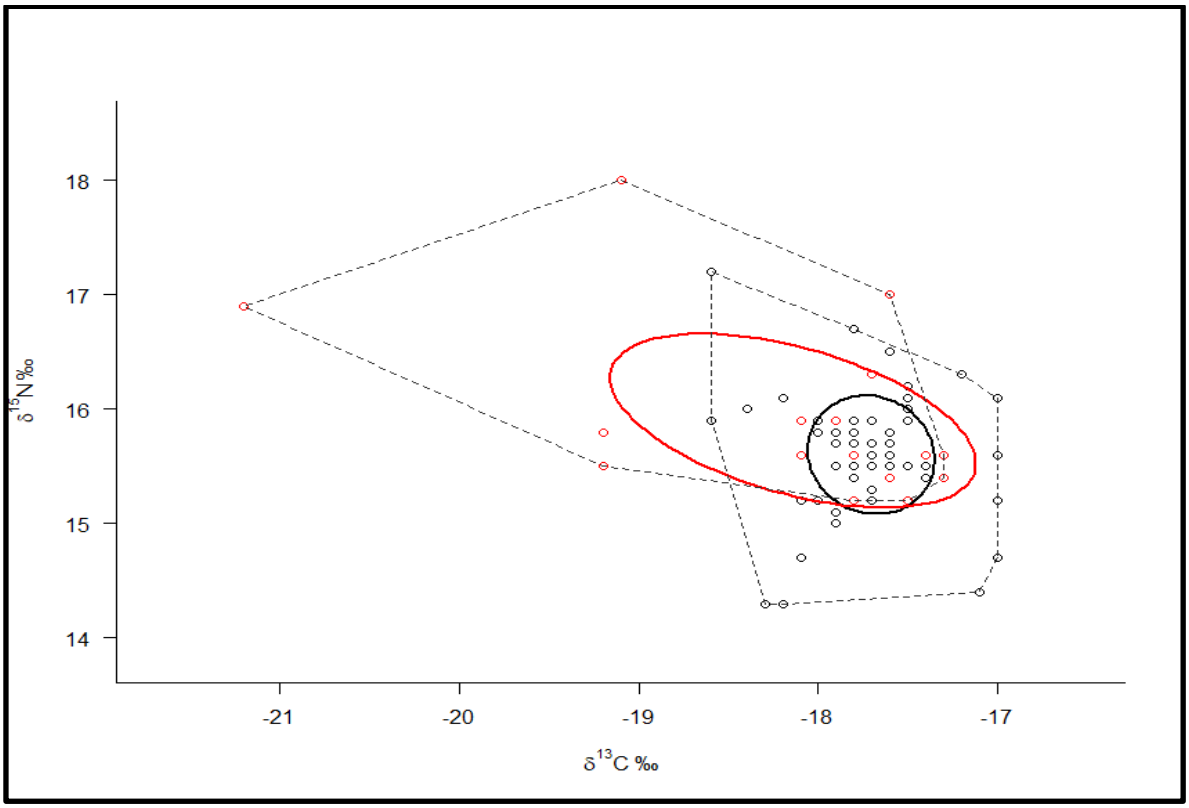

Fig. 6. Standard Ellipse Area (solid lines) and convex hull (dashed lines) for 2015 Age-0 Atlantic Croaker for isotopes $\delta^{13}C$ and $\delta^{15}N$. Black SEA= normoxic, red SEA= hypoxic

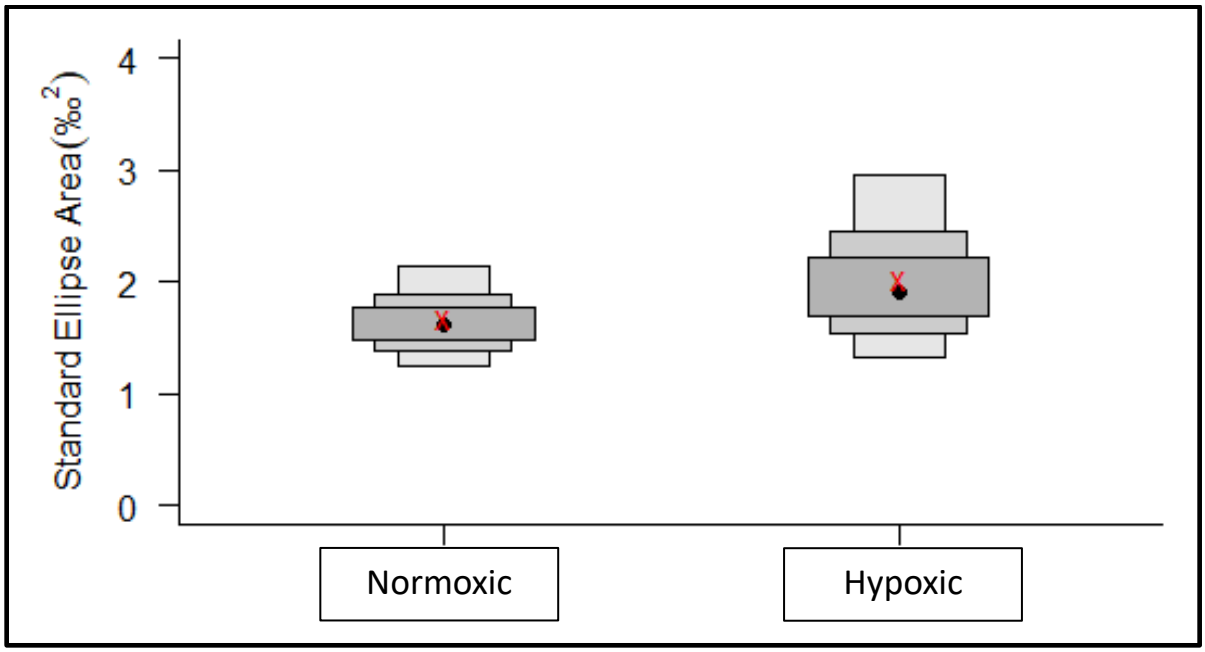

Fig. 7. Standard Ellipse Areas for isotope niches of 2014 Age-0 Atlantic Croaker from hypoxic or normoxic exposed groups. The black dot indicates the mode while the red x denotes group mean estimates. Shaded boxes represent 50, 75, and 95% credible intervals from dark to light shades, respectively.

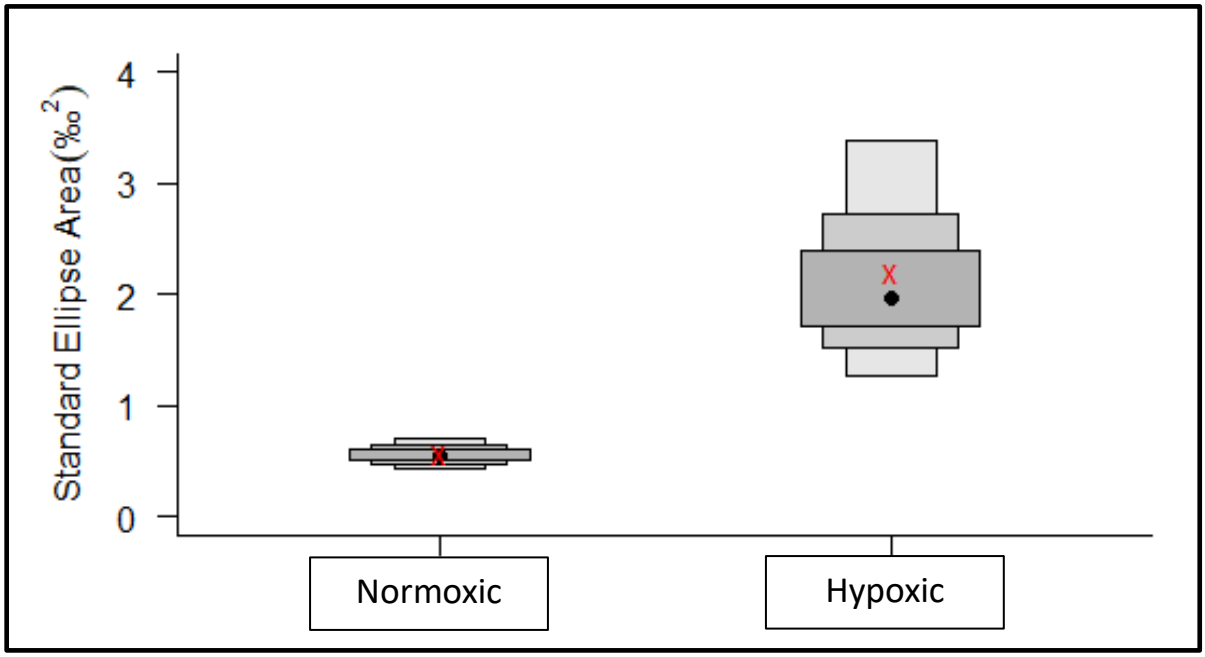

Fig. 8. Standard Ellipse Areas for isotope niches of 2015 Age-0 Atlantic Croaker from hypoxic or normoxic exposed groups. The black dot indicates the mode while the red x denotes group mean estimates. Shaded boxes represent 50, 75, and 95% credible intervals from dark to light shades, respectively.

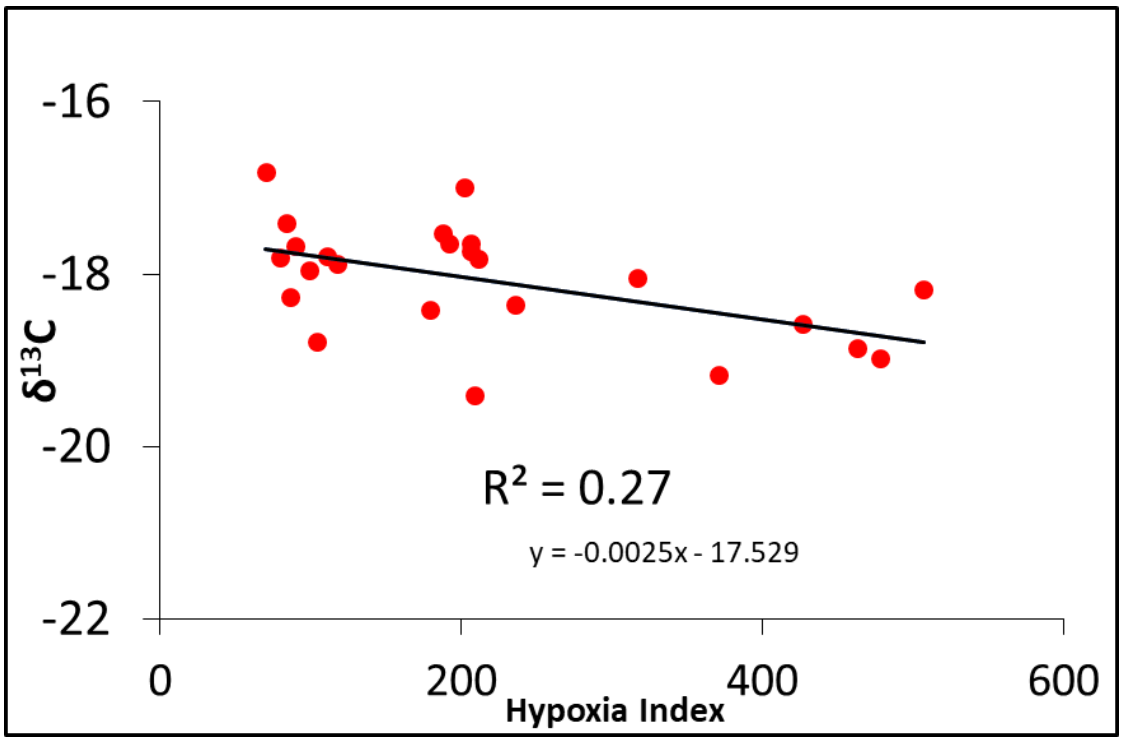

Fig. 9. Values of $\delta^{13}C$ and hypoxia exposure indices for 2014 Age-0 Atlantic Croaker

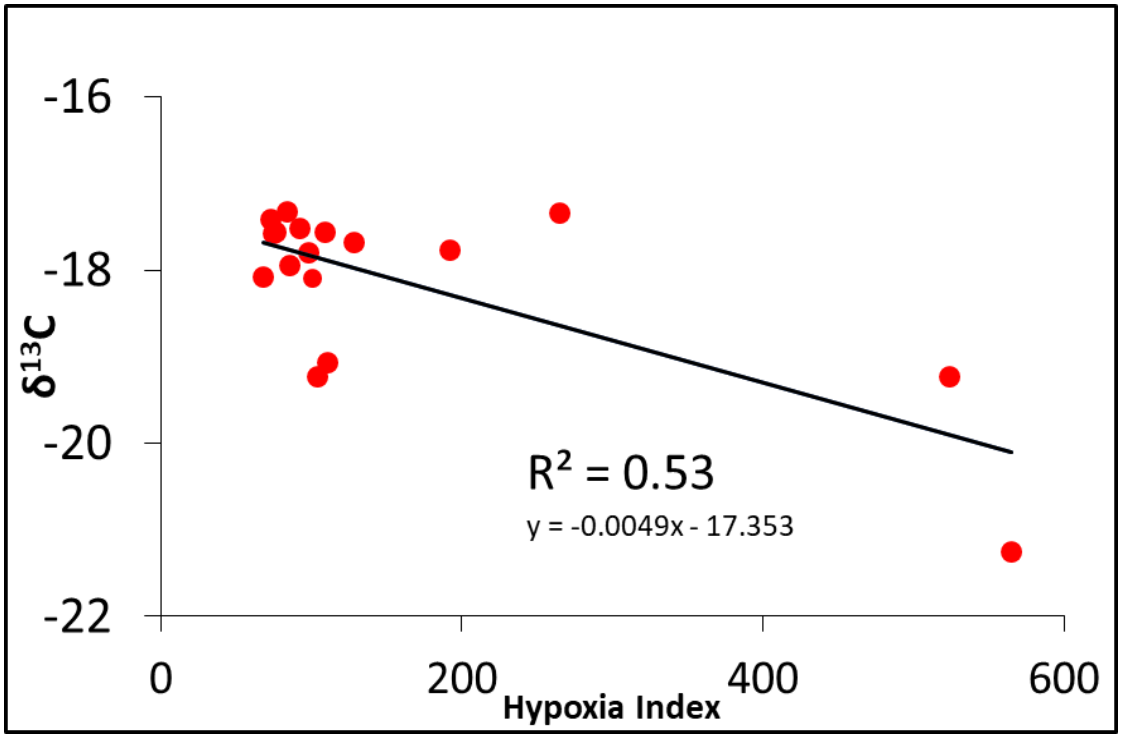

Fig. 10. Values of $\delta^{13}C$ and hypoxia exposure indices for 2015 Age-0 Atlantic Croaker

DISCUSSION

Benthic derived carbon is enriched in $\delta^{13}C$ relative to pelagic derived carbon (Post 2002). Compared to normoxic individuals, fish exposed to hypoxia have depleted $\delta^{13}C$ and expanded niche widths defined by standard ellipse area (SEA) indicating they feed more on pelagic prey. Normoxic fish showed tissue $\delta^{13}C$ values expected for individuals feeding more frequently on benthic prey, while hypoxic fish displayed significantly depleted mean $\delta^{13}C$ values expected from feeding more frequently on pelagic prey. This research combined otolith microchemistry with tissue stable isotopes to measure hypoxia-induced trophic shifts the Northern Gulf of Mexico. While individual variability in the response to hypoxia was observed in both otolith and stable isotope values amongst hypoxic and normoxic Atlantic Croaker, significant differences in trophic shifts existed each year between exposure groups. These findings support the hypothesis Atlantic Croaker experience trophic displacement from seasonal bottom water hypoxia.

Isotopic and trophic displacement was observed to be individually variable. The shape and direction of SEAs reveal individuals from normoxic and hypoxic groups both consumed benthic and pelagic prey. On average, isotope ellipses for hypoxic fish were displaced compared to ellipses from normoxic fish, yet substantial overlap between ellipses indicated that some hypoxic-exposed individuals continued to feed on benthic prey. Some individuals from the hypoxic group were displaced more towards the pelagic isotope endmember, indicating individual variability in benthic to pelagic trophic shifts is affected by the magnitude of hypoxia induced displacement. A potential explanation for this pattern is that Atlantic croaker are relatively tolerant to hypoxia exposure for at least short periods of time (Thomas and Rahman 2009, Mohan and Walther 2014). Thus, some individuals may be vertically displaced from benthic habitat temporarily, but still maintain the ability to forage on benthic prey and therefore

retain an isotopic signature of benthic food webs. Other individuals may be more severely displaced and forced to switch to pelagic diets. Evidence for this phenomenon was observed in the correlations between $\delta^{13}C$ and the hypoxia exposure index, suggesting individuals with more severe hypoxia exposure are more displaced to pelagic food webs. Thus, heterogeneous exposure histories may explain the variable displacement response observed in this study.

Ward's cluster revealed the majority of Atlantic Croaker collected in this study were grouped normoxic, with smaller groups of hypoxic and estuarine fish determined by otolith Mn:Ca ratios. Discounting estuarine fish, between 22-30% of Age-0 fish were exposed to offshore hypoxia, representing a significant proportion of the population. Previous research has indicated up to 34% of Atlantic Croaker in the nGoMex have previous exposure to hypoxia within their first year (Altenritter et al. 2018). Atlantic Croaker have shown relatively high tolerance to bottom water hypoxia. In the current study, 25% of individuals collected showed survival despite offshore hypoxia exposure in their first year of life. Thus, sublethal hypoxia exposure is not rare, and observed impacts on food web interactions could have significant implications for the structure and function of communities adjacent to hypoxia. Previous research has detailed hypoxia tolerance among Atlantic Croaker where they aggregate along the edges of hypoxic zones of estuaries off Chesapeake Bay, benefiting from stressed benthic prey (Long and Seitz 2008). This behavior is believed to favor increased consumption rates despite suboptimal oxygen conditions (Brady and Targett 2013). This study found Atlantic Croaker in the nGoMex have variable hypoxic response, which corresponds to variable dependency on benthic prey. Of note, individuals with the most severe hypoxia exposure were found to have the least proportion of benthic derived prey. Clearly hypoxia exposure is common for this species in

the Gulf of Mexico, and can manifest as sublethal effects in growth, habitat displacement and reproduction.

In both years, Atlantic Croaker grouped hypoxic showed reduced standard length (mm) and wet mass (g) compared to normoxic croaker. Reduced size following hypoxia exposure has been recorded by previous research (Rahman and Thomas 2011, Mohan et al. 2014, Altenritter et al. 2018). However, after return to normoxia, previously hypoxic individuals showed no significant difference in body size relative to normoxic fish (Rahman and Thomas 2011). The most commonly observed hypoxia adaptation resulted in damaged gametes (Thomas and Rahman 2010, Thomas and Rahman 2012). Thus, among Age 0 individuals, slight reductions in body length and mass have unknown impacts on long term condition, survival and reproductive fitness.

Hypoxia induced benthic habitat displacement was supported in this study using otolith microchemistry and tissue isotopes. Although some predators have shown benthic foraging on stressed prey without discernable harm, such as Lake Erie yellow perch (Roberts et al. 2011), energetic consequences of hypoxic hunting are most prevalent in respiration and reproductive organs of Atlantic Croaker (Thomas and Rahman 2012). Modeling reproductive impairment on the population level (Rose et al. 2009, Diamond et al. 2013, Rose et al. 2018a, Rose et al. 2018b) determined hypoxia exposure would decrease croaker stocks 25% over 40 years in the northern Gulf of Mexico. Population resiliency could be explained by the type of hypoxic conditions present in the Gulf of Mexico. Exposing fish to periodic hypoxic conditions has shown to be more stressful than continual exposure (Mohan et al. 2014). Once exposed to hypoxia, croaker likely avoid constant up- and down activation of molecular pathways. However, cellular adaptations for hypoxia tolerance are likely favored in a predator benefiting from hypoxia

exposed prey (Wu 2002), and Atlantic Croaker in the Gulf of Mexico experience energetic benefits and cost from seasonal hypoxia. The ultimate implication of benthic food web displacement following sub-lethal hypoxia is magnified predation of pelagic prey. Demersal fishes have shown resiliency and trophic flexibility with expanded foraging into the pelagic food web with unknown ecological consequence.

Hypoxia is implicated by two major ecological stress hypotheses, the Consumer Stress Model (CSM) and the Prey Stress Model (PSM)(Long and Seitz 2008). Outcomes of each model depend on the relative resilience of prey and predator to hypoxia. Lower consumer hypoxia tolerance and lower feeding rates of predator species will create prey refugia. Higher tolerance from consumers will elevate prey exposure and predation rates. Evidence from this study show pronounced individual hypoxic exposure and trophic variability in Atlantic Croaker populations of the northern Gulf of Mexico. This variation lends support to both CSM and PSM, but understanding the spread of hypoxia induced trophic behavior is more complex.

The trophic flexibility of Atlantic Croaker is a major factor in understanding food web shifts. Croaker are benthic omnivores with known predation of pelagic diet items (Sheridan et al. 1984, Nye et al. 2011). Pelagic and benthic prey consumption was supported in both groups by observed stomach contents (Altenritter et al. 2018, A Cohuo and T. Steube per comm) and total area dimensions. Research in the Chesapeake Bay (Long and Seitz 2008), Lake Erie (Roberts et al. 2011) and Gulf of Mexico (Craig 2012) found consumers will occupy margins of hypoxic zones, yet continue to feed on stressed benthic prey. Other hypoxic exposed individuals may have responded with partial shifts to pelagic food webs. With the larger proportion of their diet remaining associated with benthic sources, bulk $\delta^{13}C$ would still reflect benthic food web reliance. Individuals with partial shifts to pelagic food sources, but still feeding on benthic prey

have depleted $\delta^{13}C$ and are located near the hypoxic group SEA means. Individuals with the most depleted $\delta^{13}C$ values represent fish with the greatest proportion of pelagic prey and the largest observed trophic shifts. Isotopic mixing between food web source pools will combine into average bulk $\delta^{13}C$ values. The preference for benthic prey observed by demersal Atlantic Croaker is not expected to match pelagic endmember $\delta^{13}C$. More conservative mixing between pools would favor 1.0‰ to 2.0‰ shifts between benthic and pelagic sources rather than previously reported 4.0‰ shift between endmembers. Despite source pool mixing, observed benthic to pelagic shifts provides strong evidence hypoxia induced trophic shifts occur in demersal fish in the Gulf of Mexico.

Stable isotopes from muscle tissue represent a time integrated measure of trophic shifts. Where gut contents and HIF-1a can only determine instantaneous hypoxia exposure, stable isotopes allow investigation are highly effective at discerning environmental variation months prior to capture and supported by intensive use of $\delta^{13}C$ and $\delta^{15}N$ in trophic research (Layman et al. 2012). Studies featuring Atlantic Croaker in other locations have taken advantage of stable isotope food web tracers. For instance, Hazen et al. (2009) and Nye et al. (2011) tracked trophic shifts of Chesapeake Bay croaker to pelagic food webs in response to summer hypoxia. Other research has characterized the temporal and spatial variability of $\delta^{13}C$ and $\delta^{15}N$, deemed "isoscapes", in the Gulf of Mexico (Radabaugh et al. 2013). This current study found that niche width expansion of hypoxia exposed Atlantic Croaker supports bottom water displacement. Further, using species-specific tissue turnover rates established in previous research (Mohan et al. 2016), $\delta^{13}C$ reveals up to three months of variable trophic response.

For the current interpretation of isotope variability to be reflective of benthic-to-pelagic food web shifts, robust assessments of baseline isotope values for these two food webs must be

known. Many factors can affect $\delta^{13}C$ values. Hypoxia in the nGoMex is irregular during spring months and most prevalent June, July and August (Rabalais et al. 2001). Although hypoxia presented with a similar footprint in 2014 and 2015, precipitation, freshwater discharge, and other meteorological disparity could change the carbon baseline in benthic communities (Rabalais et al. 2007). The $\delta^{13}C$ baseline is determined from the photosynthetic fractionation of benthic algae and phytoplankton (Post 2002, Fry 2006). Any alteration to terrestrial carbon cycling could influence available marine dissolved organic carbon and dissolved inorganic carbon in turn shifting baselines (Moyer et al. 2015). Despite this potential for temporal and spatial variability, broad patterns in isotope differences between benthic and pelagic food webs in the system have been observed. Although diet items themselves were not directly measured for isotope composition, a series of prior measurements of benthic and pelagic endmember values have been made in the northern Gulf of Mexico that support the current interpretations. Observed values of $\delta^{13}C$ from offshore Atlantic Croaker ranged of -16.82‰ to -21.24‰ with a mean of -17.80‰ which falls within the range of previously recorded benthic derived values for the Gulf of Mexico (Radabaugh et al. 2013). Values $\delta^{13}C$ from hypoxic groups in this study measured 1.0‰ to 2.0‰ lower than normoxic groups. Previous research reports a -4.0‰ shift in $\delta^{13}C$ from benthic to pelagic webs along the Eastern continental shelf of the Gulf of Mexico (Radabaugh et al. 2013), and this magnitude of offset between benthic and pelagic food webs has been observed in other marine systems (Davenport and Bax 2002, Tamelander et al. 2006, Cherel and Hobson 2007, Miller et al. 2008, Woodland and Secor 2013, Kopp et al. 2015). Although a full shift of 4.0‰ between the hypoxic and normoxic groups was not observed, a smaller isotopic difference in consumers would be expected if individuals are continuing to feed at least partially in both food webs. Future work that directly measures isotope composition of

benthic and pelagic prey items for Atlantic Croaker would increase the confidence of the current interpretations, and could be used to parameterize mixing models that directly estimate the relative contribution of these food webs to consumer biomass.

For this study, $\delta^{15}N$ was used as a second trophic marker to help distinguish differences in food web participation. $\delta^{15}N$ is enriched 3.4‰ on average between trophic levels and is often used for separating primary from higher order consumers (Post 2002). Both years showed minimal (0.5- 1.0‰) differenced in $\delta^{15}N$ between hypoxic and normoxic fish. Age-0 Atlantic Croaker were assumed to feed at similar trophic levels and shifts in $\delta^{15}N$ were largely unknown because pelagic food webs differ from benthic webs in length and endmember values due to isotopic fractionation. However, increasing the number of trophic markers in stable isotope analysis allows for better resolution between source pools (Fry 2006), and $\delta^{15}N$ was valuable for quantifying the trophic shifts.

Management Implications

Management of Atlantic Croaker stocks in the nGoMex remain largely absent. The species currently has no Fisheries Management Plan in the Gulf of Mexico. Commercial fisheries harvested 737.5 metric tons worth $8,285,152 from 2000-2016 (NMFS 2018a). Over the same period recreational harvest exceeded 4,545.1 metric tons (NMFS 2018b). Commercial shrimp bycatch is another cause of mortality and between 2007 and 2010, 342 metric tons of croaker were harvested in coastal Louisiana and Texas (Scott-Denton et al. 2012). Although the current stock status of croaker in the Gulf of Mexico remains unknown, stock biomass has been determined to fall below maximum sustainable yield (Porch 2009). Effective management of this stock will require extensive knowledge of ongoing environmental stress. This study finds nGoMex Atlantic Croaker have highly variable trophic response to seasonal hypoxia that is

currently not addressed by density dependent mortality models. Assumptions that decreased available oxygenated bottom water habitat will increase density dependent mortality may not hold for individuals displaced to pelagic habitat (Brietburg et al. 2018). Predicting population-level effects of hypoxia on stocks is complicated, and often carries limited evidence for direct effects on production (Rose et al. 2009). Sustainability is likely determined by several interacting effects instead of direct hypoxia exposure. In the case of the nonexistent FMP for Gulf croaker, the resolution of hypoxia induced trophic response will benefit estimates of population productivity (Rose et al. 2009).

Seasonal hypoxic episodes are predicted to worsen in magnitude and area in the future (Rabalais et al. 2014). Oxygen availability is key in determining habitat suitability for marine fish. Because of increased thermal stratification and changes to freshwater input expected from climate change, low oxygen conditions are predicted to occur more frequently and over a greater geographic extent (Townhill et al. 2017). This may have negligible effect compared to continued anthropogenic nutrient input to the Gulf of Mexico. Without viable solutions to reducing the drivers of the northern Gulf, consumer species will be increasingly exposed to bottom water hypoxia. Results of this study ultimately ask what are the consequences of increased hypoxia exposure on consumer feeding ecology?

One major concern is the increased predation of pelagic prey by displaced demersal fishes. Several predictive models have been created to determine the long-term effects of hypoxia (Diamond et al. 2013, Rose et al. 2018a, Rose et al. 2018b), but none have addressed the impact of predation rated following benthic fish displacement in the nGoMex. This study presents individual variation within consumers as a major variable of interest for the Ecosystem Based Fisheries Management (EBFM). If hypoxia response is a combination of exposure

severity and individual variation, EBFM models will need to integrate consumer stress and prey stress models for demersal fish stocks in the northern Gulf of Mexico. Mechanisms controlling trade-offs between benthic trophic behavior and reproductive damage are complex, yet may have direct effects on pelagic food webs. Assuming uniform behavior in the face of hypoxia to build simple models may not be supported with the level of variation measure in this research. This data supplies the missing the linkage between individual resilience, stock effects, and broader ecosystem impact (Townhill et al. 2017).

Validated tissue isotope turnover of consumers provides a robust tool for establishing timelines of population response. Improving the quality of explanatory variables in predictive models will advance our understanding of hypoxia trophic interactions. As a recreationally and commercially important species (Diamond et al. 2013), investigation of hypoxia and population productivity can be established by integrating environmental stress with life history and known reproductive deficiencies (Rose et al. 2018a, Rose et al. 2018b). Improved model output will benefit EBFM management of Atlantic Croaker and enhance population productivity and stock health of additional recreational and commercial fisheries of in the Gulf of Mexico.

Future Directions

Atlantic Croaker consume a variety of benthic and pelagic prey in the Chesapeake Bay watershed including invertebrates, with polychaetes, mysids, amphipods and crustacea (Nye et al. 2011). Identification of nGoMex Atlantic Croaker prey item isotope $\delta^{13}C$ and $\delta^{15}N$ are currently underway for benthic and pelagic food webs. Tissue from benthic bivalves (*Amusium papyraceum, Macoma brevifrons)* and crustacea *(Callinectes similis, Penaeaus aztecus, Portunus gibesii, Rimapanaeus similis, Sicyonia dorsalis, Squilla Chydaea, Squilla empusa*) will represent

benthic endmembers and measured for $\delta^{13}C$ and $\delta^{15}N$. Prey items *Anchoa mitchilli, Anchoa hepsetus* and *Harangula jaguana* will represent pelagic endmembers. Together, characterizing the spatial and temporal isotopic diet signatures will provide higher resolution of trophic interactions in the Gulf of Mexico.

Summary

Investigation into the trophic response of fishes exposed to environmental stress is key to understanding population dynamics, which impacts effective fisheries management. Hypoxia is a known cause of sublethal stress among ground-fishes in the northern Gulf of Mexico, affecting nearly a quarter of Atlantic Croaker in 2014. Hypoxia exposed Croaker displayed significant shifts in baseline $\delta^{13}C$ relative to normoxic individuals, which indicated greater predation on pelagic prey following bottom water displacement. Croaker were also shown to respond to hypoxia with individually variable trophic shifts with some individuals found associated with benthic $\delta^{13}C$ while others showed pelagic $\delta^{13}C$ signatures. The largest pelagic shifts were found in fish with maximal hypoxia exposure. Displacement from benthic food webs may have deleterious effects on population growth, migration and reproduction. Likewise, the increased predation of pelagic prey on broader ecological interactions remains unknown. Understanding interaction between hypoxia and feeding ecology response in Atlantic Croaker will inform sustainable fisheries and EBFM in light of anticipated environmental stress and long-term ecosystem change affecting the Gulf of Mexico.

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APPENDIX

Walther TAMU-CC (Steube) Tissue Samples				
Sample analyzed: July 10, 2017				
Total Samples: 272				
Correlates to Data Sheets: Steube EA-DeltaV-CN-17-01				
Comments:				
Unique ID	**Year**	**Tissue**	**δ15N (AIR)**	**δ13C (VPDB)**
174	2014	Muscle	15.9	-17.4
148	2014	Muscle	16.5	-17.3
52	2014	Muscle	16.1	-18.3
58	2014	Muscle	16.0	-18.1
106	2014	Muscle	17.0	-17.5
156	2014	Muscle	14.5	-17.7
156 (duplicate)	2014	Muscle	14.6	-17.8
71	2014	Muscle	15.3	-17.9
103	2014	Muscle	16.3	-18.0
17	2014	Muscle	14.9	-17.4
14	2014	Muscle	18.3	-17.7
15	2014	Muscle	15.3	-17.6
192	2014	Muscle	14.4	-17.1
424	2014	Muscle	15.8	-17.5
325	2014	Muscle	14.1	-19.8
190	2014	Muscle	15.6	-17.4
263	2014	Muscle	19.3	-18.7
248	2014	Muscle	15.8	-17.3
210	2014	Muscle	15.7	-17.3
268	2014	Muscle	18.5	-18.0
363	2014	Muscle	14.9	-16.8
246	2014	Muscle	15.7	-18.2
288	2014	Muscle	12.7	-18.5
178	2014	Muscle	15.3	-17.9
267	2014	Muscle	16.5	-17.6
59	2014	Muscle	16.0	-17.7
105	2014	Muscle	16.9	-17.2
95	2014	Muscle	14.6	-18.1
184	2014	Muscle	14.6	-17.2
184 (duplicate)	2014	Muscle	14.7	-17.3

193	2014	Muscle	15.6	-17.7
107	2014	Muscle	15.9	-17.7
248 (duplicate)	2014	Muscle	15.8	-17.8
324	2014	Muscle	14.1	-17.6
253	2014	Muscle	16.0	-18.1
213	2014	Muscle	15.1	-17.6
112	2014	Muscle	16.4	-17.3
110	2014	Muscle	14.8	-17.5
169	2014	Muscle	16.6	-17.6
319	2014	Muscle	14.3	-18.0
56	2014	Muscle	15.8	-17.6
102	2014	Muscle	15.9	-17.7
96	2014	Muscle	16.0	-17.7
6	2014	Muscle	14.4	-17.2
142	2014	Muscle	15.6	-17.6
142 (duplicate)	2014	Muscle	15.6	-17.5
70	2014	Muscle	15.9	-17.9
97	2014	Muscle	16.0	-17.6
92	2014	Muscle	15.3	-17.9
67	2014	Muscle	15.0	-17.5
4	2014	Muscle	15.6	-17.1
10	2014	Muscle	15.4	-17.5
7	2014	Muscle	15.5	-17.0
1	2014	Muscle	15.4	-17.7
250	2014	Muscle	15.7	-17.5
320	2014	Muscle	13.5	-18.6
172	2014	Muscle	15.1	-17.8
182	2014	Muscle	14.9	-17.6
264	2014	Muscle	16.7	-17.3
244	2014	Muscle	16.1	-17.4
187	2014	Muscle	15.7	-17.6
266	2014	Muscle	16.6	-17.5
285	2014	Muscle	16.0	-18.5
285 (duplicate)	2014	Muscle	16.0	-18.3
429	2014	Muscle	15.6	-17.7
249	2014	Muscle	15.9	-18.8
171	2014	Muscle	15.9	-18.0
136	2014	Muscle	15.2	-17.6
162	2014	Muscle	14.6	-17.8

188	2014	Muscle	15.8	-17.9
261	2014	Muscle	17.6	-18.2
261 (duplicate)	2014	Muscle	17.5	-18.2
270	2014	Muscle	18.1	-19.0
251	2014	Muscle	15.8	-18.6
262	2014	Muscle	18.0	-19.4
421	2014	Muscle	18.1	-18.4
48	2014	Muscle	15.3	-17.0
48	2014	Muscle	15.3	-17.1
94	2014	Muscle	15.0	-17.7
198	2014	Muscle	15.0	-17.7
155	2014	Muscle	15.9	-18.9
99	2014	Muscle	16.9	-17.7
422	2014	Muscle	16.1	-17.8
422 (duplicate)	2014	Muscle	16.0	-17.8
423	2014	Muscle	15.3	-17.4
154	2014	Muscle	15.1	-17.4
147	2014	Muscle	16.5	-17.5
152	2014	Muscle	15.9	-18.3
65	2014	Muscle	15.5	-18.1
65 (duplicate)	2014	Muscle	15.6	-18.1
57	2014	Muscle	15.4	-16.8
100	2014	Muscle	16.4	-18.4
104	2014	Muscle	16.8	-17.8
5	2014	Muscle	19.7	-19.2
85	2014	Muscle	13.9	-18.6
164	2014	Muscle	14.0	-18.9
53	2014	Muscle	15.0	-18.2
143	2014	Muscle	15.1	-18.1
85 (duplicate)	2014	Muscle	15.3	-20.5
164 (duplicate)	2014	Muscle	15.3	-20.6
53 (duplicate)	2014	Muscle	14.6	-20.7
143 (duplicate)	2014	Muscle	14.6	-20.7
1140	2015	Muscle	15.9	-17.7
1274	2015	Muscle	16.3	-17.7
1119	2015	Muscle	15.7	-17.6
1093	2015	Muscle	15.8	-17.7
883	2015	Muscle	15.5	-17.5
1272	2015	Muscle	15.8	-19.2

958	2015	Muscle	15.3	-17.7
912	2015	Muscle	16.0	-17.5
912 (duplicate)	2015	Muscle	16.0	-17.5
485	2015	Muscle	15.7	-17.5
680	2015	Muscle	16.1	-17.0
1258	2015	Muscle	15.5	-17.6
819	2015	Muscle	15.6	-17.8
1257	2015	Muscle	15.2	-17.5
566	2015	Muscle	15.5	-17.7
1081	2015	Muscle	15.9	-17.9
1098	2015	Muscle	16.1	-17.5
1197	2015	Muscle	15.0	-17.9
492	2015	Muscle	15.6	-17.6
818	2015	Muscle	15.4	-17.6
1124	2015	Muscle	15.5	-17.4
1295	2015	Muscle	15.6	-17.1
895	2015	Muscle	15.5	-17.6
1085	2015	Muscle	17.2	-18.6
890	2015	Muscle	15.1	-17.9
1273	2015	Muscle	16.8	-18.7
568	2015	Muscle	15.6	-18.1
975	2015	Muscle	15.2	-18.1
926	2015	Muscle	15.9	-17.8
1291	2015	Muscle	15.4	-17.3
811	2015	Muscle	15.7	-19.9
856	2015	Muscle	15.4	-17.4
884	2015	Muscle	15.9	-18.6
552	2015	Muscle	15.2	-18.0
535	2015	Muscle	15.8	-18.0
529	2015	Muscle	15.5	-17.6
477	2015	Muscle	16.0	-18.4
1289	2015	Muscle	15.6	-17.4
531	2015	Muscle	15.5	-17.7
1083	2015	Muscle	15.5	-17.9
939	2015	Muscle	15.7	-17.8
809	2015	Muscle	16.1	-18.2
815	2015	Muscle	13.7	-19.7
1261	2015	Muscle	16.2	-17.5
962	2015	Muscle	15.8	-17.9

1196	2015	Muscle	15.2	-17.0
584	2015	Muscle	16.9	-21.2
1073	2015	Muscle	15.9	-17.5
545	2015	Muscle	15.6	-17.3
896	2015	Muscle	15.9	-18.0
544	2015	Muscle	15.7	-17.8
1133	2015	Muscle	15.7	-17.9
488	2015	Muscle	15.9	-17.9
954	2015	Muscle	15.8	-17.6
543	2015	Muscle	15.7	-17.7
1141	2015	Muscle	15.9	-17.8
625	2015	Muscle	14.6	-17.2
1091	2015	Muscle	16.5	-17.6
632	2015	Muscle	15.6	-17.6
595	2015	Muscle	15.5	-17.5
1275	2015	Muscle	15.7	-17.3
1210	2015	Muscle	14.7	-17.0
516	2015	Muscle	15.4	-17.4
961	2015	Muscle	15.1	-17.9
827	2015	Muscle	15.9	-17.7
976	2015	Muscle	15.5	-17.6
956	2015	Muscle	14.3	-18.3
542	2015	Muscle	15.5	-17.5
1049	2015	Muscle	14.4	-17.1
540	2015	Muscle	15.7	-17.8
1123	2015	Muscle	15.6	-17.0
1296	2015	Muscle	15.5	-17.8
489	2015	Muscle	15.6	-17.8
857	2015	Muscle	15.2	-17.7
639	2015	Muscle	17.0	-17.6
1136	2015	Muscle	15.6	-17.4
1277	2015	Muscle	15.9	-17.5
536	2015	Muscle	15.4	-17.6
878	2015	Muscle	15.6	-17.4
880	2015	Muscle	15.5	-17.5
824	2015	Muscle	15.5	-17.7
567	2015	Muscle	15.4	-17.8
974	2015	Muscle	14.9	-16.9
521	2015	Muscle	18.0	-19.1

478	2015	Muscle	15.8	-17.8
822	2015	Muscle	15.6	-17.7
469	2015	Muscle	15.5	-19.2
574	2015	Muscle	15.6	-17.6
674	2015	Muscle	15.5	-17.5
821	2015	Muscle	15.9	-18.1
1088	2015	Muscle	15.4	-17.6
1106	2015	Muscle	16.1	-17.6
666	2015	Muscle	15.2	-17.8
1209	2015	Muscle	15.3	-17.7
1101	2015	Muscle	16.7	-17.8
851	2015	Muscle	14.3	-18.2
1300	2015	Muscle	16.3	-17.2
863	2015	Muscle	14.7	-18.1
270	2014	Liver	15.2	-19.0
182	2014	Liver	13.2	-18.4
263	2014	Liver	15.6	-19.7
96	2014	Liver	13.3	-19.2
250	2014	Liver	14.4	-19.3
429	2014	Liver	13.5	-19.6
110	2014	Liver	13.1	-18.5
324	2014	Liver	12.7	-19.2
261	2014	Liver	15.2	-19.3
264	2014	Liver	14.5	-19.7
99	2014	Liver	15.6	-19.8
210	2014	Liver	13.8	-19.2
15	2014	Liver	14.4	-18.5
213	2014	Liver	13.3	-19.7
320	2014	Liver	12.2	-19.5
94	2014	Liver	13.6	-19.4
105	2014	Liver	14.6	-18.9
246	2014	Liver	14.8	-20.3
363	2014	Liver	13.0	-18.1
422	2014	Liver	13.6	-19.4
423	2014	Liver	13.3	-19.5
249	2014	Liver	14.1	-19.6
192	2014	Liver	13.4	-18.0
102	2014	Liver	14.2	-18.9
253	2014	Liver	14.4	-20.0

267	2014	Liver	14.7	-19.5
5	2014	Liver	16.5	-20.7
6	2014	Liver	12.8	-18.8
10	2014	Liver	13.1	-18.5
268	2014	Liver	15.4	-19.1
266	2014	Liver	14.4	-19.4
104	2014	Liver	15.1	-20.0
171	2014	Liver	14.0	-19.7
251	2014	Liver	14.2	-19.4
198	2014	Liver	13.2	-19.9
178	2014	Liver	13.9	-18.3
1141	2015	Liver	14.2	-19.9
567	2015	Liver	13.3	-19.3
1300	2015	Liver	13.8	-18.7
1273	2015	Liver	14.4	-19.7
595	2015	Liver	12.7	-19.8
956	2015	Liver	12.9	-19.6
1295	2015	Liver	13.1	-19.3
1196	2015	Liver	12.2	-18.2
822	2015	Liver	13.3	-19.8
809	2015	Liver	14.0	-20.9
1091	2015	Liver	13.4	-19.2
1257	2015	Liver	13.0	-19.4
569	2015	Liver	12.9	-19.0
1210	2015	Liver	12.2	-18.0
961	2015	Liver	13.4	-19.7
1274	2015	Liver	14.8	-19.5
566	2015	Liver	13.1	-19.5
545	2015	Liver	14.1	-19.4
516	2015	Liver	13.2	-19.0
1124	2015	Liver	14.1	-19.5
1127	2015	Liver	13.7	-18.6
680	2015	Liver	14.9	-18.9
1197	2015	Liver	12.7	-19.4
544	2015	Liver	13.0	-19.3
639	2015	Liver	14.7	-19.7
1073	2015	Liver	13.9	-19.0
1136	2015	Liver	13.8	-19.5
625	2015	Liver	12.1	-18.5

552	2015	Liver	14.4	-19.0
912	2015	Liver	14.1	-19.5
1209	2015	Liver	12.8	-19.5
1083	2015	Liver	13.4	-19.9
1119	2015	Liver	13.3	-19.4
895	2015	Liver	13.4	-19.6
585	2015	Liver	15.8	-17.9
469	2015	Liver	13.8	-20.1