THE EFFECT OF SEASONAL HYPOXIA
ON GROUNDFISH IN THE NORTHERN GULF OF MEXICO

AN ABSTRACT
SUBMITTED ON THE TWENTY NINTH DAY OF JULY 2020
TO THE DEPARTMENT OF ECOLOGY AND EVOLUTIONARY BIOLOGY
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
OF THE SCHOOL OF SCIENCE AND ENGINEERING OF
TULANE UNIVERSITY
FOR THE DEGREE
OF
DOCTOR OF PHILOSOPHY
BY

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ABSTRACT

The goal of this dissertation was to identify the large scale effects that seasonal hypoxia has on the benthic community, with a particular focus on groundfish, in the Gulf of Mexico (GOMEX) while also examining ecological and physiological factors that could explain how hypoxia alters benthic communities. Seasonal hypoxia is a rapidly growing threat, not just in the GOMEX, but globally. Hypoxic conditions are known to impact marine organisms at the individual level by altering behavior and reproductive physiology while also impacting marine communities by disrupting predator prey interactions, community biomass, community composition, and community spatial dynamics. Research into hypoxia in the GOMEX has historically been localized, focusing on specific sub-regions with temporally limited sampling. While this approach has produced findings of the highest quality and importance, it has also shown that the impacts of hypoxia can be variable making the overall impact of hypoxia on GOMEX benthic communities difficult to discern. In taking both a geographically and temporally broad approach in comparing the abundances of marine organisms between hypoxic sites and normoxic (normal levels of dissolved oxygen, not hypoxic) sites I found that hypoxic sites had significantly lower biodiversity compared to normoxic sites and that 102 out of 465 examined species had significantly lower abundances in hypoxic areas compared to normoxic areas. When I compared the diets of common groundfish species from hypoxic areas to the diets of the same species from normoxic areas a few key differences were noted for some species, while the diets of other species remained relatively unchanged. After comparing the reproductive condition and presence/absence of ovarian masculinization between hypoxic areas and normoxic areas in three species of groundfish, I found evidence of ovarian masculinization in all three species, and evidence of reproductive impairment in two species. In this dissertation I

showed that hypoxia in the GOMEX alters the community composition and biodiversity of the benthic community, additionally finding evidence that hypoxic conditions alter the diets and reproductive biology of several fishes.

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INTRODUCTION

In the last century, coastal hypoxia has emerged as one of the biggest threats to coastal ecosystems. Anthropogenic coastal hypoxia has been observed as early as the 1930's [1] and occurrences of coastal hypoxia have been exponentially increasing since the 1960's [1,2]. Aquatic hypoxia refers to areas of a water body with low levels of dissolved oxygen. The range of dissolved oxygen values used to describe an area as "hypoxic" is not universal; but it has been typically used to describe dissolved oxygen values between 0.2-3.0 mg/L [3]. Coastal hypoxia is caused when organic material is broken down by microorganisms in the lower strata of a stratified water column. The increased microbial respiration depletes the dissolved oxygen in the lower strata; and the dissolved oxygen is unable to diffuse into these lower strata due to density differences in the water column. Once these lower strata are depleted of oxygen they will not be replenished unless the water column is physically mixed or all strata reach the same density.

The global increase in the occurrence of coastal hypoxia can mostly be attributed to the augmented use of synthetic nitrogen fertilizers since their discovery in the 1940's [1,4]. The increased Nitrogen runoff from these fertilizers results in large algal blooms in coastal waters. When these algal blooms die, their decomposition uses up much of the dissolved oxygen in the water column. This process can result in hypoxia if the water column is sufficiently stratified. It is worth noting that coastal hypoxia can be a naturally occurring phenomenon; areas of high nutrient upwelling in locations such as the eastern Pacific, southeast Atlantic, and the northern Indian ocean create large areas of hypoxia [5].

The Gulf of Mexico (GOMEX) is one of the most important and productive bodies of water in the world. In 2014 the GOMEX was responsible for over one billion dollars in overall fisheries landing revenue, over 11 billion dollars in recreational fishing equipment revenue, and nearly 28 billion dollars in seafood processing/sales revenue [6]. The GOMEX also holds a great deal of cultural and spiritual value to many people in the region. Unfortunately, the GOMEX also seasonally experiences the second largest zone of hypoxia in the world [3]. This zone of hypoxia is fueled by intense nutrient pollution fueled by non-point source agricultural runoff, feedlot operations, and point-source pollution from industrial plants, and municipal waste treatment facilities throughout the Mississippi river watershed [7]. The amount of nutrient pollution reaching the GOMEX is not surprising given the size of the Mississippi River Basin, which drains approximately 41% of the continental United States [8,9].

Hypoxia in the northern GOMEX is typically a summer phenomenon, however hypoxic zones can begin to form as early as February and persist into October [10]. GOMEX hypoxia has been known to occur in depths between four and 60 meters, however it is most common between five and 30 meters. The offshore extent of hypoxia in the northern GOMEX is dependent upon the slope of the continental shelf, thus hypoxia typically occurs farther offshore in the northwestern and northcentral Gulf (as far as 130km) where the shelf is more gradually sloped than in the more steeply sloped northeastern Gulf (55km). While hypoxia typically impacts the bottom most layers of the water column, the vertical extent of hypoxia in the water column can be variable, with the main determinates being the depth of the water and the pycnocline [10].

Since elemental oxygen is essential for cellular respiration in most organisms, it is not surprising that hypoxia is known to have a litany of effects on marine organisms. Aquatic hypoxia is known to reduce the activity level of many marine organisms. Atlantic cod, for example, exposed to hypoxic conditions showed a reduction in swimming behavior [11]. The onset of hypoxia has also been linked to a reduction in the movement of aquatic invertebrates [12] as observed in the Norway lobster [13].

The effect that hypoxia has on the behavior of marine organisms is well documented. Marine fishes and invertebrates are known to reduce their feeding in response to hypoxia [12] as observed in the European seabass [14], the lesser blue crab, the blue crab, oyster drills [15], and tubicolous polychaetes [16]. Field and laboratory experiments suggest that fish and crustaceans [12,17] are able to detect hypoxic conditions and often respond by migrating to areas of higher dissolved oxygen. In response to hypoxia, infaunal marine invertebrates have been observed to migrate up in the sediments or, in the case of bivalves, extend their siphons into the water column in an effort to reach higher concentrations of dissolved oxygen [12]. Examples of these migrations include Chesapeake bay polychaetes and bivalves [18], Swedish soft bottom macrofauna [19], and hypogean amphipods and epigean isopods [20].

The effects of hypoxia can extend far beyond individual organisms; a variety of ecological level effects having been described in the literature. Species interactions, for example, have been shown to be impacted by hypoxia. Hypoxic conditions have been experimentally shown to alter the functional response of a predatory isopod to amphipod prey [21]; and increased predation and predation risk has been associated with the hypoxia avoidance behavior in Chesapeake bay polychaetes and bivalves [18]. The opposite situation was observed in a field experiment by Nestlerod and Diaz (1998). Tethered annelids were found to be approximately 82 times less likely to be consumed by predators in hypoxic waters compared to normoxic water [22].

Hypoxia has also been shown to alter species composition and community biomass. Areas of the Lower Chesapeake bay exposed to hypoxia, for example, contained communities made up of more opportunistic species compared to the upper bay [23]. In the Kattegat Bay, hypoxia has been linked to changes in the benthic fauna, which is evident in the diet of demersal fish from the region [24]. Reduced fish biomass in response to hypoxia has been well documented in the Chesapeake bay [23,25,26], and the Swedish Kattegat [27-29].

Since coastal hypoxia is typically a seasonal phenomenon, hypoxia exposed communities often have opportunities to recover from the hypoxic event. However, community recovery can be highly variable and depends on a variety of environmental and biological factors [12], such as community complexity and the severity/duration of hypoxia [30]. The variability of community recovery following a hypoxic event is evident when comparing case studies from different communities. For example, an experimental study in Hong Kong showed that community composition of defaunated sediment reached 60 percent similarity with the local community within five months of return to normoxic conditions, and 94 percent similarity within 15 months [31]. High mortality from an 8000 km^2 hypoxic event in New Jersey that lasted two months was followed quickly by the recolonization of the larvae of opportunistic species [32]. In contrast, the macrobenthic fauna of the Swedish Gullmarsfjord had not recovered 18 months after an extreme hypoxic event eliminated all macrobenthic fauna below 115m [33] and the nematode community structure in the same area had failed to return to the pre-hypoxia state a year after the same hypoxic event [34]. Similarly, the benthic community in the Adriatic sea was found to have only returned to 36% of its original biomass three years after a multi-day anoxic (no dissolved oxygen) event killed almost 90% of all benthic fauna [35].

The collective body of research focusing on hypoxia’s effect on marine ecosystems underscores the complexity and variability associated with how hypoxia impacts marine organisms. The goal of this dissertation is to better understand how hypoxia impacts marine communities in the GOMEX, specifically by addressing how the GOMEX effects the community composition while also exploring potential ecological and physiological factors that might contribute to changes in community composition in hypoxic areas. This dissertation is organized into three chapters, with the first chapter serving as a broad comparison of species composition/abundances and biodiversity between hypoxic sampling sites and normoxic sampling sites with the second and third chapters focusing on ecological and physiological factors, respectively, that could be used to explain differences in species abundance/community composition observed in the first chapter.

In my first chapter I compare the abundances of species caught during annual Southeast Area Monitoring and Assessment Program (SEAMAP) bottom trawl surveys between hypoxic and normoxic sampling sites between 2001 and 2011 with the goal of identifying the species most effected by hypoxic conditions. I also compared the average biodiversity at each sampling site between hypoxic and normoxic sites from 1988 to 2011. For my second chapter I compare the diets and diet overlap of common groundfish species between fishes sampled from hypoxic sites and fishes sampled from normoxic sites. Additionally, I use metrics of diet diversity and diet individualism to further identify any changes in diet associated with hypoxia. My third chapter focuses on how the reproductive biology of fishes is affected by hypoxia exposure. In this chapter I compare the overall condition, gonadal health, and presence/absence of ovarian masculinization in three species of common GOMEX groundfish sampled at hypoxic sites with fish sampled at normoxic sites

CHAPTER 1

INTRODUCTION

Despite its economic and ecological importance, research on how seasonal hypoxia impacts the northern GOMEX is relatively sparse and mostly focuses on economically important species. The occurrence of Atlantic croaker and Brown shrimp, for example, has been shown to be significantly impacted by seasonal hypoxia, with the occurrences of both species declining in waters with dissolved oxygen below 2mg/L. Hypoxia was also shown to have altered the water temperatures and depth distribution in which Brown shrimp were found most frequently with shrimp occupying inshore and offshore waters over moderate depths in the presence of severe hypoxia, potentially exposing them to sub-optimal temperature regimes [36]. Severe hypoxia has also been found to reduce the available suitable habitat available for flatfish in the northern GOMEX, particularly for inshore species [37]. Severe hypoxia in the northern GOMEX has also been associated with decreases in macrobenthic (invertebrates collected with sediment corer) diversity and increases in megabenthic diversity (invertebrates and fish collected via trawl) [38]. The response of benthic communities in the northern GOMEX has led some to suggest that cumulative annual hypoxia might be more important than individual incidences of hypoxia in structuring benthic invertebrate communities in the Northern Gulf of Mexico [38,39].

In the northern GOMEX, hypoxia has been shown to impact the abundances and distribution of many different groundfish and benthic invertebrates. In a study of four benthic sites in the northern GOMEX with different dissolved oxygen regimes, the site that experienced the lowest average dissolved oxygen between 2000-2010 was found to contain fewer of the normally more common sciaenid and larger crustacean species, while containing more of the normally less common small groundfish species. In the same study, fish at the site with the lowest historic dissolved oxygen values tended to be smaller than fish sampled from sites with higher historic dissolved oxygen values [38]. In a different three year study of the spatial distribution/abundance of several GOMEX groundfish species, the majority of the studied species displayed oxygen avoidance thresholds at or near published species specific lethal limits [40]. In the same three year study, oxygen avoidance thresholds for Atlantic croaker were found to be below levels that are experimentally shown [41] to impair gametogenesis and gonadal growth in Atlantic croaker [40].

While avoidance of hypoxic waters is a well-documented response of some aquatic organisms, others show a preference for areas of hypoxia. Cownose rays in the northern GOMEX are known to congregate in pelagic waters above hypoxic benthic conditions [42]. In the Chesapeake bay, benthic invertebrate predation was found to be higher after hypoxic events [43]. An acoustic study of anchovy movement in the Neuse River estuary, observed rapid forays by anchovies into the hypoxic zone at the same time that zooplankton were aggregated there, suggesting that anchovies were foraging in the hypoxic zone [44]. This phenomenon is not limited to marine waters as mudminnows in the stratified Trout Lake are known to forage in the hypoxic bottom waters during the day in pursuit of phantom midge larvae [45].

Previous work on hypoxia in the GOMEX, has been more localized in sampling location and sampling year producing results that highlight the variability and complexity of hypoxia’s effect on benthic communities. These data are invaluable, enlightening, and of the utmost importance in advancing the collective understanding of how hypoxia effects the ecology of the GOMEX, however the more localized scale of much of this research makes drawing broader conclusions regarding hypoxia’s impact on GOMEX benthic communities difficult. A broader understanding of how a phenomenon like hypoxia impacts important natural resources is crucial in communicating with stakeholders, policy makers, and fisheries managers. My first chapter aims to develop a better understanding of the large-scale impacts that hypoxia has on the GOMEX benthic community. In this chapter I utilized fishery independent bottom trawl data from SEAMAP to understand how hypoxia effects the species composition and biodiversity of GOMEX benthic communities. This chapter is broad in its scale, both temporally and geographically, so that overarching effects of hypoxia on species composition in the GOMEX can be assessed.

HYPOTHESES

Biodiversity

H_0: The average biodiversity at hypoxic sites and the average biodiversity at normoxic sites will be equal, as either DO has no effect on biodiversity, or my definition of hypoxia (2 mg/L) is too high to observe a difference in biodiversity.

H_A: The average biodiversity at hypoxic sites will be significantly lower than the biodiversity at normoxic sites because marine organisms are known to avoid hypoxic conditions. Additionally, hypoxic conditions are known to reduce the abundance and diversity of benthic and infaunal invertebrates which make up the diets of much of the groundfish species in the GOMEX. I do not anticipate edge effects or potential foraging opportunities to significantly impact this prediction because, despite the fact that the phenomena of organisms aggregating at the edges of hypoxic sites and organisms utilizing the hypoxic zone as a foraging opportunity are well documented, the number of species utilizing hypoxic zones is likely in the minority.

Abundance

H_0: Hypoxic conditions will have no effect on the abundance of species caught in SEAMAP summer groundfish cruises, with the average yearly Catch per Unit Effort (CPUE) at hypoxic sites being indistinguishable from the average yearly CPUE from normoxic sites. In other words, the average biomass of a species caught per square Kilometer sampled will be the same from hypoxic sites and normoxic sites.

H_A: Due to species differences in motility, physiological tolerances, trophic ecology, and behavior differences in average CPUE between hypoxic and normoxic sites will be variable at the species level. I predict that many species, due to a combination of the factors mentioned above, will have lower average yearly CPUE values at hypoxic sites compared to normoxic sites. I also predict that some species will have higher average yearly CPUE values at hypoxic sites compared to normoxic sites. I believe that these species will be in the minority, but that the potential increased foraging opportunities and other edge effects at some hypoxic sites either attract or promote higher abundances of “hypoxia specialists” or hypoxia tolerant species.

METHODS

Source Data

I utilized the Southeast Area Monitoring and Assessment Program (SEAMAP) summer groundfish survey as the source of fishery-independent catch data and environmental/geographical data for this study, using both extrapolated count and catch data (calculated by SEAMAP), because SEAMAP sampling protocols involve extrapolating fish counts and weights by subsampling from larger catches. Extrapolated counts and weights for species are calculated using the total catch weight (always recorded regardless of the catch size) and the sub-sampled counts and catch weights for each species. The exact methodology of calculating the extrapolated catch values can be found in the SEAMAP Trawl and Plankton survey operations manual. For comparisons and analyses comparing the abundances or occurrences of individual species and overall species composition, I used sampling events between 2001 and 2011 as these years occur after the most recent changes to the survey methodology standards. Statistical tests for any comparisons or analyses regarding biodiversity or species counts utilized years before 2001. These analyses were always run a second time, excluding the data prior to 2001, to ensure that the addition of years before the most recent sampling standardization did not drive any statistically significant differences.

Hypoxia Definition

I defined two levels of DO to compare, hypoxic and normoxic. I defined hypoxic stations as stations with DO values less than or equal to 2.0 mg/L and I defined stations as normoxic if DO values were above 2.0 mg/L. 2.0 mg/L has been demonstrated to be the threshold at which marine benthic communities begin to change rapidly [46] as well as the threshold at which fishery collapses begin to occur [47]. Additionally, a review of marine biodiversity thresholds reported that 55 percent of studies used 2.0 mg/L or lower as the definition of hypoxia with the average value used being 2.28 mg/L DO [2].

Species Composition

In order to compare species composition between hypoxic and normoxic sites, I filtered the SEAMAP data using Non-Metric Multidimensional Scaling (NMDS) Analysis, Breiman's random forest model algorithm [48] (referred to as Random Forest Modelling hereafter), and PERMANOVAs. I used the biomass catch per unit effort (CPUE) for all species found at minimum at one percent of stations either east or west of Mobile Bay as the variables, with sites corresponding to sampling events (stations). These data were used to run a NMDS Analysis (k=4, random starts=100) and a Random forest model (562 species tried per node, number of trees=1000) with the categorical "east or west" of Mobile Bay as the dependent variable. Additionally, I ran a PERMANOVA using the same dependent and independent variables as the Random forest model. I used a combination of visual inspection of the NMDS plot, ability of the Random forest model to identify stations as either "east or west" of Mobile Bay, and the results of the PERMANOVA to determine if the species compositions were different enough "east or west" of Mobile Bay in order to justify the exclusion the eastern stations from analysis. After geographical filtering I employed the same methodology of using NMDS analysis, Random forest modelling, and PERMANOVA to further filter the data along different environmental gradients (i.e., depth).

Biodiversity Comparisons

I employed the Shannon diversity index as the measure of yearly species diversity. The Shannon Index is based on the idea of uncertainty, functioning as a measure of the ability to predict the species of an individual, randomly drawn from a community. The index value increases as a community gains more individuals and as the abundances of different species become more similar to one another. I chose to utilize the Shannon index because it takes into account both species richness and evenness [49], and has been used by others to describe groundfish community diversity [50-52]. I used the R package vegan to calculate the Shannon diversity index.

In order to generate a single average Shannon index value for both hypoxic and normoxic sites, I utilized the average Shannon index value for hypoxic or normoxic sites for each sampling year as replicates. I generated the average Shannon index value for each sampling year by averaging the Shannon Diversity Index value for each sampling event for each year between 1984 and 2011 by DO (hypoxic or normoxic sites). This method essentially treats individual stations as sub-replicates within their respective sampling year, with the sampling year serving as the replicate. I used a Kruskal-Wallis tests to compare Shannon diversity between hypoxic and normoxic sites, using years as replicates, with significance defined at the 0.05 level in the r Stats package. I used the vegan package in R to calculate rarefaction curves for each year and DO level (hypoxic or normoxic) between 2001 and 2011 using pooled yearly species count data, splitting the species count data into fishes, crustaceans, and mollusks so that differences in these taxonomic groupings could be visualized. I utilized years prior to 2001 in these biodiversity analyses in order to gain a wider temporal perspective. Additionally, the ability to utilize rarefaction curves to assess potential under sampling related biases mitigates potential confounding factors related to changes in sampling protocols.

Catch Per Unit Effort (CPUE) and Percent Occurrence at Depth Calculation and Comparison

Species abundance was assessed using biomass as opposed to species count. Due to the number of species included in this study, biomass offers a better measure of abundance, as biomass requires less interpretation relative to individual species which can vary a great deal in size. I expressed species abundances in the terms of catch per unit effort (CPUE) in order to account for slightly different sampling times and sampling gear sizes (length of net opening), inherent in the SEAMAP sampling protocols. I defined CPUE as the biomass of a species caught per Km^2 as this accounted for both differences in sampling time and the smaller net used by Texas Wildlife and Fisheries. In order to compare species abundances between hypoxic and normoxic sites I summed the total yearly species catch for each year for stations between 0-25m for both hypoxic sites and normoxic sites, taking the mean of the years and comparing them using a Kruskal-Wallis test ($p<0.05$, years as replicates $n=11$).

I calculated the percent occurrence for a species at a given depth by dividing the number of stations (between 2001 and 2011) at which the species was encountered by the total number of stations at that depth.

$$Occurance\ Species\ A_{depth\ i} = \frac{Number\ of\ stations\ containing\ Species\ A\ _{depth\ i}}{Total\ Number\ of\ Stations_{depth\ i}}$$

Relative occurrence, was thereby defined by the percent occurrence divided by the maximum observed percent occurrence for that species (both hypoxia and normoxia), multiplied by 100.

$$Relative\ Occurance\ Species\ A_{depth\ i} = \frac{Occurance\ Species\ A_{\ depth\ i}}{Maximum\left(Occurance\ Species\ A_{all\ depths}\right)} \times 100$$

Area Under the Curve (AUC) Analysis

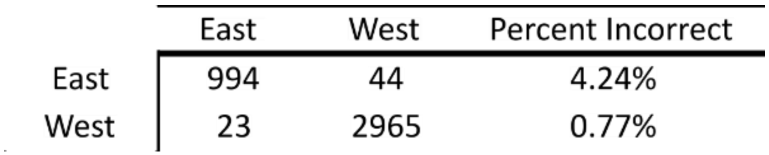
In order to quantify the magnitude of the overall difference between a species' occurrence at hypoxic and normoxic sites across a depth gradient I first subtracted the relative occurrence in normoxic conditions at depth from the relative occurrence in hypoxic conditions at depth. I then used these points to purposefully overfit a 6^{th} order polynomial function. When this function is plotted, the curve, and the area under the curve, corresponds to depths at which hypoxic sites had higher relative abundances, with the inverse being true for lines, and areas under the curve, below zero. The total value for the area under the curve thus reflects both how great the difference between hypoxic and normoxic percent occurrence was, as well as how large of a depth range this difference occurred in. An overall negative value suggests a lower overall affinity or tolerance for hypoxia while a positive value suggests some affinity for hypoxia or some degree of hypoxia tolerance.

I utilized a depth range of 10-40 m with depth bins of 5m for this analysis. Hypoxia is uncommon deeper than 40m, and stations below 10m are less represented than stations between 10-40m. Preliminary tests on the AUC protocol suggested that stations shallower than 10m could have been biasing the analysis towards higher AUC values for hypoxic sites due to the way the AUC is calculated in the r coding, so the shallower station bins were dropped. The bin size of 5m was chosen so that enough stations fell within each depth bin between 10-40 m and so that there were enough depth bins to generate the AUC curve. The choice to overfit the AUC curves helps to alleviate potential differences in outcome due to alterative depth bin choices as the overall trend remains the same.

RESULTS

Gulf Wide Species Composition and Geographic Filtering

Using the initial station grouping criteria of east and west of -88.1 degrees of longitude (the approximate western edge of Mobile Bay) resulted in a Random forest model that was able to predict whether a station was located east or west of Mobile Bay with and 98.2 percent accuracy (1673/1703) given test data (Table 1). Additionally, a PERMANOVA comparing the species composition between east and west of Mobile Bay sites found the species compositions to be significantly different ($p<0.001$, $n=5679$). The



	East	West	Percent Incorrect
East	994	44	4.24%
West	23	2965	0.77%

Table 1. Results of random forest model run on the test data (number of trees= 1000, number of variables tried at each node=562) using SEAMAP summer groundfish data from 2001 to 2011 to separate stations based on their location (either east or west of Mobile Bay). Biomass of species appearing in at least 1% of stations (either east or west of the Mobile Bay) were input as independent variables with the dependent variable being the station's location (categorical variable, input as either east or west). The training data comprised a random sampling of 70% of the total number of stations with the test data comprising the remaining 30% of the stations. A PERMANOVA of the same data used in the random forest model found there to be a significant relationship between species composition and location ($p<0.001$).

species most important to accurately predicting a station's location relative to Mobile Bay were found to be sand perch, *Diplectrum formossum,* and the dusky flounder, *Syacium papillosum*, with removal of either species from the model corresponding to an average of 19.8% and 6.7% of identifications being incorrect (of the station being east or west of Mobile Bay) respectively (Table 2).

NMDS analysis of the same data used in the gulf wide Random forest model above showed depth and longitude to be the two most highly correlated (Pearson) environmental variables with any of the NMDS axes. The NMDS axes represent the collapsed ranks of species biomass for all species collapsed into four axes minimizing correlation between axes. The first two NMDS axes, MDS1 and MDS2, were significantly correlated with depth and longitude (Pearson, $p<0.05$) with the other two axes not correlating as highly with any available environmental or geographic data. MDS1 had a correlation coefficient of 0.49 with depth and 0.66 with longitude with MDS2 having a correlation coefficient of -0.59 with depth and 0.39 with longitude. *D. formossum* and *S. papillosum*, the species with the highest importance in the accuracy of the Random forest model are more correlated with the eastern Gulf than areas west of Mobile Bay (Table 2).

When all of the station NMDS values of MSD1 and MSD2 are plotted, two groups corresponding to east and west of Mobile Bay are clearly discernable (Figure 1). An NMDS analysis of only stations west of Mobile Bay showed species composition to be highly correlated ($r^2=0.63$) with depth (Figure 2) with the other two axes not correlating highly with any available environmental or geographic data.

Species	Importance	MDS1	MDS2
Diplectrum formosum	796.96	0.85	0.54
Syacium papillosum	269.49	0.88	0.07
Penaeus aztecus	77.80	-0.35	-0.20
Stenotomus caprinus	68.27	0.03	-0.38
Callinectes similis	32.71	-0.51	-0.25
Eucidaris tribuloides	29.09	1.29	0.08
Acanthostracion quadricornis	18.99	0.99	0.67
Trachinocephalus myops	18.04	1.06	0.07
Centropristis ocyura	10.99	1.04	0.07
Calamus proridens	10.64	0.96	0.84

Table 2. Species with an importance greater than 10 of the most important species from the random forest model used to distinguish stations from east of Mobile bay from stations west of Mobile bay using species and the total catch weight as independent variables and the station location (categorical, east or west) as the dependent variable. Importance value corresponds to the average number of incorrect classifications resulting from removing the variable (species) from the model. MDS1 and MDS2 correspond to the first two axes, out of 4, from the NMDS of all stations and all species with percent occurrences greater than one percent. MDS1 and MDS2 both correlated highly with depth and longitude, with the other two axes not correlating strongly with any of the environmental or geographic variables.

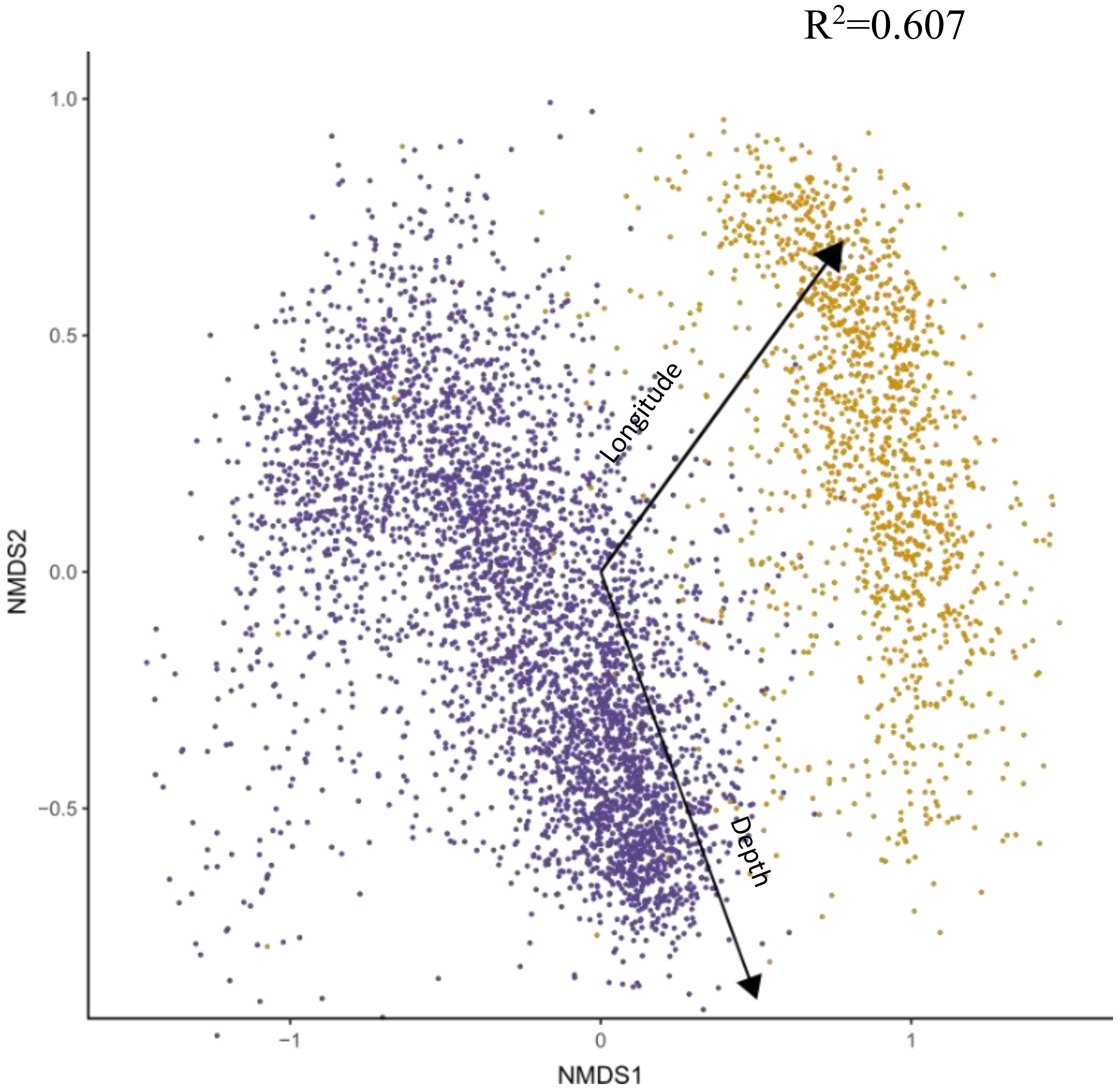


Figure 1. The two NMDS axes most correlated with longitude and depth from the NMDS analysis of all SEAMAP data between 2001 and 2011. Purple points represent stations west of Mobile Bay and yellow stations represent stations east of Mobile Bay. The length of the arrow lines corresponds to their r square value, with longer segments corresponding to a higher proportion of the variation being explained.

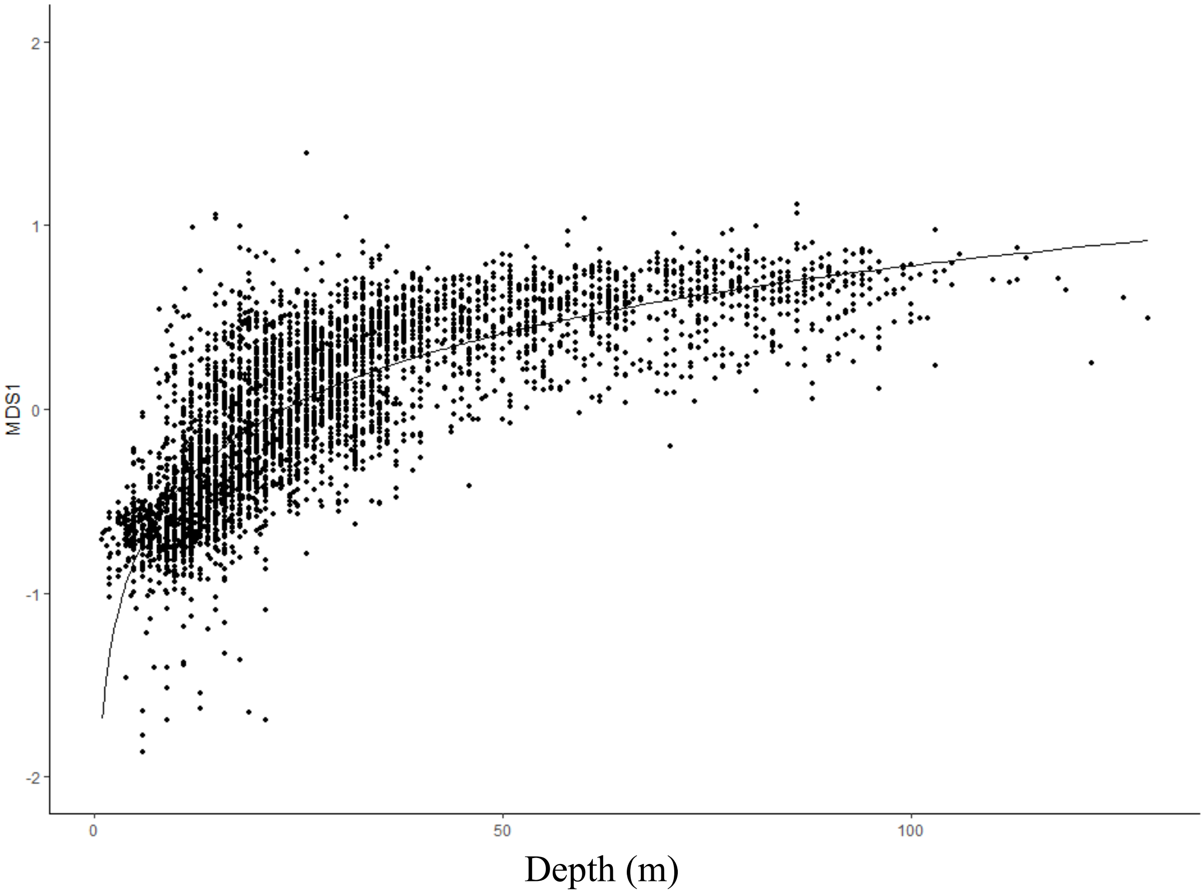


Figure 2. MDS1 plotted against station depth (m) for the NMDS analysis of all stations west of Mobile Bay. No other axis correlated highly with any other available environmental or geographic variable.

A random forest model using west of Mobile Bay data showed 64.4% training accuracy in assigning stations to depth bins of 10 meters (with stations deeper than 90 meters being grouped in a single bin). After changing the depth bins to 0-25 m, 25-45 m, 45-65 m, and greater than 65m the random forest model training accuracy increased to 85.1 percent with accuracies of 94 percent and 76.9 percent for the 0-25 m and 25-45 m bins respectively (Table 3).

Biodiversity, Abundance, and Species Composition Relative to Hypoxia

Between 1982 and 2011, at stations west of Mobile Bay and between 0 m and 25 m of depth normoxic stations had a higher average Shannon index value (Kruskal Wallis $p<0.05$) and higher average number of species (Kruskal Wallis $p<0.05$) compared to hypoxic stations (Figure 3). Rarefaction curves using pooled data for each year between 1984 and 2011 for fishes, crustaceans, and mollusks show that for the majority of years more species were found in normoxic conditions compared to hypoxic conditions, despite an apparent under sampling of hypoxic sites (Figure 4.)

Of the 465 species caught within 25m of depth West of Mobile Bay between 2001 and 2011, 102 had significantly lower average annual CPUE in hypoxic areas compared to normoxic areas (Kruskal Wallis $p<0.05$) (Appendix Table 1). Some of the most abundant species at normoxic stations including *L. xanthurus*, *L. fasciatus*, and *C. similis* had significantly lower average yearly CPUE at hypoxic sites with hypoxic catch of *L. xanthurus* and *C. similis* being 45 percent lower than at normoxic sites while the average yearly CPUE at hypoxic sites was 82 percent lower for *L. fasciatus*. (Figure 5). The sea pansy, *Renilla mulleri*, pink shrimp, *Penaeus duorarum*, and the inshore

	0-25m	25-45m	45-65m	>65m	Percentage Incorrect
0-25m	1562	98	0	1	6%
25-45m	127	564	39	4	23.2%
45-65m	5	71	207	29	34%
>65m	0	13	57	210	25%

Table 3. Results of random forest model (number of trees= 1000, number of variables tried at each node=561) using SEAMAP summer groundfish data west of Mobile Bay (-88.1 degrees longitude) from 2001 to 2011 to separate stations based on their depth (depth bins combined from depth bins of 10m). Biomass of species appearing in at least 1% of stations were input as independent variables with the dependent variable being station depth (categorical variable, input as depth bin). The training data comprised a random sampling of 70% of the total number of stations with the test data being comprising the remaining 30% of the stations. A PERMANOVA of the same data used in the random forest model found a significant relationship between species composition and depth bin ($p<0.001$).

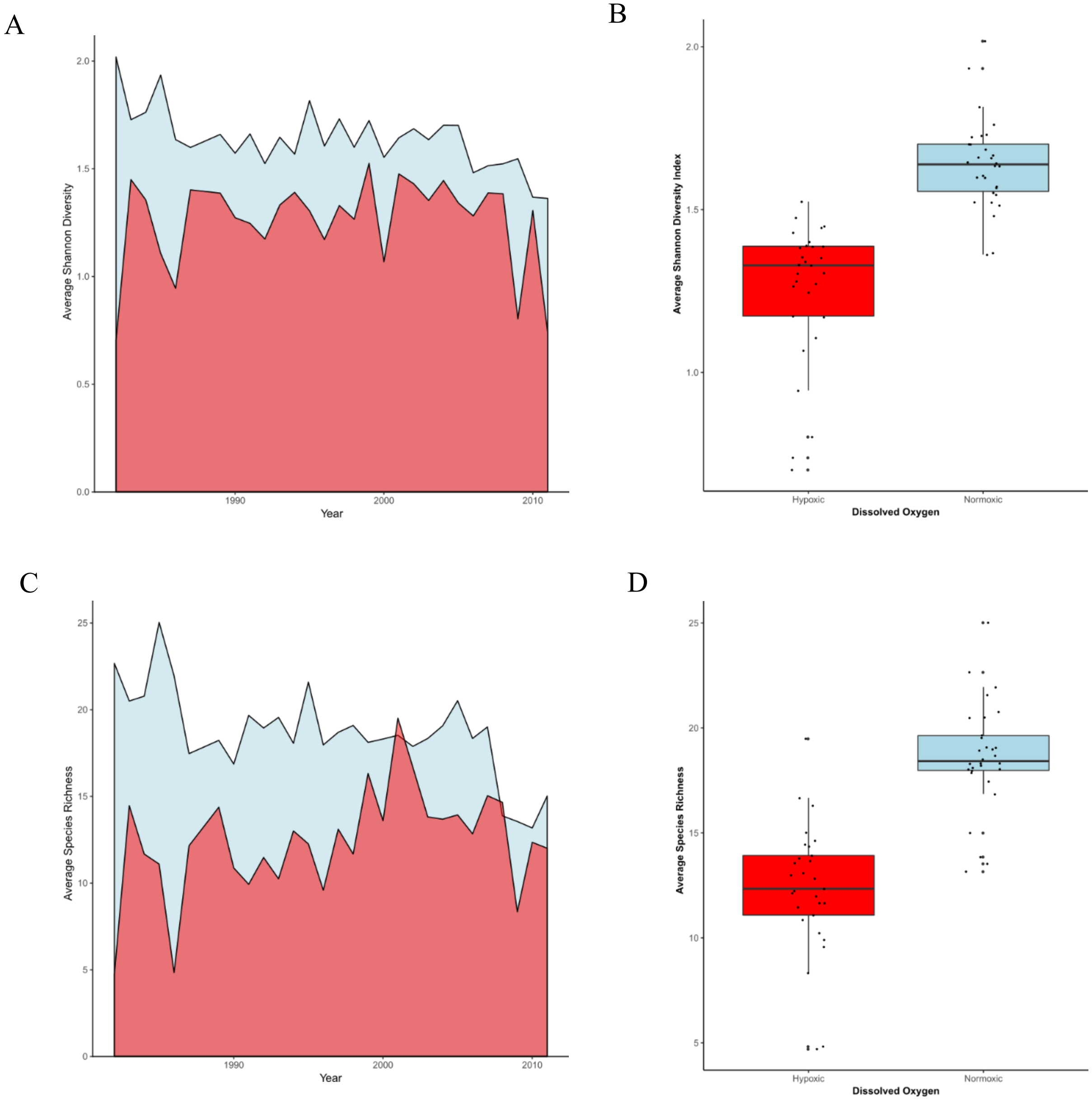


Figure 3. Yearly (A) and average (B) Shannon diversity of SEAMAP summer groundfish trawls from 1985 to 2011 at normoxic and hypoxic sites less than 25 meters west of Mobile Bay. Yearly (C) and average (D) number of species caught in SEAMAP summer groundfish trawls from 1985 to 2011 at normoxic (blue) and hypoxic (red) sites less than 25 meters west of Mobile Bay. Both average Shannon index value and average number of species were significantly lower for hypoxic stations compared to normoxic stations (Kruskal Wallis $p<0.05$, $n=27$)

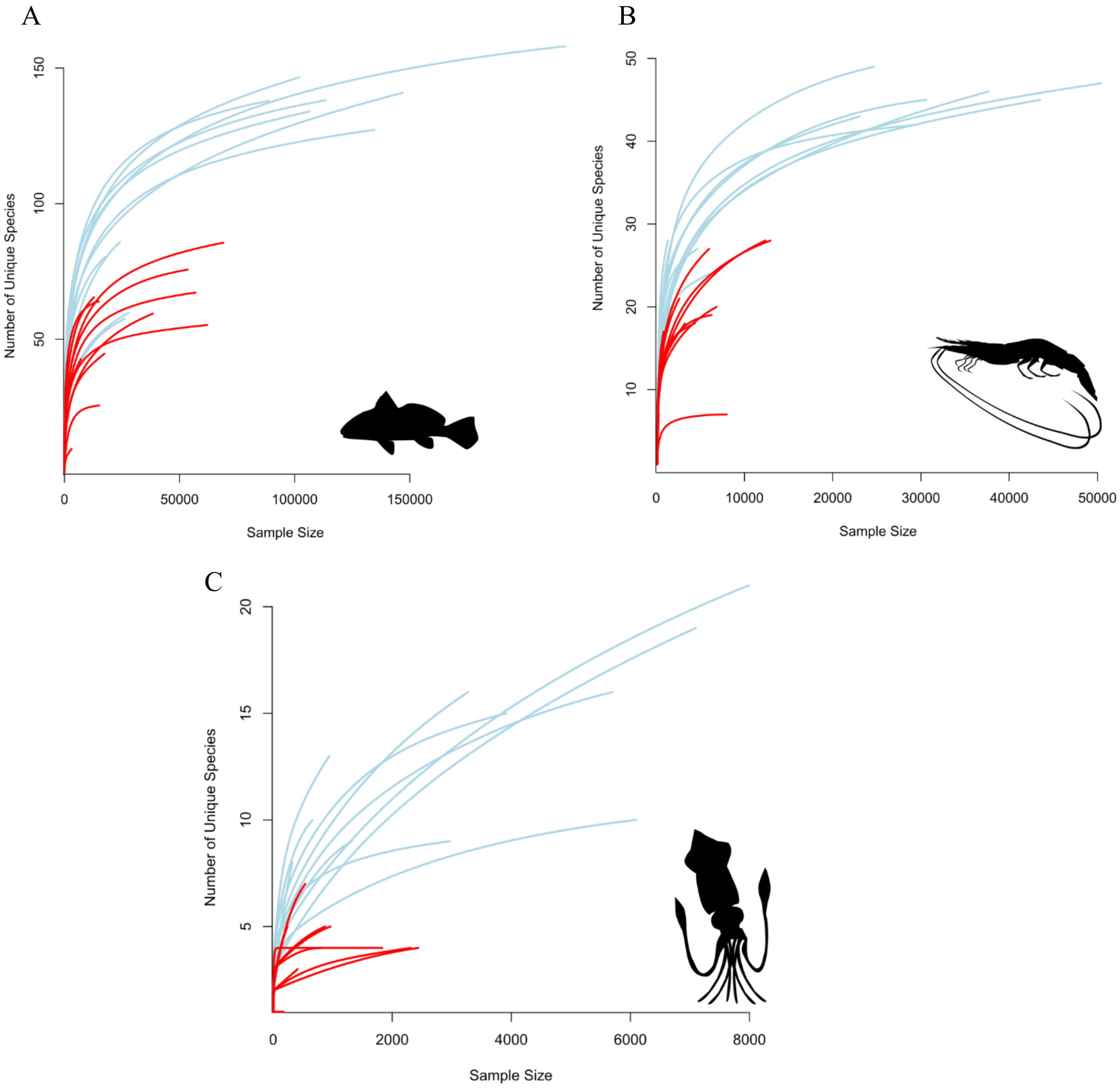


Figure 4. Yearly pooled species accumulation curves for all species west of mobile bay and between 0 and 25m for fishes (A) crustaceans (B) and mollusks (C). Data was obtained from SEAMAP summer groundfish cruises between 1984 and 2011. Each blue line represents a species accumulation curve for all normoxic stations within a year and each red line represents a species accumulation curve for all hypoxic stations within a year. All lines terminate at the total number of unique species encountered (y axis) and total number of extrapolated animals caught (x axis).

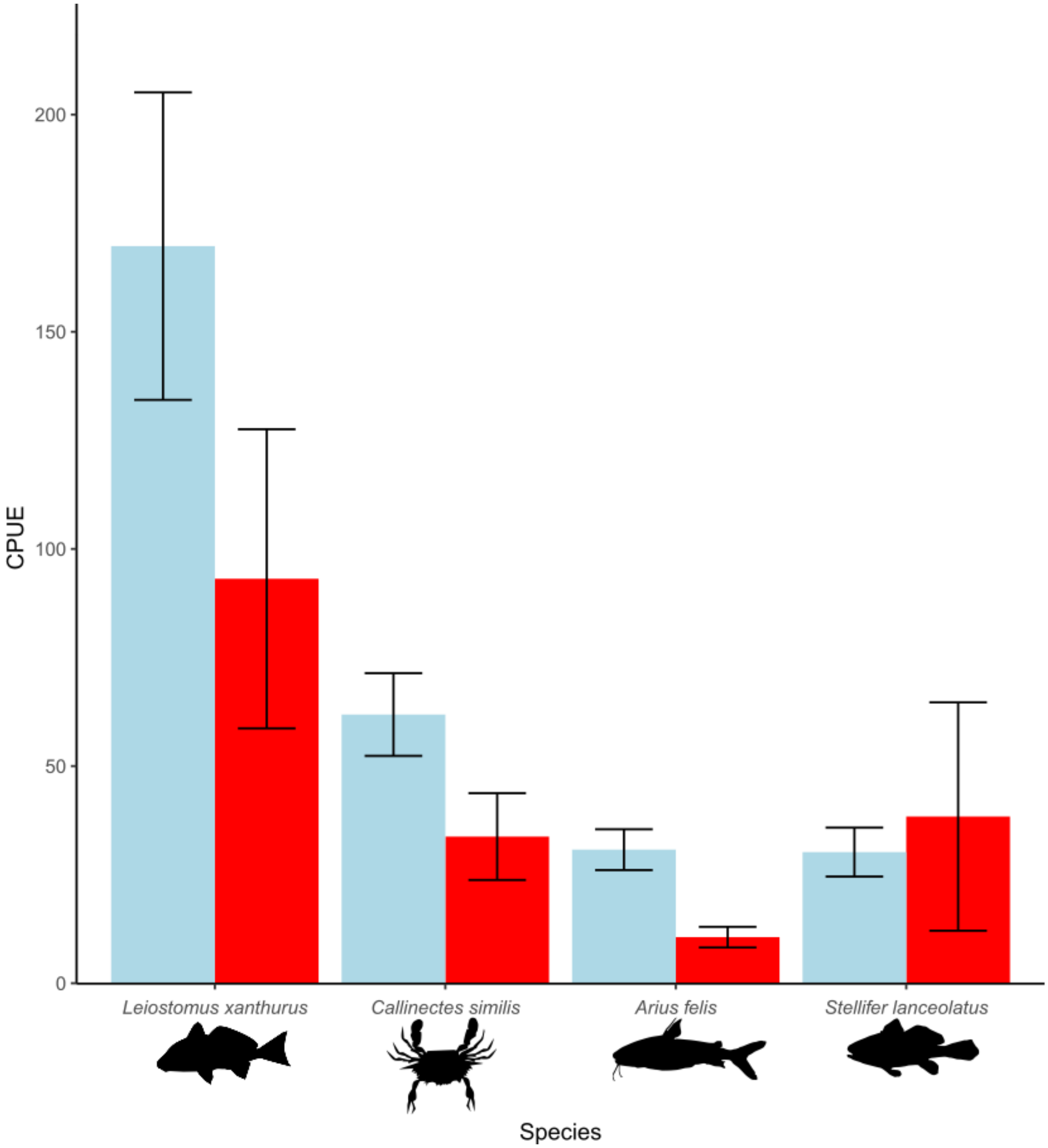


Figure 5. Mean annual CPUE (kg/km^2) of select groundfish and invertebrates caught in either hypoxic or normoxic conditions in which the difference in mean annual CPUE is significantly different. (±SEM, Kruskal Wallis, p value <0.05, n(years)=11) Catch and effort data were obtained from SEAMAP summer groundfish cruises between 2001 and 2011 from stations between 0m and 25m west of Mobile Bay, AL.

lizardfish, *Synodus foetens*, have average annual CPUE values from hypoxic sites that are more than 80 percent lower than average annual CPUE values from normoxic sites (Figure 6). The average yearly CPUE from hypoxic sites in some species, including *U. parvus,* the yellow box crab, *Callapa sulcata,* and the silver jenny, *Eucinostomus gula*, is more than 90 percent less than the average yearly CPUE from normoxic sites (Figure 7). The average percent difference between average CPUE between hypoxic and normoxic sites for all species, in which the difference was significant ($p<0.05$), is 85 percent. The star drum, *Stellifer lanceolatus,* is the only species with a significantly greater average CPUE from hypoxic sites compared to normoxic sites, with the average CPUE from hypoxic sites being 27 percent greater compared to normoxic sites.

There was a significant relationship between the percent occurrence of a species in 5m depth bins between 10m and 45m and whether the stations were hypoxic or normoxic (Chi-square $p<0.05$) for 51. 2 percent of species (271/529). Some species exhibited higher percent occurrences at normoxic sites compared to hypoxic sites across all depth bins such as in the dwarf goatfish, *Upeneus parvus*. Other species, such as the mantis shrimp *Squilla empusa*, showed percent occurrences to be higher for the majority of depth bins at hypoxic sites. Species such as *M. undulatus* and *Prionotus longispinosus* showed lower percent occurrences at shallower depths at hypoxic sites compared to normoxic sites, while showing higher percent occurrences at hypoxic sites compared to normoxic sites in deeper waters (Figure 8).

Of the 271 species with a significant relationship between occurrence in 5m depth bins and dissolved oxygen (hypoxic or normoxic), 67.2 percent (182/271) have negative area under the curve (AUC) values and 32.8 percent (89/271) have positive AUC values

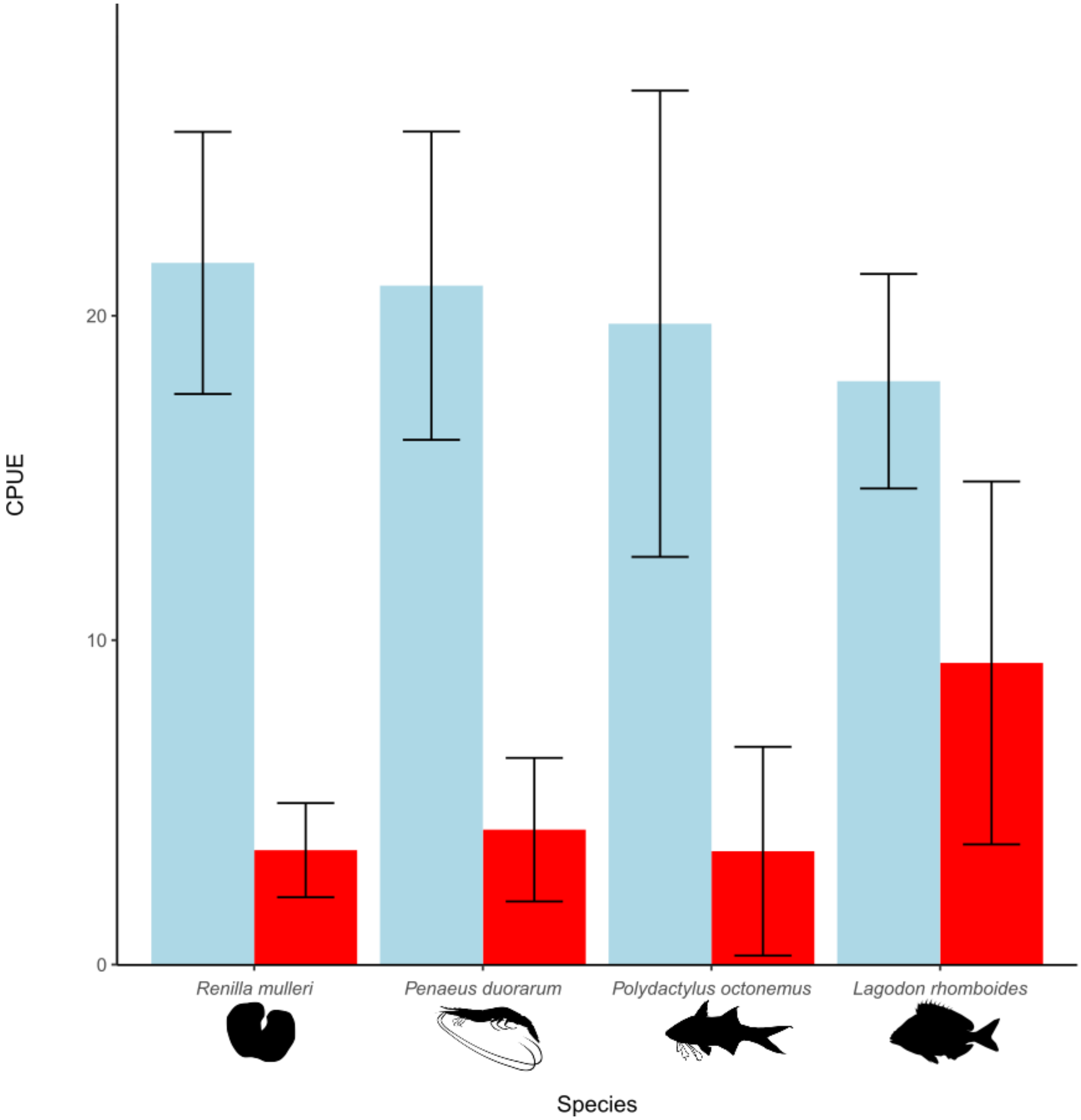


Figure 6. Mean annual CPUE (kg/km^2) of select groundfish and invertebrates caught in either hypoxic or normoxic conditions in which the difference in mean annual CPUE is significantly different. (±SEM, Kruskal Wallis, p value <0.05, n(years)=11) Catch and effort data were obtained from SEAMAP summer groundfish cruises between 2001 and 2011 from stations between 0m and 25m west of Mobile Bay, AL.

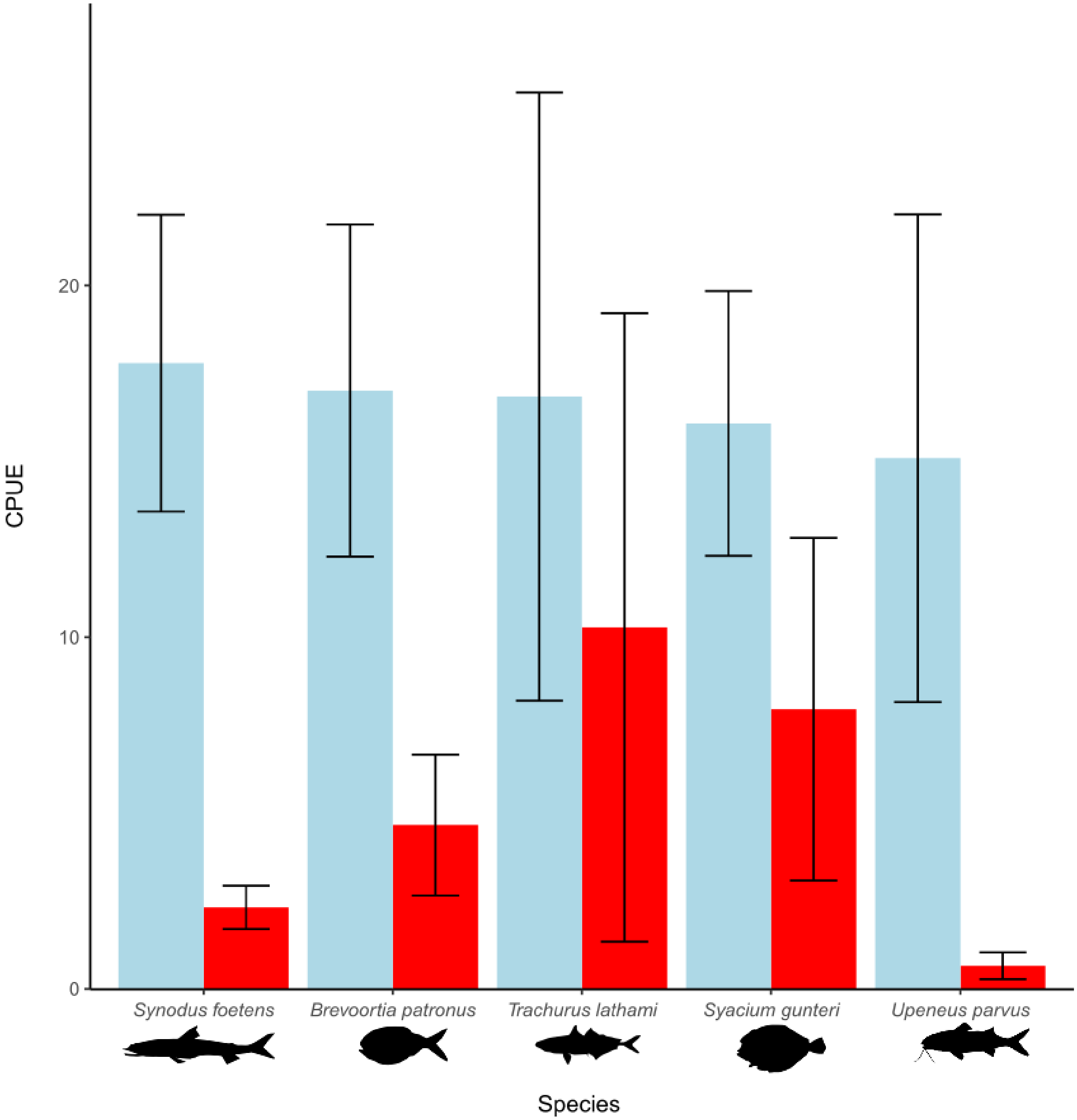


Figure 7. Mean annual CPUE (kg/km^2) of select groundfish and invertebrates caught in either hypoxic or normoxic conditions in which the difference in mean annual CPUE is significantly different. (±SEM, Kruskal Wallis, p value <0.05, n(years)=11) Catch and effort data were obtained from SEAMAP summer groundfish cruises between 2001 and 2011 from stations between 0m and 25m west of Mobile Bay, AL.

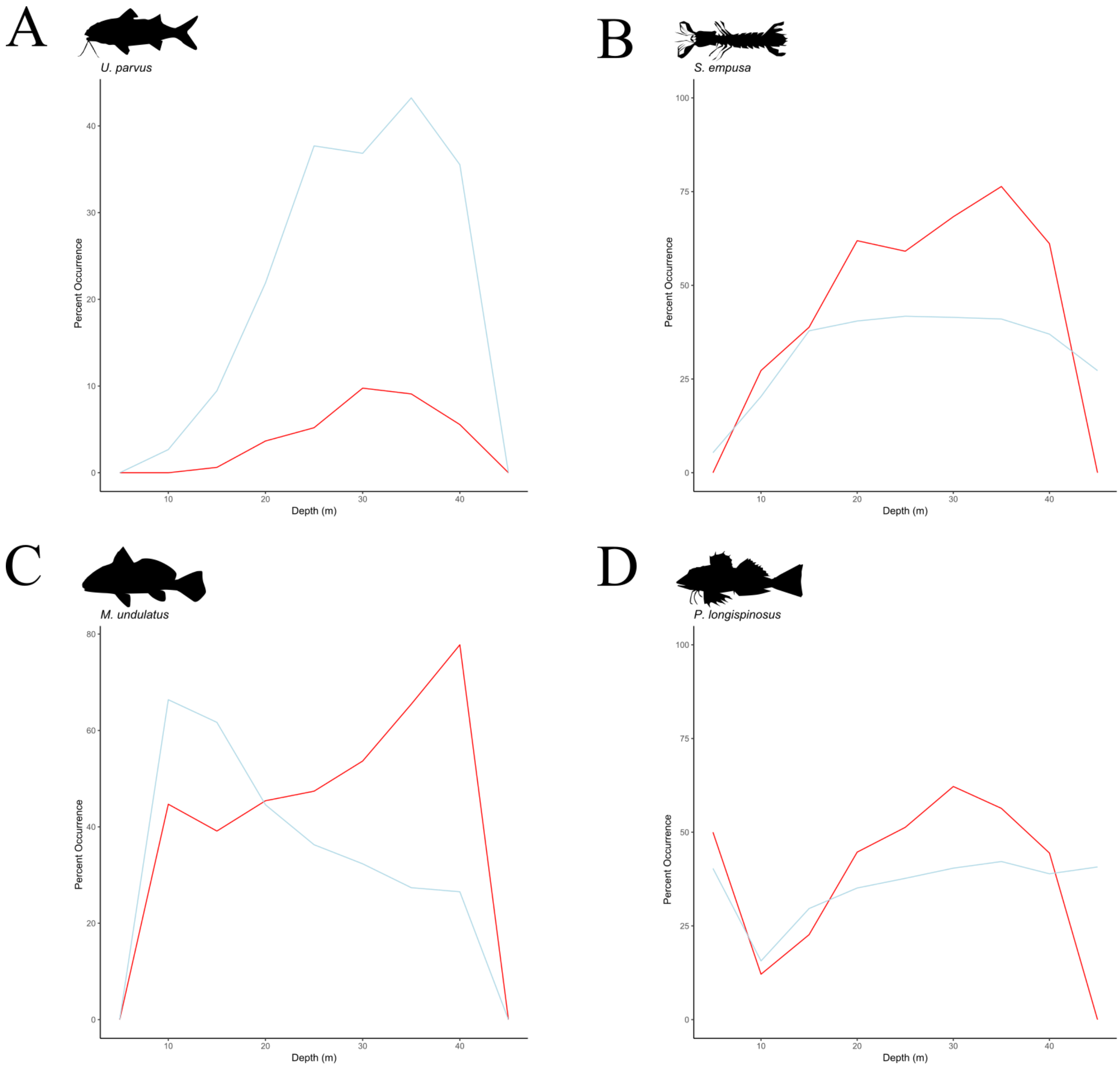


Figure 8. Percent occurrence at 5m depth intervals between 10m and 45m for *Upeneus parvus* (A), *Squilla empusa* (B), *Micropogonias undulatus* (C), and *Prionotus longispinosus* (D) between 2001 and 2011 west of Mobile bay. 271 out of 529 species analyzed showed a significant relationship between occurrence at depth and dissolved oxygen (hypoxic or normoxic), including the four species shown above.

(Figures 9-15). A negative AUC value denotes a higher relative percent occurrence across all depth ranges for normoxic sites, while positive AUC values denote higher relative percent occurrences for hypoxic sites across all depth ranges. Some species, such as, *Cynoscion nothus*, while displaying higher relative percent occurrences at shallower depths for normoxic sites (negative AUC), show nearly equal lower percent occurrences at deeper depths at normoxic sites. This near equality in the difference between the relative percent occurrence between hypoxic and normoxic sites at shallow versus deeper stations is reflected in the overall AUC value of 57 being relatively low for *C. nothus*. Other species, while having high relative percent occurrences at shallow stations (negative AUC), showed positive AUC values at deeper stations that are an order of magnitude greater than the negative AUC values at the shallow stations, highlighted by *Prionotus longispinosus* (Figure 12).

Random Forest Model: Dissolved Oxygen and Species Composition

A random forest model comparing the species composition of stations between 0m and 25m west of Mobile bay showed a training accuracy of 72.8% for hypoxic sites and 83% for normoxic sites while correctly identifying 99/131 hypoxic stations correctly and 1322/1629 normoxic stations correctly. The species found to be the most important in distinguishing hypoxic stations from normoxic stations were *Synodus foetens, Luidia clathrata, Renilla mulleri, Penaeus aztecus, Chloroscombrus chrysurus, Prionotus longispinosus, Lolliguncula brevis, Penaeus setiferus, Lagodon rhomboides, Squilla empusa,* and *Leiostomus xanthurus* (Table 4).

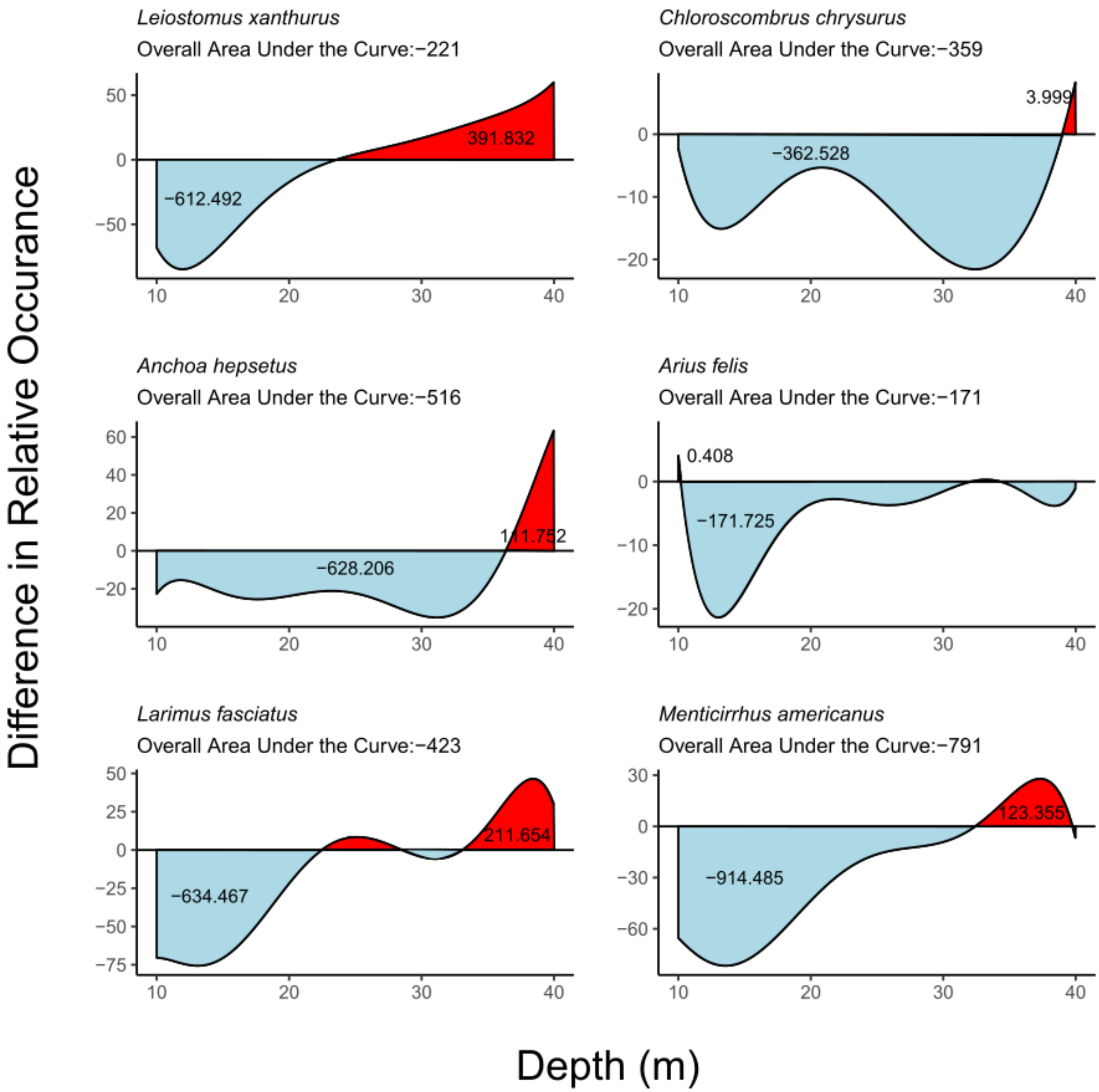


Figure 9. Area under the curve (AUC) plots at 5m depth intervals between 10m and 45m for select GOMEX groundfish caught during SEAMAP summer groundfish surveys between 2011 and 2011. Blue shading denotes AUC corresponding to greater relative percent occurrences at normoxic sites compared to hypoxic sites with red shading corresponding to the opposite condition. Relative occurrence was calculated by dividing all of the percent occurrence values (across all depth bins) by the highest relative percent occurrence value and multiplying by 100, thus a value of 100 (for relative occurrence) denotes the highest percent occurrence observed.

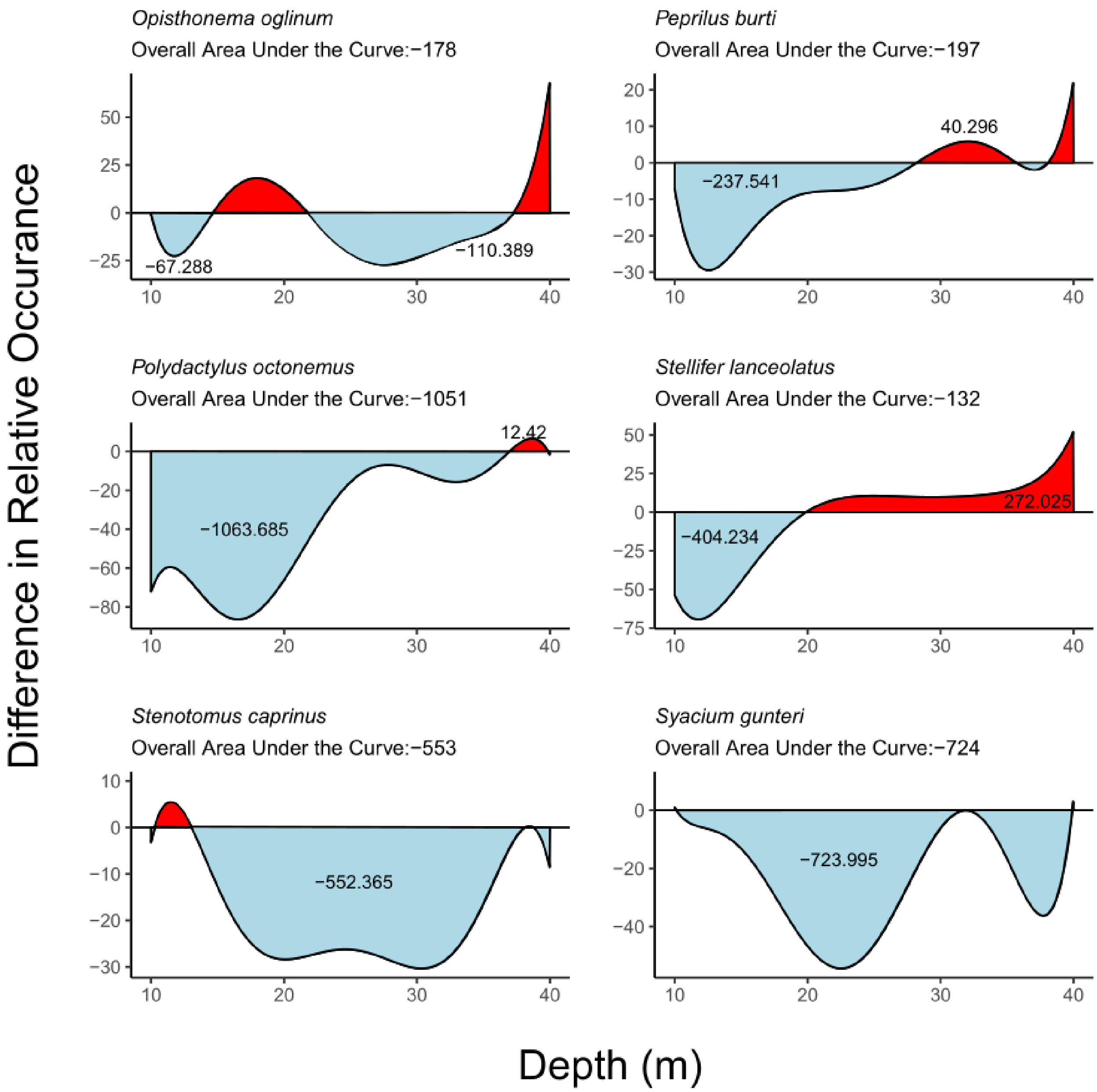


Figure 10. Area under the curve (AUC) plots at 5m depth intervals between 10m and 45m for select GOMEX groundfish caught during SEAMAP summer groundfish surveys between 2011 and 2011. Blue shading denotes AUC corresponding to greater relative percent occurrences at normoxic sites compared to hypoxic sites with red shading corresponding to the opposite condition. Relative occurrence was calculated by dividing all of the percent occurrence values (across all depth bins) by the highest relative percent occurrence value and multiplying by 100, thus a value of 100 (for relative occurrence) denotes the highest percent occurrence observed.

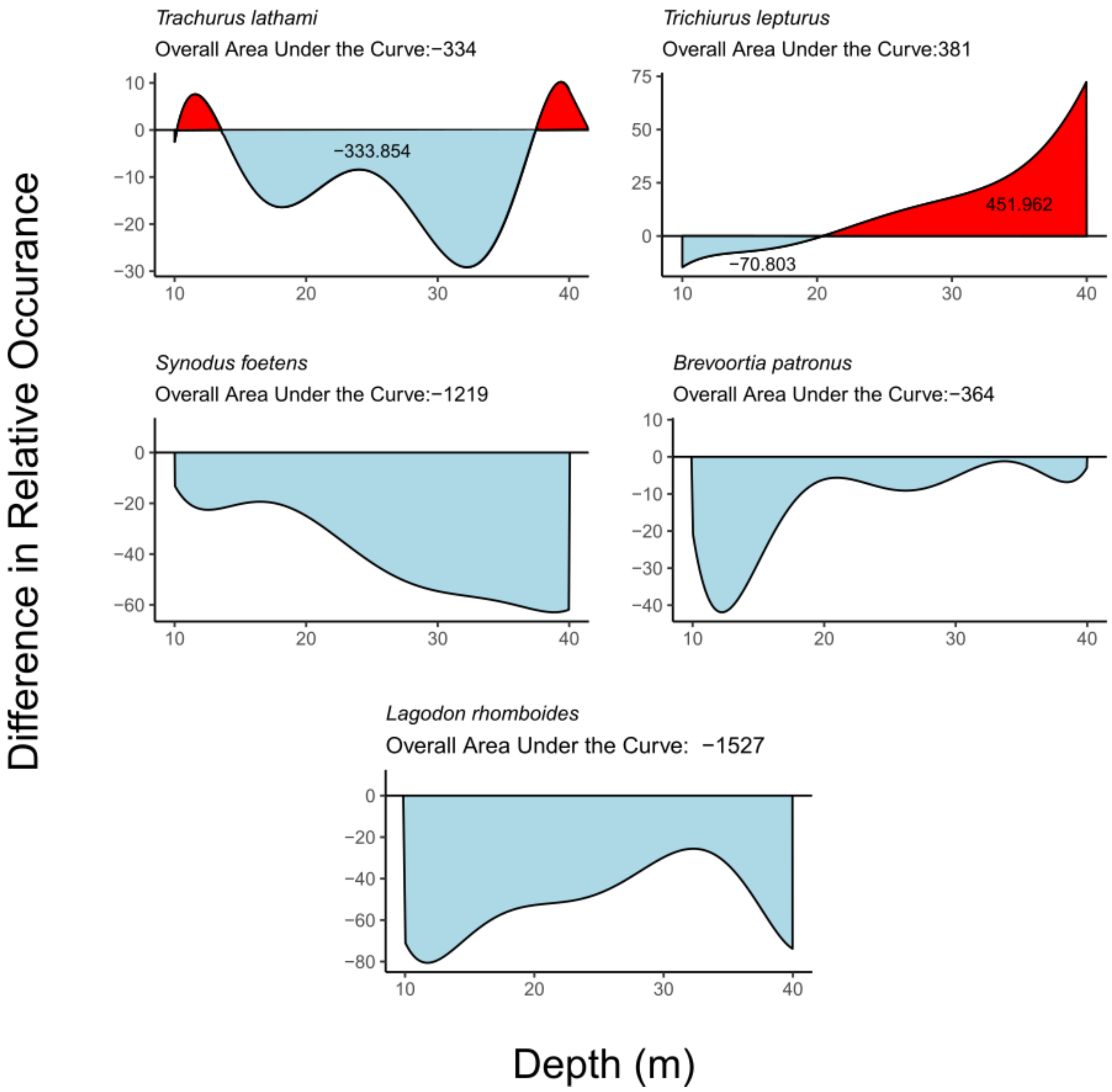


Figure 11. Area under the curve (AUC) plots at 5m depth intervals between 10m and 45m for select GOMEX groundfish caught during SEAMAP summer groundfish surveys between 2011 and 2011. Blue shading denotes AUC corresponding to greater relative percent occurrences at normoxic sites compared to hypoxic sites with red shading corresponding to the opposite condition. Relative occurrence was calculated by dividing all of the percent occurrence values (across all depth bins) by the highest relative percent occurrence value and multiplying by 100, thus a value of 100 (for relative occurrence) denotes the highest percent occurrence observed.

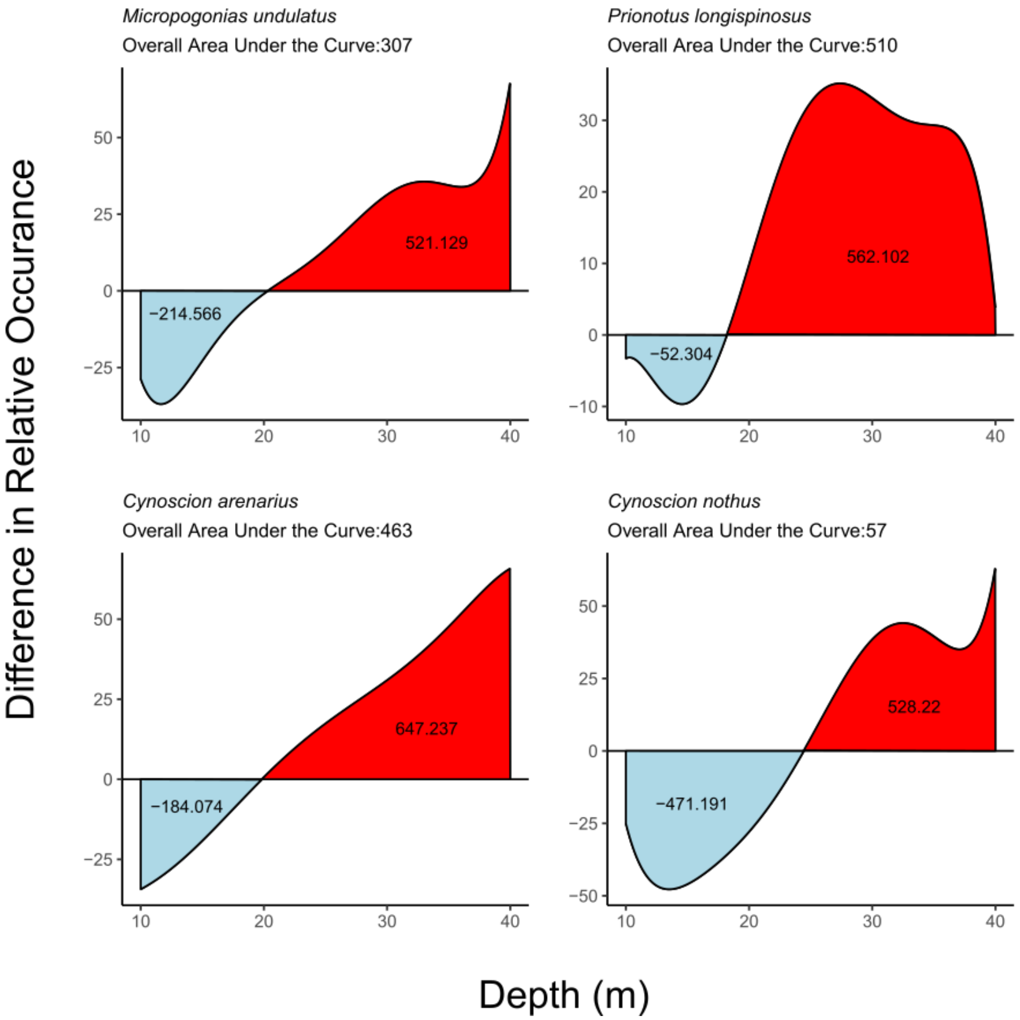


Figure 12. Area under the curve (AUC) plots at 5m depth intervals between 10m and 45m for select GOMEX groundfish caught during SEAMAP summer groundfish surveys between 2011 and 2011. Blue shading denotes AUC corresponding to greater relative percent occurrences at normoxic sites compared to hypoxic sites with red shading corresponding to the opposite condition. Relative occurrence was calculated by dividing all of the percent occurrence values (across all depth bins) by the highest relative percent occurrence value and multiplying by 100, thus a value of 100 (for relative occurrence) denotes the highest percent occurrence observed.

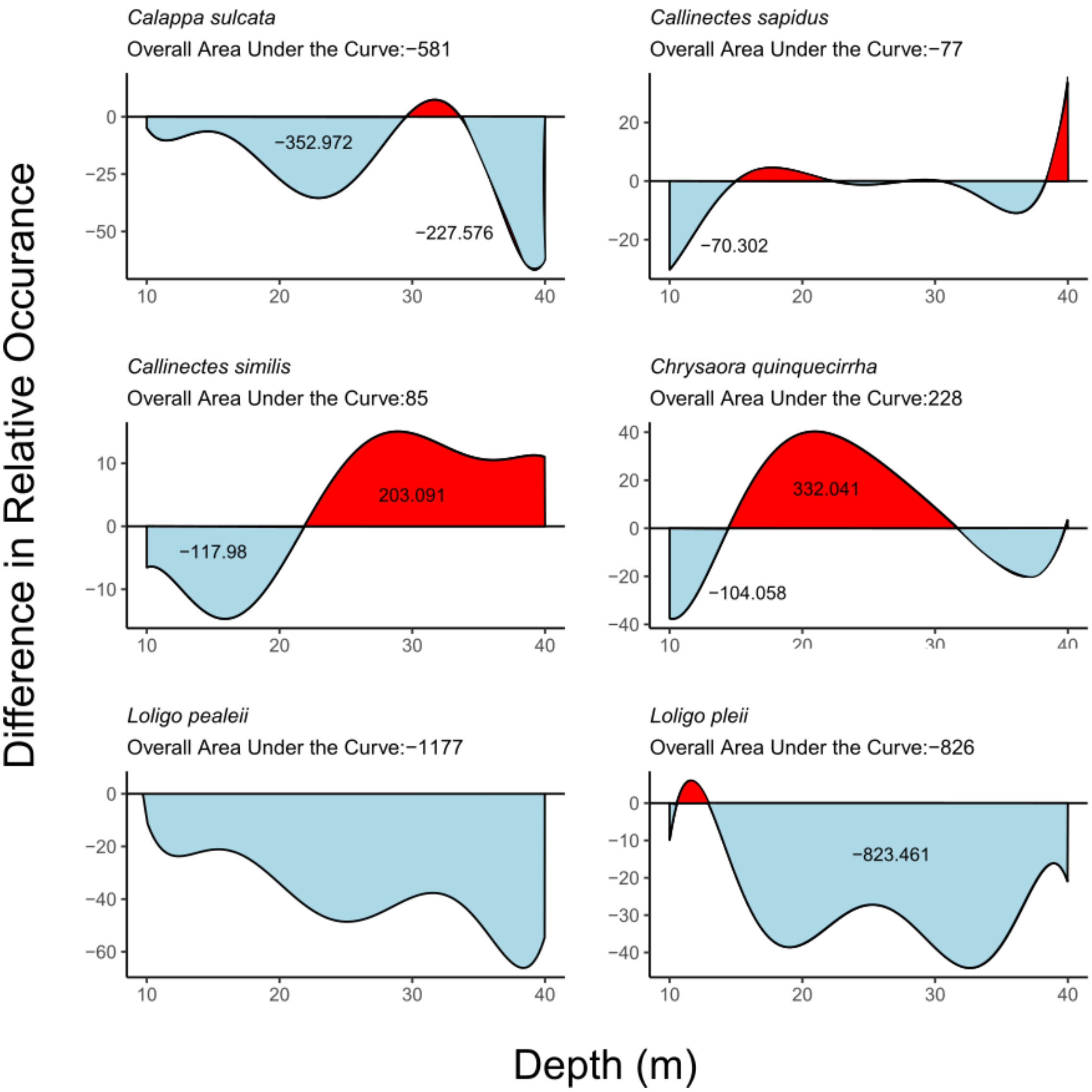


Figure 13. Area under the curve (AUC) plots at 5m depth intervals between 10m and 45m for select GOMEX invertebrates caught during SEAMAP summer groundfish surveys between 2011 and 2011. Blue shading denotes AUC corresponding to greater relative percent occurrences at normoxic sites compared to hypoxic sites with red shading corresponding to the opposite condition. Relative occurrence was calculated by dividing all of the percent occurrence values (across all depth bins) by the highest relative percent occurrence value and multiplying by 100, thus a value of 100 (for relative occurrence) denotes the highest percent occurrence observed.

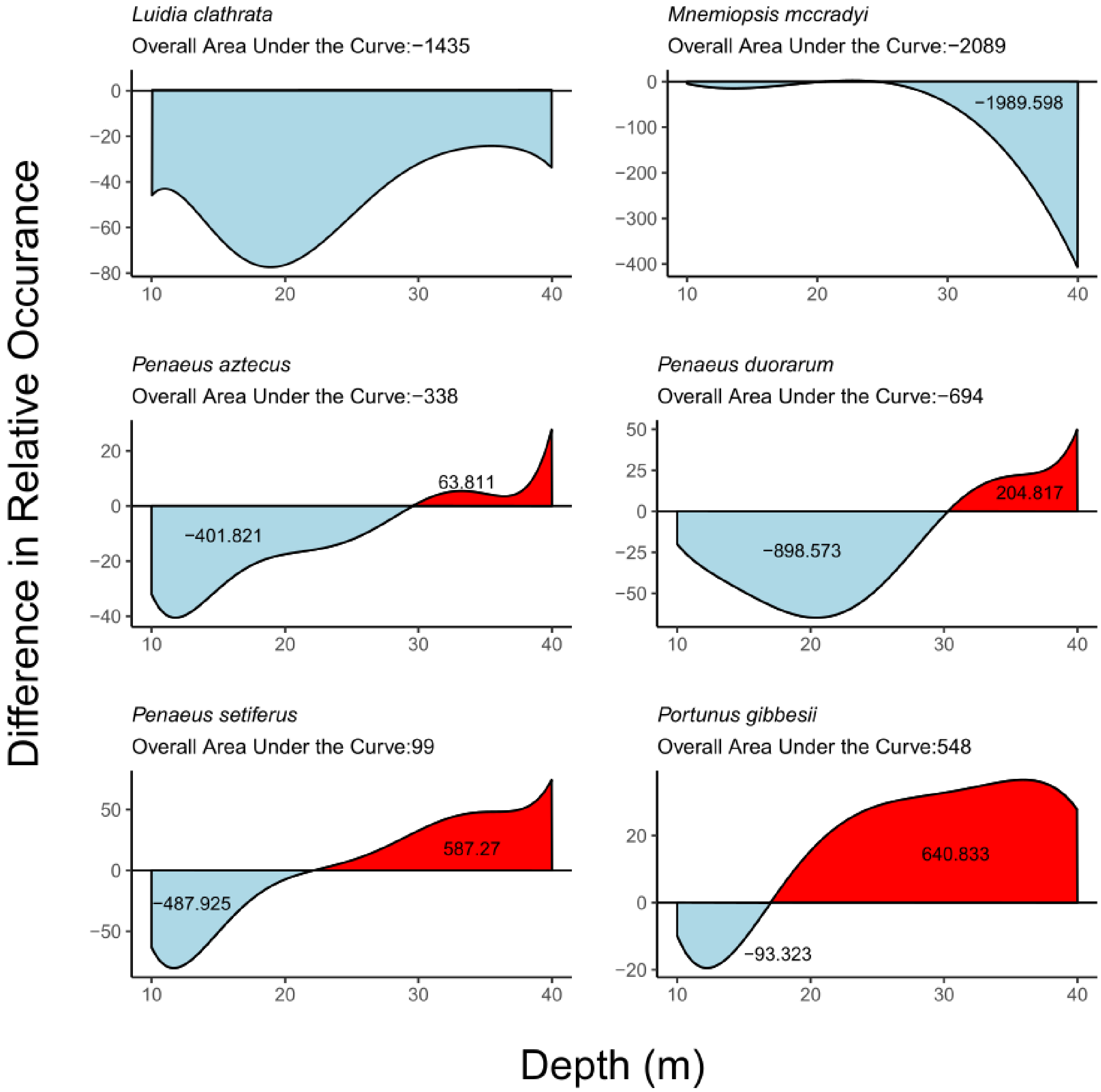


Figure 14. Area under the curve (AUC) plots at 5m depth intervals between 10m and 45m for select GOMEX invertebrates caught during SEAMAP summer groundfish surveys between 2011 and 2011. Blue shading denotes AUC corresponding to greater relative percent occurrences at normoxic sites compared to hypoxic sites with red shading corresponding to the opposite condition. Relative occurrence was calculated by dividing all of the percent occurrence values (across all depth bins) by the highest relative percent occurrence value and multiplying by 100, thus a value of 100 (for relative occurrence) denotes the highest percent occurrence observed.

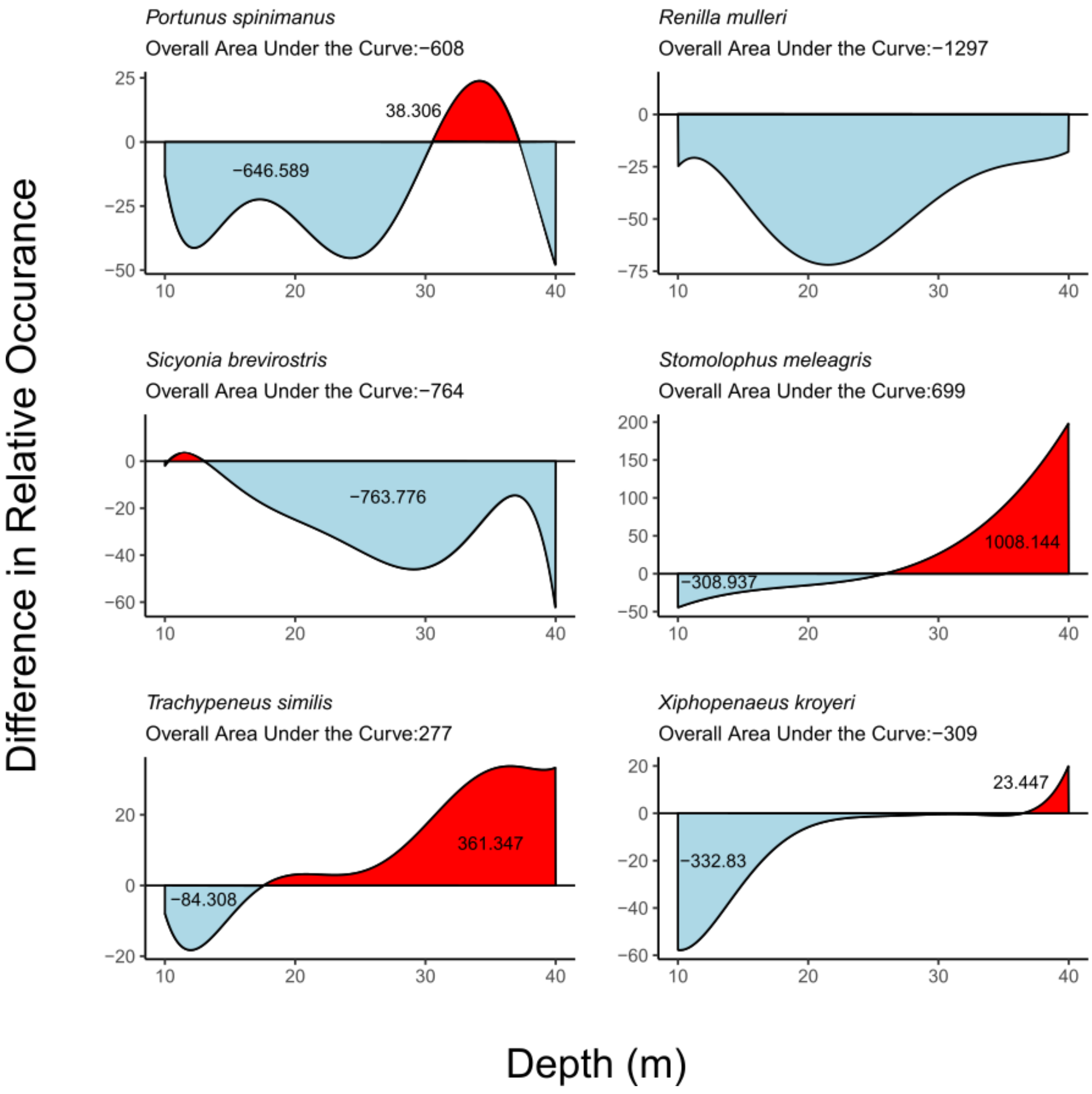


Figure 15. Area under the curve (AUC) plots at 5m depth intervals between 10m and 45m for select GOMEX invertebrates caught during SEAMAP summer groundfish surveys between 2011 and 2011. Blue shading denotes AUC corresponding to greater relative percent occurrences at normoxic sites compared to hypoxic sites with red shading corresponding to the opposite condition. Relative occurrence was calculated by dividing all of the percent occurrence values (across all depth bins) by the highest relative percent occurrence value and multiplying by 100, thus a value of 100 (for relative occurrence) denotes the highest percent occurrence observed.

Species	Importance	p value	$\mathrm{CPUE}_{\mathrm{Hypoxic}}$·/$\mathrm{CPUE}_{\mathrm{Normoxic}}$
Renilla mulleri	35.35	0.00	*0.16*
Arius felis	14.43	0.00	*0.35*
Synodus foetens	14.37	0.01	*0.13*
Penaeus aztecus	14.15	0.11	*0.79*
Chloroscombrus chrysurus	10.40	0.97	*1.25*
Micropogonias undulatus	9.38	0.53	*1.69*
Prionotus longispinosus	8.07	0.72	*2.06*
Luidia clathrata	7.07	0.00	*0.18*
Penaeus setiferus	7.01	0.06	*0.53*
Lolliguncula brevis	6.08	0.21	*0.74*
Polydactylus octonemus	5.80	0.00	*0.18*
Lagodon rhomboides	5.61	0.01	*0.52*
Squilla empusa	5.57	0.11	*2.88*
Chrysaora quinquecirrha	5.27	0.01	*0.35*

Table 4. Importance value (average number of incorrect identifications by the model when variable is excluded from model), and the average cpue (biomass per square km) of hypoxic sites reported as a percentage of the average normoxic cpue (n=11, n=years) as well as the p value (Kruskal-Wallis) comparing the average cpue of normoxic and hypoxic sites for the random forest model using species as independent variables and whether a site was hypoxic or normoxic as the dependent variable.

DISCUSSION

Geographic Filtering

I chose the western edge of Mobile bay (-88.1°) to define stations as eastern or western gulf due to the decreasing impact of the Mississippi river on the benthos moving east towards Florida. It is known   that the majority of the Mississippi river's freshwater discharge is directed westward towards Louisiana and Texas [53]. It is also well established that the bottom type of the eastern GOMEX is dominated by sand [54] with western GOMEX being dominated by finer sediments like silt and clay [55]. The combined results of the NMDS, random forest model, and PERMANOVA analyses of all SEAMAP groundfish stations between 2001 and 2011, utilizing the western edge of Mobile Bay as the line of demarcation between the eastern or western gulf, justifies excluding eastern gulf stations from further analysis. The random forest model's ability to accurately predict a station's location as being east or west of Mobile bay was 98.2%, and was based on abundance, biomass, of two fish species, *Diplectrum formossum* and *Syacium papillosum*. The NMDS analysis and PERMANOVA run on the same data also support the conclusion that the benthic communities east and west of Mobile Bay are quantifiably different. Plotting the first two NMDS axes (axes highly correlated with depth and longitude) from NMDS analysis of the random forest model data shows two clear clusters of points that are explained almost exclusively by longitude (Figure 1). Given that the proximity of points in this plot corresponds to similarity in species composition, this result also supports excluding eastern Gulf stations from further analysis. Additionally, the PERMANOVA analysis detected a significant difference in species composition between SEAMAP stations east and west of Mobile Bay.

NMDS analysis on only western gulf data did not yield any discernable grouping of stations by their species composition for any combination of axes. One axis, however, did correlate highly (r^2=0.607) with depth, and plotting this axis against depth (Figure 2) it is clear that species composition in the western Gulf changes rapidly with increasing depth to around 50m where it starts to level off. Despite this change in species composition, a random forest model based on the same data as the NMDS analysis successfully predicted 94% of stations as being from between 0-25m. Given that this depth range is within the range in which hypoxia most frequently forms in the GOMEX [10] and that a PERMANOVA of the same data found a significant ($p<0.05$) interaction between species composition and depth range, the use of 0-25m as the depth range for the majority of my analyses is appropriate.

Biodiversity, Abundance, and Species Composition Relative to Hypoxia

My results clearly show that seasonal hypoxia in the GOMEX west of the Mississippi River reduces diversity of the megabenthic fauna. Hypoxic stations had significantly lower average Shannon index values (Kruskal Wallis $p<0.05$) and the pooled yearly diversity of hypoxic sites never exceed or equaled the diversity of pooled normoxic sites (Figure 3). Additionally, 171 species found between 0-25 m at normoxic sites were never caught in hypoxic conditions and although many of these 171 species are relatively rare in SEAMAP summer groundfish trawls, the absence of 31 of these species in hypoxic sites compared to their average yearly CPUE at normoxic sites was significantly different (Kruskal-Wallis, $p<0.05$). Two of these 31 species, the lesser electric ray, *Narcine brasiliensis,* and the red goatfish, *Mullus auratus* are not uncommon.

Because the Shannon index is known to place a greater weight on rarer species than other diversity indices, Simpson indices were also calculated and were also found to be significantly lower in hypoxic sites compared to normoxic sites (Appendix Figure A1) Finally, the trajectories of rarefaction curves of pooled yearly data between 2001 and 2011 of fishes, crustaceans, and mollusks suggest that hypoxic sites, if sampled more, would not reach the species totals of normoxic sites (Figure 4).

My findings differ, to some degree, from those of Briggs et al. (2017), who found that a site with the lowest average summer DO between 2000 and 2010 showed the highest macrobenthic diversity. This increase in biodiversity was attributed to the exodus of common sciaenid fishes and the retention of rarer species at the station [38]. This is in direct contrast, not only the lower average biodiversity observed at hypoxic sites, but also to the species composition observed in my results. In addition to lower species richness at hypoxic sites, I found hypoxic sites to be dominated by common sciaenids, most notably *M. undulatus*, and fewer occurrences of rare species. This discrepancy between my results and those of Briggs et al. (2017) can likely be explained by three main factors: sampling depth, the timeline of hypoxia formation, and the definition of hypoxic sites[38].

The sampling stations of Briggs et al. (2017) were located along the 30m isobath, 5m deeper than the deepest extent of our study range (0-25m). Despite the difference between my maximum sampling depth and the sampling depth of Briggs et al (2017) being only 5m, most of my samples did not occur near 25m (average depth = 15m). Briggs et al. (2017) having higher abundances of rare species like batfishes and lower abundances of common sciaenids like *M. undulatus* also makes sense in the context of depth. *M. undulatus* is more common at shallower depths than deeper, and batfishes are more common in deeper waters. Additionally, Briggs et al. (2017) noted that their moderately affected hypoxia site likely became hypoxic only eight days prior to sampling and that this could have increased the catch of many of their species [38].

The measure of hypoxia was also different between my study and that of Briggs et al. (2017). The hypoxic sites used by Briggs et al. (2017) were defined by their historic exposure to hypoxia, and not the immediate DO readings at the time of sampling, as hypoxic sites were defined in my study. Thus, the findings of Briggs et al. (2017) reflect the chronic impacts of repeated hypoxia at a given site, while my study focuses more on the broader impact of hypoxia on the northern GOMEX.

The lower diversity of hypoxic sites, coupled with the fact that 23.7 percent of species caught between 0-25m from 2001 and 2011 showed significantly lower yearly CPUE (biomass per km^2) at hypoxic sites compared to normoxic sites within the same depth range shows that hypoxia alters not only community composition but also biomass of a wide range of species in the GOMEX. The negative relationship between hypoxia and fish diversity and biomass is a serious concern for the GOMEX. Diversity and biomass are important to maintaining community resilience and resisting invasions by non-native species. Loss of biodiversity also destabilizes food webs [56]. It is important to place hypoxia impacts on GOMEX biotic resources in the context of other deleterious anthropogenic activities affecting the ecosystem services and economically and culturally important fisheries of the northern GOMEX.

Species Occurrence at Depth Relative to Hypoxia

My results suggest that depth plays an important role in how hypoxia impacts species composition and species occurrences. Significant relationships between occurrence and DO were observed for 51.2 percent of species at stations between 10-45m depth (Chi-Square $p<0.05$). Among these species, 77.2 percent had lower overall occurrences in hypoxic conditions (as determined by my AUC analysis) with many of these species having percent occurrences in hypoxic sites that never exceeded their percent occurrences in normoxic sites. This large percentage suggests that many species show either a high propensity to avoid hypoxia or a low tolerance for hypoxic conditions, and depth has little influence on how hypoxia impacts these species. For example, percent occurrence vs depth lines for *U. parvus* at hypoxic and normoxic sites (Figure 8) are approximately the same shape, both peaking around the same depth range. In this case hypoxic conditions are not altering the relationship between percent occurrence and depth of *U. parvus,* simply reducing the percent occurrence nearly uniformly across the depth ranges. Conversely, the relationship between percent occurrence and depth in *M. undulatus* is altered by hypoxia. At normoxic sites between 0-20m the percent occurrence of *M. undulatus* is at its peak, declining as the depth increases, whereas at hypoxic sites, deeper stations have the highest percent occurrences with some of the lowest percent occurrences occurring between 0-20m (the opposite of the pattern at normoxic stations).

It is difficult to say with any amount of certainty what is driving the relationship between depth occurrence and hypoxia in species like *M. undulatus* without further research. One possible explanation that should be further explored centers on the fact that hypoxia in the GOMEX typically occurs between 5-30m [10]. Since hypoxia is more common in these shallower depths, shallower relative to 100m (the approximate maximum sampling depth of SEAMAP summer groundfish surveys), it can be assumed that species with shallower depth preferences are more exposed to hypoxia than species with deeper depth preferences and because of this frequent exposure, or historic exposure, some are more hypoxia tolerant. If hypoxia were to occur in deeper water then it is likely to assume that much of the fauna typical of deeper water would migrate or be eliminated due to their low hypoxia tolerance and that that their migration or elimination from deeper hypoxic sites could allow species with shallower/ broader depth preferences and higher hypoxia tolerances, that are residing in shallower water, to invade these deeper sites due to the elimination or easing of predation or competition.

Several potential competitors and predators of shallow water groundfish with depth preferences deeper than 30m (depth bin with highest normoxic occurrence) show significant decreases in yearly average CPUE at hypoxic sites compared to normoxic sites as well as lower percent occurrences at hypoxic sites. *S. foetens*, for example, is a known predator of smaller groundfish species [57] and larger individuals in the northern GOMEX are known to feed extensively on *M. undulatus* [58]. *U. parvus*, comprises approximately 1 percent of the average yearly community biomass at sites below 30m, and is nearly excluded from hypoxic sites between 0-25m. The diet of *U. parvus* in the GOMEX consists mainly on decapod crustaceans, but also includes polychaetes, echinoderms, and mollusks. With a diet of mainly benthic invertebrates, the diet of *U. parvus* overlaps with the diets of several shallow water species that show an increased AUC in deeper water under hypoxic conditions including *M. undulatus* and *C. nothus* (Cyrana et al in prep). The longspine porgy, *Stenotomus caprinus*, which makes up 12.5

percent of the average yearly community biomass at sites deeper than 30m, has a percent occurrence at hypoxic sites that is 30 percent lower than that of normoxic sites between 25-30m (Figure 10). As such a major component of the community, changes in the abundance or occurrence of *S. caprinus* likely effects the abundance of its competitors.

The examples given above are not meant to argue for the proposed explanation of the observed relationship between occurrence and depth relative to hypoxia but are meant to demonstrate its plausibility and highlight the importance of future work on the subject. The idea that species interactions, such as competition, are impacted by depth has been explored in multiple systems. In a study investigating competition between *Mysis* shrimps and *kokanee* salmon in Lake Chelan, depth was found to be important in regulating apparent competition while temperature was found to be important in regulating resource competition [59]. Additionally, a study of two California reef fishes with similar diets and morphologies dispersed along a depth gradient found that the removal of the competitor with the shallower depth preference resulted in an influx of the deeper competitor into shallower water with the inverse not being true [60]. These studies further highlight the importance of future research investigating how hypoxia's impact on the benthic community in the GOMEX changes with depth.

Species Composition at Hypoxic Sites

Results from my random forest model of western gulf stations within 0-25m gives insight into the species composition of hypoxic sites compared to normoxic sites, particularly in identifying the species most impacted by hypoxia. Of the fourteen species most important in determining whether a station was from hypoxic or normoxic conditions, seven had significantly (Kruskal-Wallis $p<0.05$) lower average yearly CPUE values at hypoxic sites. All seven of these species had yearly average hypoxic CPUE values that were at least 58 percent lower than the yearly average normoxic CPUE values with the lowest (*S. foetens*) being 86.98 percent lower. These seven species are important to the model because, not only is their yearly average CPUE so much lower at hypoxic sites, but because they are also relatively common. 171 species were never found in hypoxia between 1984 and 2010, but since most of these species are never common at stations, they are not useful for determining whether a station is hypoxic or normoxic. Thus, having high importance in the random forest model and low average CPUE at hypoxic sites suggest that a species is a relatively common member of the community that is negatively impacted by hypoxia.

Another way to understand how hypoxia alters the benthic community in the GOMEX is to compare the relative contribution of each species to the overall community biomass. In the averaged normoxic community *M. undulatus* and *C. chrysurus* make up 47 percent of the community biomass while accounting for 55 percent of the community biomass in the averaged hypoxic community (Figure 16.) Neither species differed significantly in their average yearly CPUE between hypoxic sites and normoxic sites and, combined, the two species only account for 7 percent more of the community biomass at

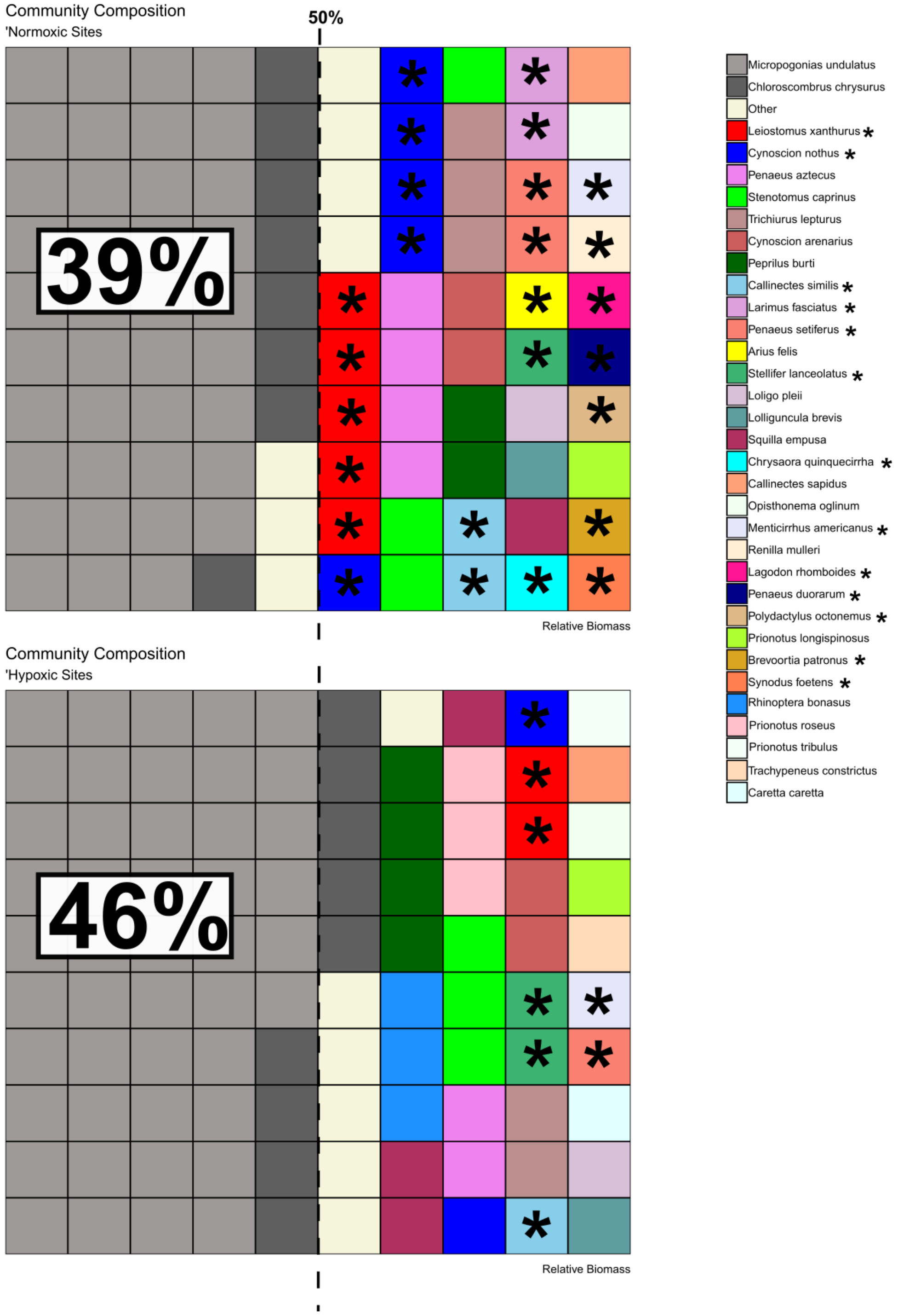


Figure 16. Waffle plot showing the average relative percent (percent of total biomass) of species accounting for at least 1 percent of the community biomass. Each color represents a different species with each square representing 1 percent of the average community biomass. Other represents the biomass of all species making up less than 1 percent of the community biomass. Asterisks indicate that the average yearly CPUE for the species was significantly different between hypoxic and normoxic sites (Kruskal Wallis, p<0.05).

hypoxic sites. The majority of the remaining components of the averaged normoxic community biomass (those that made up at least 1 percent of the community) have significantly lower average yearly CPUE at hypoxic sites compared to normoxic sites and do not make up at least 1 percent of the community biomass in the averaged hypoxic community. Additionally only 6 of the species making up at least 1 percent of the averaged hypoxic community had significantly different average yearly CPUE between hypoxic and normoxic sites suggesting that the change in their contribution to the overall hypoxic community biomass could be a result of other species becoming less abundant and boosting the percent contribution of other species. It is also worth considering that the vast majority of the 102 species found to have significantly lower yearly CPUE at hypoxic sites compared to normoxic sites make up less than 1% of the total average biomass of the average normoxic community. These observations support the lower average Shannon diversity observed at hypoxic sites in this study (Figure 3).

A decrease in the abundance of the rarer species in a community coupled with the increase in the relative abundance of the most common species corresponds to a loss of species richness, one of the factors used to calculate the Shannon index. This combined with the findings of lower numbers of species at hypoxic sites compared to normoxic sites (Figure 4.) elucidates a general trend that can describe hypoxic sites relative to normoxic sites. Hypoxic sites are categorized by a loss of the rarer members of the community and are more homogenous with *M. undulatus* and *C. chrysurus* still accounting for most of the community biomass. This generalization is purposefully broad and is meant to complement more spatially-focused work that has already been done [38,39]. Further work testing whether this generalization holds up at smaller spatial scales is important especially considering my findings that show an interaction between species occurrence at depth and hypoxia (Figures 8-15) and my previous discussion of differences in results between this study and Briggs et al (2017).

Complexities Inherent in the Results

While I am confident that my results suggest that hypoxia reduces diversity and decreases the abundance (biomass) of many species of the GOMEX megabenthos, there are factors that arise from my broad treatment of the data that should be noted. For example, depending on the ecology and physiology of a species, it is possible that hypoxic conditions could increase catchability, which would skew comparisons of the catch between hypoxic and normoxic sites because the catch would no longer be a result of only the numbers of organisms present, but also their catchability. One species that could be exhibiting this phenomenon is the mantis shrimp species, *S. empusa*. *S. empusa* showed a higher percent occurrence at hypoxic sites for all depths, and despite not being significantly different (Kruskal-Wallis, $p>0.05$), the average yearly CPUE at hypoxic sites was nearly 300 percent of the yearly average CPUE of normoxic sites. These data might suggest that *S. empusa* is, at the least, hypoxia tolerant, or even potentially taking advantage of hypoxic sites. However, the ecology of this species suggests that the boost in occurrence and abundance could be a result of catchability.

Squilla empusa is known to excavate and dwell within burrows [61], as do other species within the *Squilla* genus [62]. Studies on *Squilla mantis*, highlight how these burrowing species are most vulnerable to conventional bottom trawling when outside of their burrows [63]. A variety of infaunal marine invertebrates have been documented to migrate out of the sediments when exposed to hypoxia in an attempt to reach more favorable DO conditions [12,19,20,64]. If *S. empusa* migrates out of the sediments and onto the sediment surface in an attempt to reach more oxygenated water, this behavior could increase the likelihood of being caught in a bottom trawl compared to individuals remaining within their burrows. The fact that hypoxia exposure has been shown to result in decreases in swimming behavior and activity in marine organisms also cannot be ignored [11-13]. This leaves the possibility that observations of higher catches in areas of hypoxia could result from increases in catchability resulting from lethargy or changes in behavior. It is important to note, however, that unless a clear ecological or behavioral link can be made between catchability and hypoxia for a species, it cannot be assumed that altered catchability is the result of higher catches from hypoxic sites.

My results do not take into account fine spatial scales and thus do not capture certain phenomena such as the edge effects associated with hypoxic zones. Craig (2012) reported aggregations of marine organisms at the edge of the hypoxic zone from 2002-2004 [40] and fishes in the Chesapeake bay are known to make short foraging forays into hypoxic areas in order to take advantage of dead and dying benthic invertebrates [43]. While evidence of these edge effects are likely represented in the data or results in some form, my methodology doesn’t permit me to definitively identify them.

Interannual variations in hypoxia, and in the GOMEX in general, is also not represented in my results. The hypoxic zone is well known to be highly variable in shape, location, and severity between years and there is known to be interannual variations in the ways in which hypoxia interacts with organisms. Craig (2010) noted that DO avoidance thresholds varied in different years, suggesting that DO avoidance thresholds are context dependent [42], with studies into hypoxia’s impact on the biota of the Neuse river estuary [65] supporting this idea. By using years as replicates for many of my analyses most of my results do not highlight interannual variability.

Summary

With the finer scale effects of hypoxia on GOMEX fauna [38,39,66,67] and the larger scale impacts of hypoxia on specific GOMEX organisms [40,42,68-70] being explored in detailed studies, I endeavored to broadly explore the large scale general impacts of hypoxia on GOMEX biota. My results clearly show that when one looks across years and compares the megabenthic diversity at hypoxic sites to that of normoxic sites, hypoxic sites are less diverse than normoxic sites. Additionally, the biomass of 21.7 percent of species between 0-25 m was significantly lower in hypoxic areas with 171 species never being caught in hypoxic sites. These results suggest that when one takes a broader look at hypoxia's impact on GOMEX biota, ignoring interannual variability and spatial context, hypoxia clearly negatively impacts biodiversity and reduces the biomass of, or excludes, many species. Additionally, my results suggest that depth plays an important role in how hypoxia impacts species, with many species having different occurrence at depth relationships in hypoxic and normoxic sites.

CHAPTER 2

INTRODUCTION

Many of the most common groundfish species in the GOMEX are known to prey heavily on benthic and infaunal invertebrates. Species such as the Atlantic croaker, *Micropogonias undulatus*, and spot, *Leiostomus xanthurus*, are known target polychaetes and crustaceans, while avoiding mollusks. The diets of GOMEX seatrout in the genus *Cynoscion* are known to largely consist of benthic crustaceans [71-74] as do the diets of many of the GOMEX searobins [75-77]. Given that these common species all feed on similar prey, benthic invertebrates, and that hypoxia is known to reduce the diversity and abundance of benthic invertebrates, the existence and degree of food competition as well as mechanisms to alleviate and avoid said competition among GOMEX groundfish is an important avenue of research.

Niche partitioning studies on several different communities show that both the degree of diet overlap and evidence of resource partitioning can vary by community. The three main piscivores in the northern Scandinavian Pasvik watercourse were found to have high diet overlap and limited resource partitioning, mainly diverging in diets ontogenically [78]; whereas Venezuelan piscivore communities were shown to exhibit a high degree of diet specialization, particularly when prey became limited [79]. A study of Haplochromine cichlids in Lake Malawi produced evidence of niche differentiation in rocky shore communities while also suggesting that this differentiation was not necessary for similar species to co-exist [80]. Herder and Freyhof (2006) found no evidence of niche partitioning in a Vietnamese freshwater fish community [81]. Searobins off the West Florida shelf were found to primarily partition by macrohabitat while also displaying some evidence of resource partitioning by prey size [82], whereas three species of eastern Pacific intertidal sculpins were found to have similar niches and displayed niche partitioning, suggesting that competition between the 3 species has shaped their resource use [83].

While the results of niche partitioning studies in fishes vary, there are some general trends that have been suggested. For example, it has been argued that because of increased probability of resource limitation, due to less available habitat, resource partitioning may be more important to freshwater species than it is for marine species [84]. Ross, in a survey of niche partitioning in fishes, also suggests that biotic interactions become less important in structing fish communities in harsher environments [85]. These ideas can be applied and studied in the northern GOMEX. Recent work on diet shifts of GOMEX groundfish show that the diet of GOMEX fishes is altered by hypoxic conditions [86]. The goal of this chapter is to investigate how hypoxic conditions impact the diets and diet overlap of select GOMEX groundfish species in order to better understand how food resources are used by GOMEX groundfish faced with seasonal hypoxia.

METHODS

Sampling

I collected samples of fish specimens aboard the RV Oregon II during the 2016, 2017, and 2019 SEAMAP summer groundfish surveys. Dissolved oxygen and depth were determined using the CTD unit aboard the RV Oregon II immediately prior to sampling as outlined in the SEAMAP sampling protocol. Hypoxia was defined as DO values less than 2.0 mg/L with one station with a DO value of 2.04 rounded down and considered hypoxic. DO concentration of 2.0 mg/L is the value most often used for hypoxia studies, especially in the GOMEX [2]. Upon completion of each bottom trawl sampling station, fish specimens were immediately fixed in a 10 percent formalin solution for at least 1 week, washed and transferred to a 35% and then a 70% ETOH solution. I measured the length and weight of all specimens prior to sampling the stomach contents. The length measurements used for specimens of each species adhered to the type of length measurement utilized aboard SEAMAP cruises for each species (i.e. total length, standard length or fork length). Stomach contents were identified to the lowest possible taxonomic level with the wet weights of all identified and unidentified contents being taken.

Study Species

I chose to include the following species in this study: *Micropogonias undulatus* (subsequently referred to as croaker), *Leiostomus xanthurus* (subsequently referred to as spot), *Larimus fasciatus* (subsequently referred to as banded drum), *Prionotus longispinosus* (subsequently referred to as searobin), *Centropristis philadelphica* (subsequently referred to as rock seabass), and *Cynoscion arenarius* and *Cynoscion nothus* (subsequently referred to as seatrout). I pooled *C. arenarius* and *C. nothus* analyzing both species as a single unit. I pooled these species because I had a limited amount of *C. nothus* specimens and the diets of the two co-occurring species have been found to be similar [72-74]. These study species were chosen because they are all common and abundant species in normoxic conditions and were sampled enough in hypoxic conditions to have a sufficient sampling size. Banded drum, while only being represented from one site, were included because their abundance has been shown to be significantly lower in hypoxic sites compared to normoxic sites (Cyrana and Bart in prep). I included rock seabass in this study despite only having 3 individuals from hypoxic sites because this species is poorly studied and information on its diet and diet overlap with other species is informative.

Data Treatment, Analysis, and Measurements

Both overall and average diet composition only considered the identifiable components of the stomach contents, identifiable being defined as identified at least to phylum. For analysis, gut items were divided into 31 identifiable taxonomically grounded prey categories (taxonomically grounded as opposed to being determined by functional group i.e. zooplankton). I utilized a combination of overall and average diet as well as gut item percent occurrence to provide a more complete picture of groundfish diet than is capable by using only one measurement. Overall prey abundance treated each species, from either hypoxic or normoxic sites, as one collective diet, taking the sum of the wet weights of all the individuals and dividing it by the total wet weight of all stomach contents. This measurement provides an important broad summation of the collective diet of a species, but it can be skewed by a few individuals eating relatively large items, especially if most other individuals are feeding on smaller items with less mass.

$$Overall\%\ Prey\ Item_{ij} = \frac{\sum Mass_{ij}}{\sum Mass_{all\ items\ j}} \times 100$$

Average prey item percent was calculated for each individual by dividing the wet weight of each stomach item by the total wet weight of all identifiable stomach items, which was then averaged for the species and DO condition.

$$Average\%\ Prey\ Item_{ij} = \frac{\sum_{n1_{j} \rightarrow n_j} \frac{mass_{ij}}{mass_j}}{n_j}$$

Both average and overall diet were calculated using the *RInSp* package in R. I converted diet data into a package specific object type using the *import.RInSp* function then using the *popdiet* function to calculate either the overall or average diet per species [87]. For each diet analyzed (species and DO condition) the Levin's Diversity Index, D value, was returned [87,88] as a measure of the diet diversity. This D value was then standardized to be between 0 and 1, where 1 is a uniform diet of only one prey category and 0 is a diet containing all possible prey categories , using the transformation developed by Hurlbert (1978) [89].

$$Standardized\ D\ value = \left(\frac{Levins\ Diversity\ Index\ -1}{Number\ of\ Prey\ Catagories - 1}\right)$$

I defined the fullness measurement as the percent of the total body weight consumed by each specimen. This measurement utilized all stomach contents, not just the identifiable items.

$$Fullness = \left(\frac{Weight_{gut\ contents}}{Weight_{specimen}}\right) \times 100$$

I regressed fullness against DO values of the stations each specimen was sampled from, plotting all species together on one plot in order to see if patterns existed across species. Due to the bimodal nature of the distribution I utilized a 2nd degree polynomial regression using the *lm* function in the r stats package [90]. In addition to regression in which I pooled all species, I also conducted regressions of only croaker and only seatrout fullness against DO values as these species had the highest numbers of individuals sampled. The distributions for croaker and seatrout were also bimodal so I utilized a 2nd degree polynomial for these additional regressions.

Diet Overlap and Individualism

Both diet overlap and the individualism index were calculated using the *RInSp* package, specifically the overlap and *PSicalc* functions [87]. The individualism index represented the mean Czekanowski proportional similarity index. Both Schoener (1968) and Feinsinger et al. (1981) promoted the use of the Czekanowski proportional similarity index in niche comparison studies [91,92]. This index compares the proportion of each prey item in the stomach of an individual to the proportion of the same prey items in the population. The same proportional similarity index is utilized by the overlap function.

To calculate the average diet overlap between two species, the pairwise diet overlap of each specimen of the two species was calculated, for either hypoxic sites or normoxic sites. The average pairwise overlap was then calculated for each specimen, generating one average diet overlap value for each specimen. Since averaging the diet overlap for either species in a two species comparison results in the same mean diet overlap between the two species, the species with the lowest sample size was used for hypothesis testing. To test whether the average diet overlap was significantly different between two species, the average overlap values for the species with the lowest number of individuals was compared between hypoxic and normoxic sites with individuals as replicates (Table 5).

	SpeciesX_Fish_1	SpeciesX_Fish_2	SpeciesX_Fish_3	SpeciesX_Fish_4	SpeciesX_Fish_5	SpeciesX_Fish_6	SpeciesX_Fish_7	SpeciesX_Fish_8	Row Mean
SpeciesY_Fish_1	0.4	0.8	0.16	0.05328	0.15984	0.128	0.2664	0.7992	0.34584
SpeciesY_Fish_2	0.5	1	0.2	0.0666	0.1998	0.2	0.333	0.999	0.4373
SpeciesY_Fish_3	0.43	0.86	0.172	0.057276	0.171828	0.14792	0.28638	0.85914	0.373068
SpeciesY_Fish_4	0.44	0.88	0.176	0.058608	0.175824	0.15488	0.29304	0.87912	0.382184
SpeciesY_Fish_5	0.47	0.94	0.188	0.062604	0.187812	0.17672	0.31302	0.93906	0.409652
SpeciesY_Fish_6	0.67	1.34	0.268	0.089244	0.267732	0.35912	0.44622	1.33866	0.597372
SpeciesY_Fish_7	1	2	0.4	0.1332	0.3996	0.8	0.666	1.998	0.9246
SpeciesY_Fish_8	0.43	0.86	0.172	0.057276	0.171828	0.14792	0.28638	0.85914	0.373068
SpeciesY_Fish_9	0.53	1.06	0.212	0.070596	0.211788	0.22472	0.35298	1.05894	0.465128
SpeciesY_Fish_10	0.23	0.46	0.092	0.030636	0.091908	0.04232	0.15318	0.45954	0.194948
SpeciesY_Fish_11	0.55	1.1	0.22	0.07326	0.21978	0.242	0.3663	1.0989	0.48378
Column Mean	0.513636364	1.027272727	0.205454545	0.068416364	0.205249091	0.238509091	0.342081818	1.026245455	

Mean of Column Means	0.453358182
Mean of Row Means	0.453358182
Number of Columns	8
Number of Rows	11

Table 5. An example of a diet overlap matrix used to calculate the mean overall diet overlap between two species at either hypoxic or normoxic sites. Note that the mean diet overlap does not change wether its calculated from the row means (average for Species Y), or column means (average for species X). When comparing the average diet overlap between two species between hypoxic and normoxic sites the mean overlap values used in the Kruskal Wallis test were taken from the species with the lowest sample size which in the example above is Species X. For the example above, assuming the values in the table are from normoxic fishes, the column means would be used in the Kruskal Wallis test and compared against the columns means from the hypoxia overlap matrix for Species X and Species Y.

I employed NMDS analysis as a method of visualizing the diets of all specimens from all species on one plot. Using the raw wet weights of the identifiable stomach contents I ran a 2-dimensional NMDS analysis. The subsequent NMDS plot represents the similarities between diets, the closer two diets (points) the more similar the diets are in composition. The contribution of prey items to the similarity of diets was also plotted on the NMDS plot, the closer the prey item is to a point, the more that prey item is represented in that diet(point). One benefit of NMDS analysis is that it is rank based, so there are less assumptions compared to other methods such as PCA.

RESULTS

Community Diet

The study species prey on a variety of prey items, with crustaceans, fishes, and polychaetes representing some of the most common prey items (Figures 17 and 18). Polychaetes comprised larger portions of the diets of croaker and spot with amphipods and nematodes being more associated with diets of searobins and spot. Fishes were important components of the diets of seatrout and rock seabass. Banded drum had diets with items like larval fishes and crustaceans as well as sergestid shrimps, which were also associated with seatrout diets.

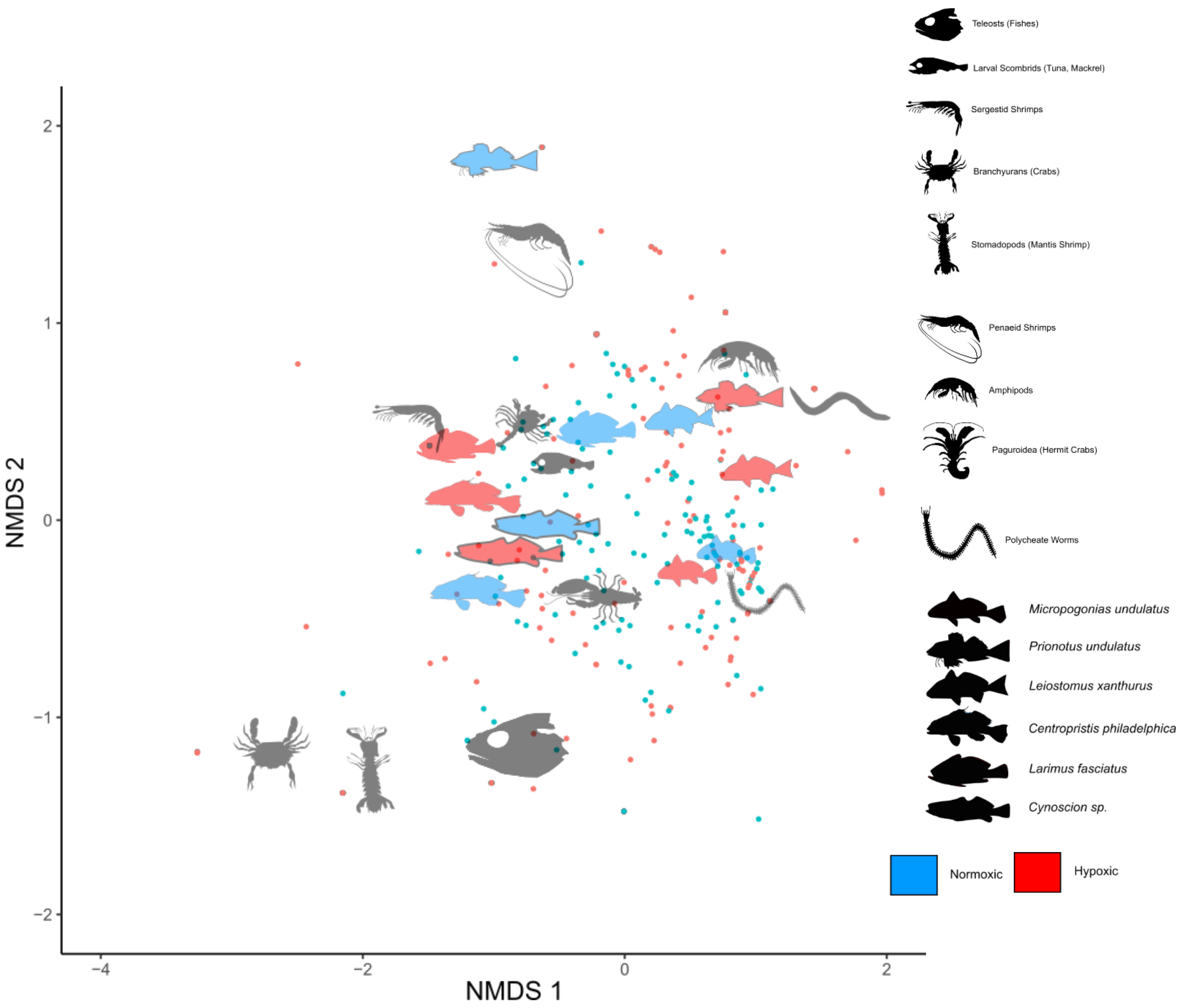


Figure 17. NMDS plot of the stomach contents (percent wet weight of prey items) of individual specimens of croaker(*M. undulatus*), sea robin(*P. longispinosus*), spot(*L. xanthurus*), rock seabass(*C. philadelphica*), banded drum(*L. fasciatus*), and seatrout (*Cynoscion sp.*) from hypoxic(red) and normoxic (blue) sites collected during the 2016, 2017, and 2019 SEAMAP summer groundfish cruise. Points that are close to one another have similar diets. The dark prey symbols denote what prey items are most associated with stomach contents of nearby points. The colored fish symbols denote the average of all points of a particular species with blue symbols representing the average for normoxic specimens and red symbols representing the average for hypoxic specimens.

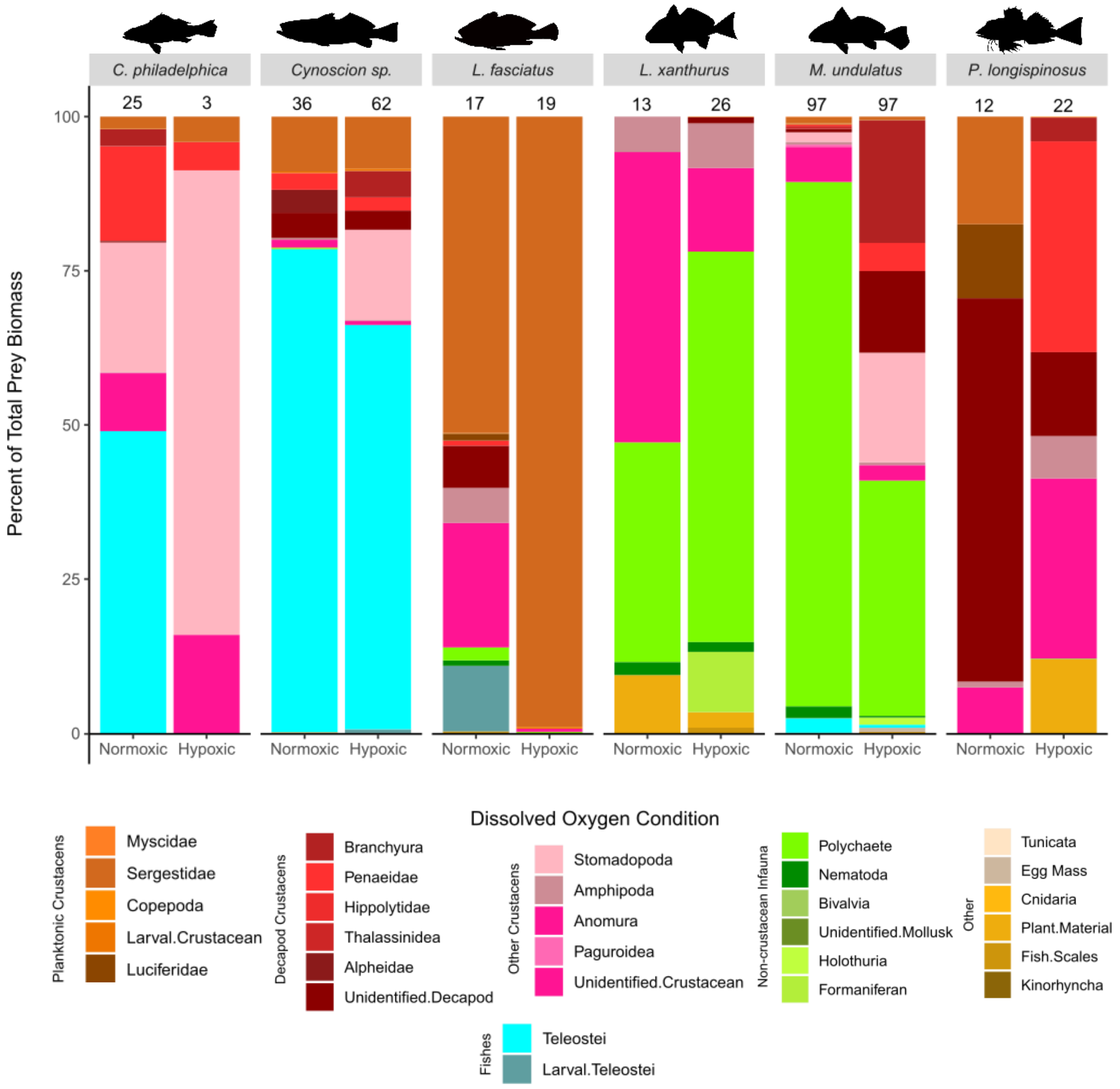


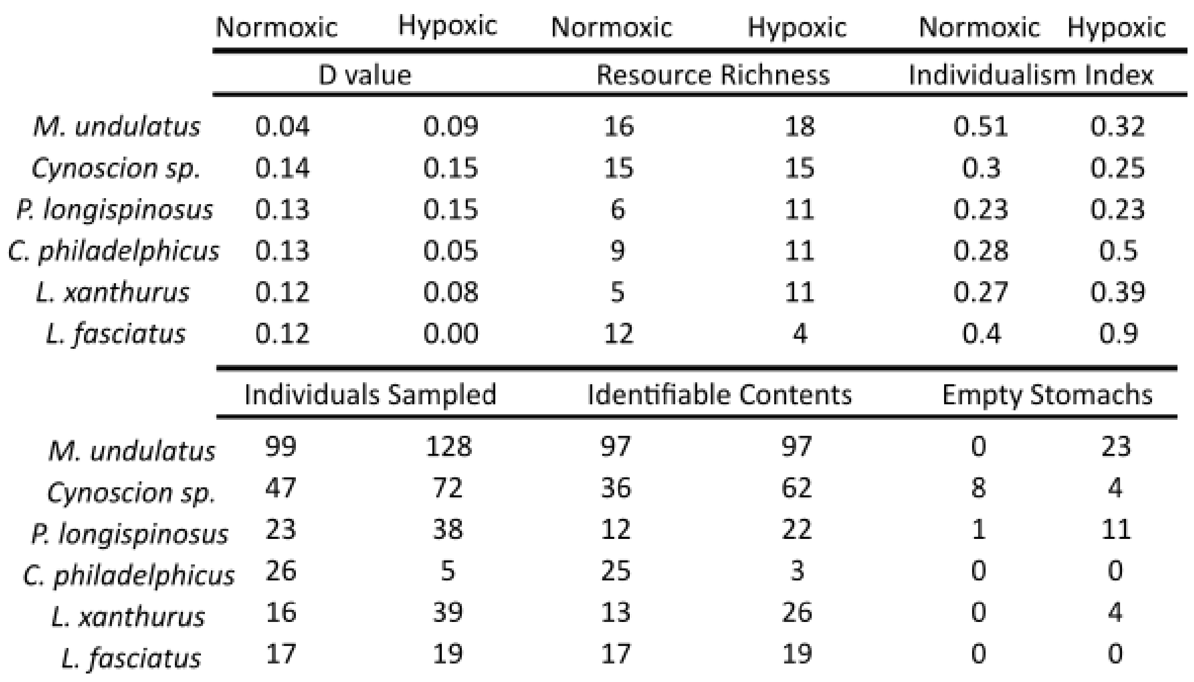
Figure 18. Total average diet composition of identifiable stomach contents of croaker(*M. undulatus*), sea robin(*P. longispinosus*), spot(*L. xanthurus*), rock seabass(*C. philadelphica*), banded drum(*L. fasciatus*), and seatrout (*Cynoscion sp.*) from normoxic and hypoxic sites in the northern GOMEX. Numbers above bars denote the number of stomachs with identifiable contents (n).

Diet croaker (M. undulatus)

The diversity values (D values) for croaker showed a higher prey diversity at hypoxic sites. Croaker from hypoxic sites also showed higher individualism (lower individualism index values) compared to normoxic sites and a higher ratio of empty stomachs (chi square, $p<0.05$) (Table 6). The identifiable component of the diet of croaker was dominated by polychaetes at both normoxic and hypoxic sites (Figure 18). Polychaetes occurred in a similar percentage of stomachs of individual croaker at both normoxic and hypoxic sites but accounted for a larger average and overall percentage of the identifiable prey biomass at normoxic sites. Decapod crustaceans occurred more frequently and accounted for a larger percent of the average and overall prey biomass at hypoxic sites compared to normoxic sites. At hypoxic sites, brachyurans only accounted for an average of 3 percent of the total prey biomass and occurred in only about 3 percent of individuals while accounting for almost 20 percent of the overall prey biomass. This suggest that a few individuals consumed the majority of the brachyuran biomass at hypoxic sites.

Diet of seatrout (Cynoscion spp.)

The D values and individualism indices did not differ greatly between seatrout from normoxic sites and those from hypoxic sites 0.14 and 0.15 respectively, with seatrout from hypoxic sites having a slightly lower, 0.25, individualism index value compared to seatrout from normoxic sites, 0.3. Seatrout from normoxic sites also had a significantly higher ratio of empty stomachs compared to seatrout at hypoxic sites, although most of these empty stomachs came from one station. (Table 5). Sergesitd shrimps, fishes, and decapod crustaceans accounted for the majority of both average and overall prey biomass in seatrout while also having similar percent occurrences in seatrout



	Normoxic	Hypoxic	Normoxic	Hypoxic	Normoxic	Hypoxic
	D value		Resource Richness		Individualism Index	
M. undulatus	0.04	0.09	16	18	0.51	0.32
Cynoscion sp.	0.14	0.15	15	15	0.3	0.25
P. longispinosus	0.13	0.15	6	11	0.23	0.23
C. philadelphicus	0.13	0.05	9	11	0.28	0.5
L. xanthurus	0.12	0.08	5	11	0.27	0.39
L. fasciatus	0.12	0.00	12	4	0.4	0.9
	Individuals Sampled		Identifiable Contents		Empty Stomachs	
M. undulatus	99	128	97	97	0	23
Cynoscion sp.	47	72	36	62	8	4
P. longispinosus	23	38	12	22	1	11
C. philadelphicus	26	5	25	3	0	0
L. xanthurus	16	39	13	26	0	4
L. fasciatus	17	19	17	19	0	0

Table 6. Standardized D value, Resource richness, individualism index, number or individuals sampled, number of empty stomachs, and the number of stomachs with identifiable stomach contents of the 6 study species. The ratio of empty stomachs to stomachs with stomach with material in them was significantly different between hypoxic and normoxic sites for *M. undulatus*, *Cynoscion spp., and P. longispinosus* (chi square, p <0.05).

diets at hypoxic and normoxic sites. Stomatopods and branchyurans were not observed in the stomachs of normoxic seatrout but accounted for 8.5 and 3.8 percent of the average and 14.7 and 4.3 percent of the overall prey biomass respectively at hypoxic sites. Stomatopods were found in the stomachs of approximately 16.6 percent of hypoxic seatrout with branchyurans being found in approximately 8 percent of hypoxic seatrout (Figure 18).

Diet of spot (L. xanthurus)

Both the D value and the individualism for spot from normoxic sites were higher compared to spot from hypoxic sites and there was no significant difference in the ratio of empty stomachs between hypoxic and normoxic sites (Table 5). Crustaceans, polychaetes, and nematodes were the only prey items identified in diets of spot from normoxic sites. Crustaceans and polychaetes were also dominant prey items in stomachs of spot from hypoxic sites with polychaetes comprising a greater average and overall percent of prey biomass at hypoxic sites while having a similar percent occurrence to spot from normoxic sites (Figure 18).

Diet of searobin (P. longispinosus)

The D value and the overall number of prey items were higher in diets of searobins from hypoxic sites compared to searobins from normoxic sites with searobins from both hypoxic and normoxic sites having identical individualism index values. The ratio of empty stomachs was significantly higher in searobins from hypoxic sites compared to those from normoxic sites (chi square, $p<0.05$) (Table 5). The most frequently encountered prey items in stomachs of searobins from normoxic sites were amphipods and sergestid shrimps, with both of these prey items accounting for approximately 18 percent of the overall prey biomass in searobins from normoxic sites. Unidentified decapod crustacean material was found in approximately 6.8 percent of stomachs of searobins from normoxic sites but accounted for approximately 62 percent of the overall prey biomass of searobin diets from these sites. Searobins from hypoxic sites also had a large percentage of their overall prey biomass composed of crustaceans, however searobins from hypoxic sites did not prey upon any sergestid shrimps (Figure 18).

Diet of rock seabass (P. philadelphica)

Rock seabass from normoxic sites fed primarily on fishes and crustaceans with stomatopods and penaeid shrimps accounting for most of the crustacean prey biomass. The few rock seabass sampled from hypoxic sites fed exclusively on crustaceans (Figure 18).

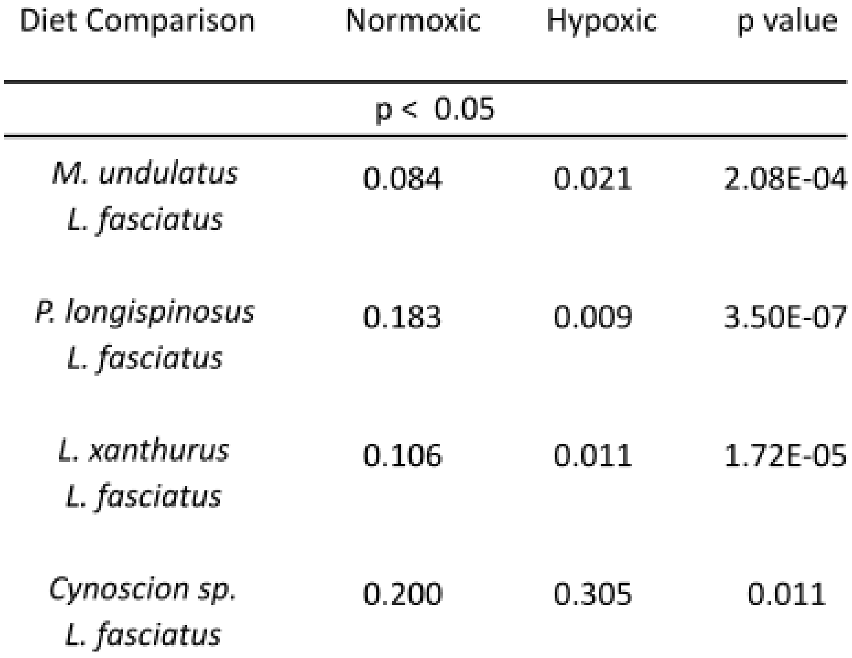
Diet of banded drum (L. fasciatus)

The diets of banded drum from normoxic sites were more diverse than banded drum diets from hypoxic sites. D values of these sites were 0.12 and 0 respectively, with the individualism index of banded drum from normoxic sites being lower than that of banded drum from hypoxic sites. The individualism index of 0.9 for banded drum from hypoxic sites corresponds to the diets of drum from these sites being nearly identical (Table 6). The diets of banded drum from normoxic sites were dominated by sergestid shrimps, with amphipods, crustacean material, larval decopods, and polychaetes also occurring in stomach contents. Banded drum from hypoxic sites fed almost exclusively on sergestid shrimps, with only small amounts of crustacean material, larval crustaceans, and polychaetes found in stomachs (Figure 18).

Diet Overlap and Fullness

The average diet overlap at normoxic sites was significantly higher (Kruskal Wallis, $p<0.05$) than average diet overlap at hypoxic sites in comparisons between banded drum and all other study species except for the rock seabass (Table 7). The average diet overlap at normoxic sites and average diet overlap at hypoxic sites were not significantly different for any other pairing of study species (Kruskal Wallis, $p>0.05$) (Table 8).

There is a significant ($p<0.05$, all variables) 2nd order polynomial relationship between specimen fullness and the DO of the sampling site from which specimens were taken, with fish from sites with intermediate DO values having lower fullness than stations with both higher and lower DO values (Figure 19). A similar and similarly statistically significant trend is also observed in regressions involving only croaker (Figure 20) and only seatrout (Figure 21).



Diet Comparison	Normoxic	Hypoxic	p value
	$p < 0.05$		
M. undulatus *L. fasciatus*	0.084	0.021	2.08E-04
P. longispinosus *L. fasciatus*	0.183	0.009	3.50E-07
L. xanthurus *L. fasciatus*	0.106	0.011	1.72E-05
Cynoscion sp. *L. fasciatus*	0.200	0.305	0.011

Table 7. Diet overlap values between pairs of study species in which the average diet overlap was significantly higher at normoxic sites (Kruskal Wallis, $p < 0.05$, n=individuals).

Diet Comparison	Normoxic	Hypoxic	p value
p > 0.05			
M. undulatus *C. philadelphicus*	0.048	0.1	0.07
M. undulatus *L. xanthurus*	0.207	0.275	0.157
M. undulatus *Cynoscion sp.*	0.065	0.042	0.307
P. longispinosus *C. philadelphicus*	0.117	0.117	0.074
P. longispinosus *L. xanthurus*	0.081	0.115	0.247
C. philadelphicus *L. fasciatus*	0.335	0.086	0.395

Diet Comparison	Normoxic	Hypoxic	p value
p > 0.05			
M. undulatus *P. longispinosus*	0.039	0.087	0.139
Cynoscion sp. *P. longispinosus*	0.136	0.047	0.055
Cynoscion sp. *C. philadelphicus*	0.120	0.145	0.193
Cynoscion sp. *L. xanthurus*	0.045	0.017	0.195
C. philadelphicus *L. xanthurus*	0.178	0.215	0.157

Table 8. Diet overlap values between pairs of study species in which the average diet overlap was not significantly different between normoxic sites and hypoxic sites (Kruskal Wallis, $p < 0.05$, n=individuals).

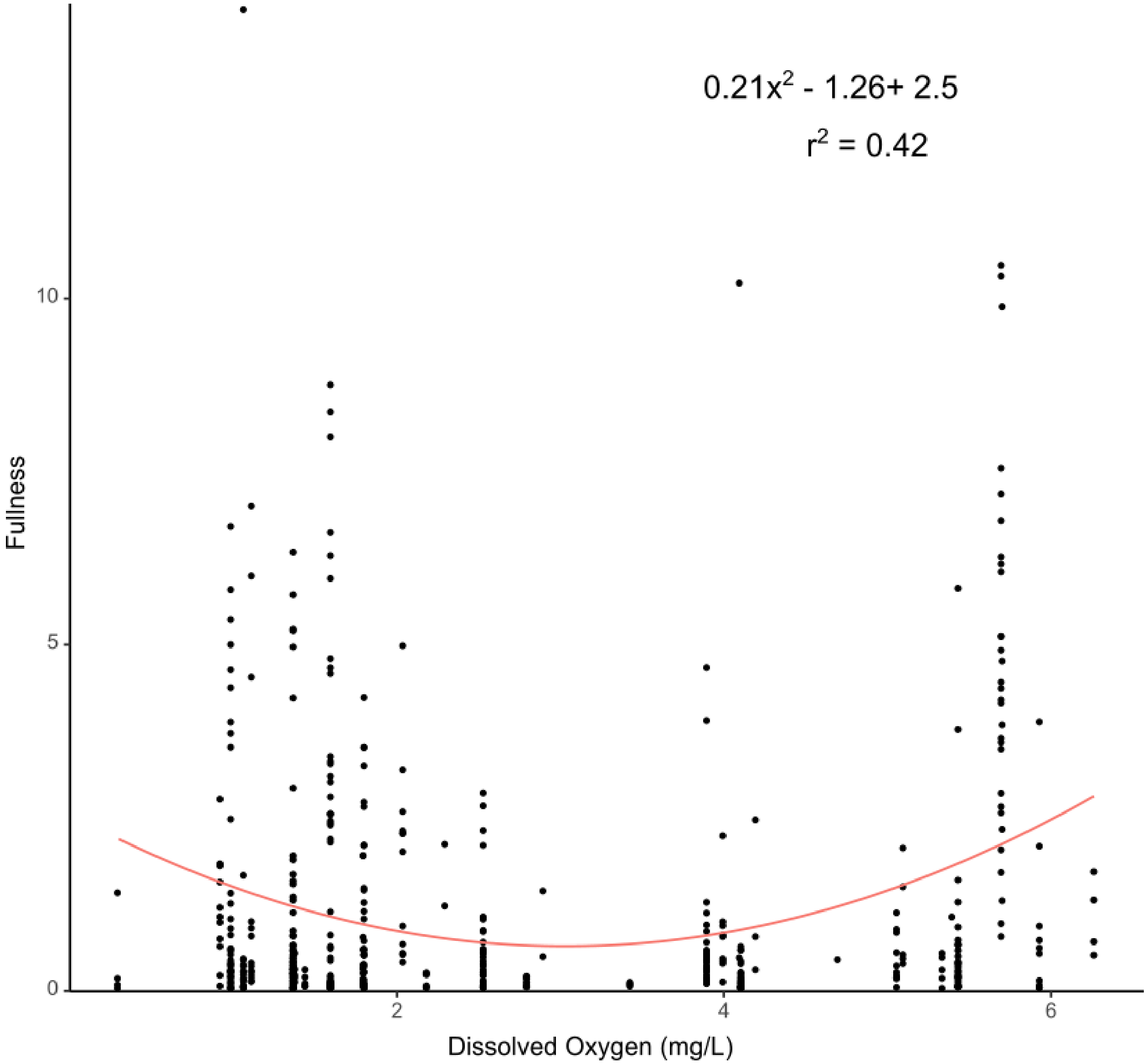


Figure 19. Polynomial regression (all variables and intercepts significant, $p<0.05$) of fullness as a function of dissolved oxygen (mg/L) for individuals for all study species.

$$Fullness = \left(\frac{Weight_{gut\ contents}}{Weight_{specimen}}\right) \times 100$$

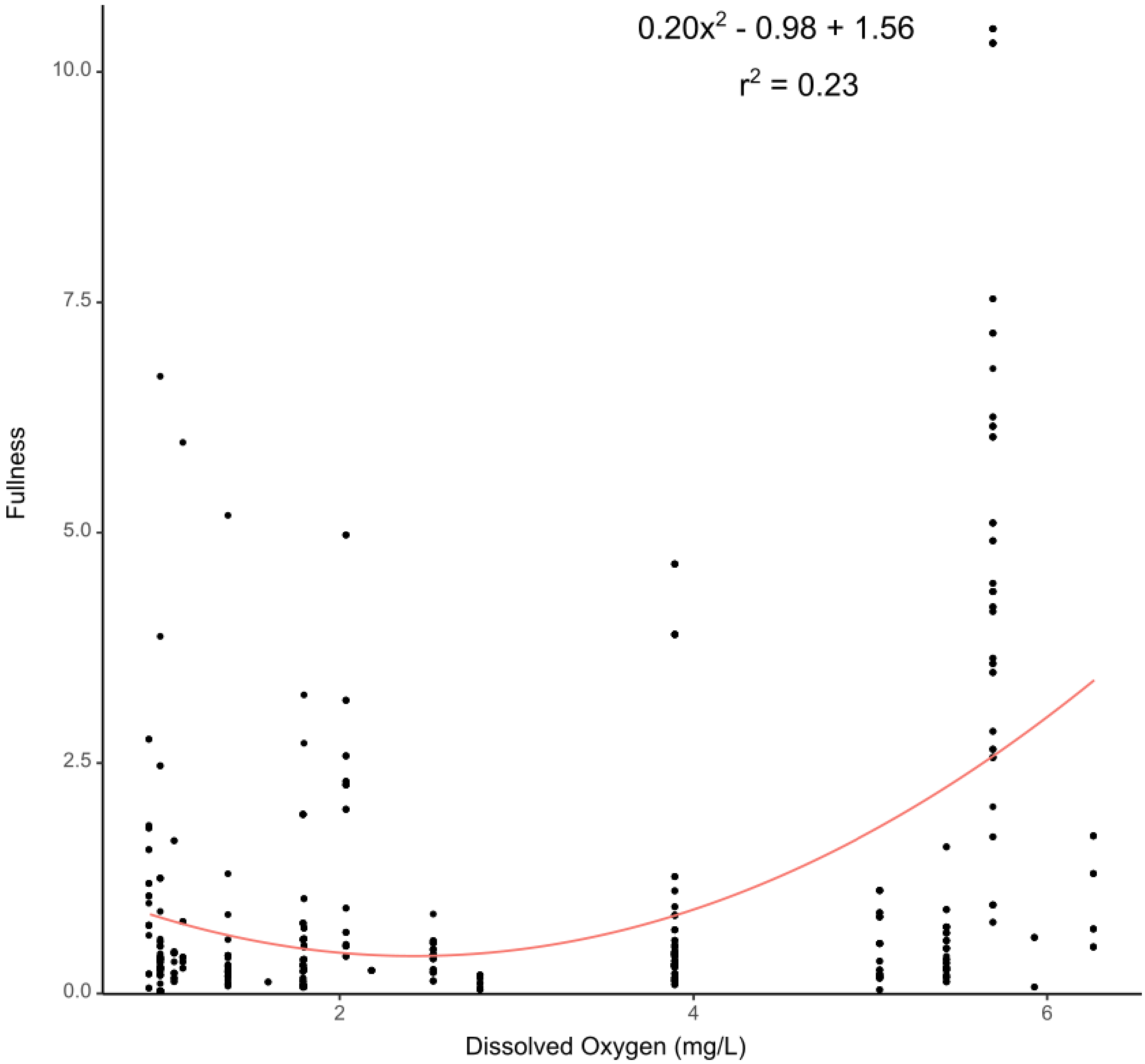


Figure 20. Polynomial regression (all variables and intercepts significant, $p<0.05$) of fullness as a function of dissolved oxygen (mg/L) for individual croaker.

$$Fullness = \left(\frac{Weight_{gut\ contents}}{Weight_{specimen}}\right) \times 100$$

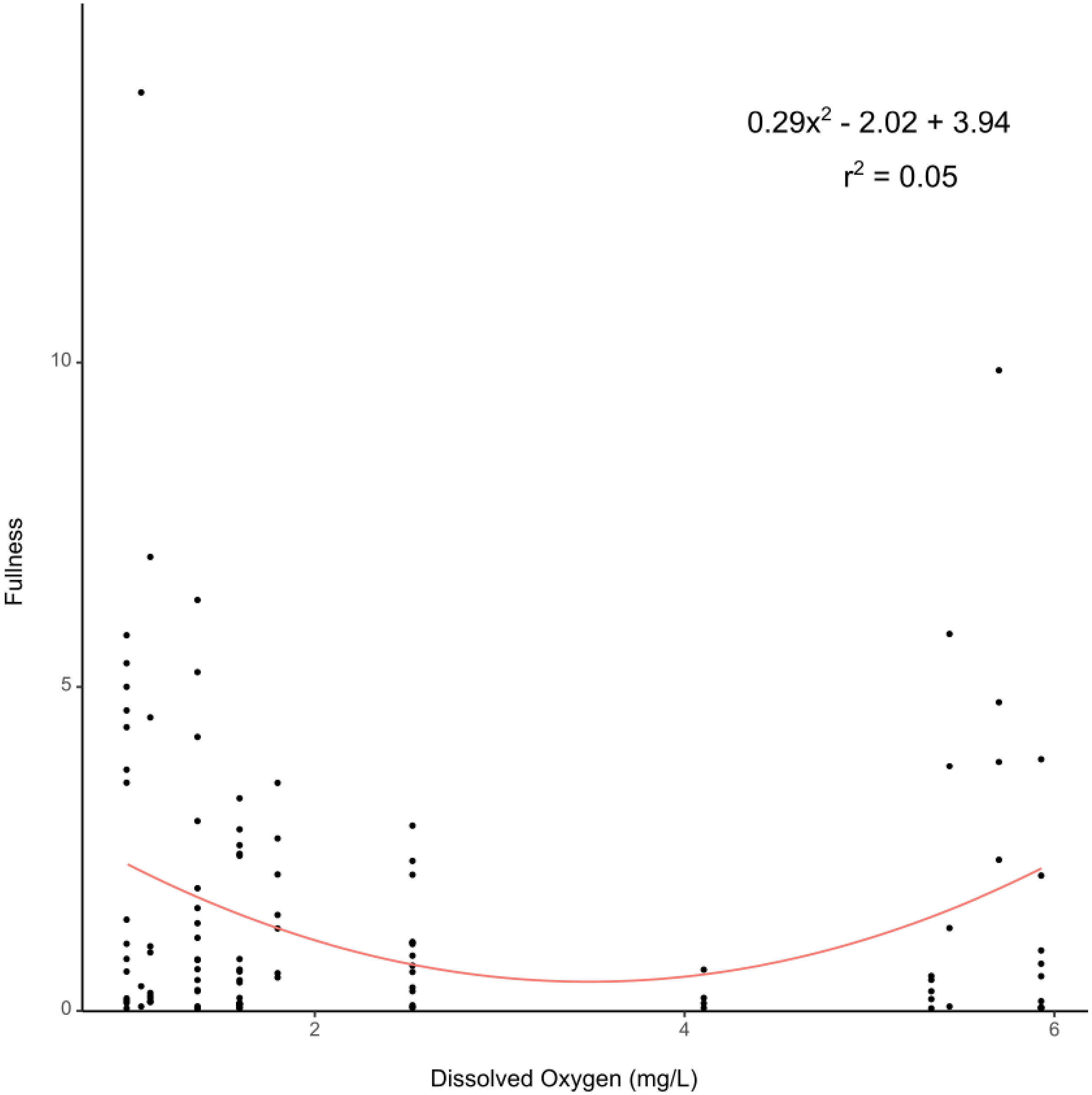


Figure 21. Polynomial regression (all variables and intercepts significant, $p<0.05$) of fullness as a function of dissolved oxygen (mg/L) for individual seatrout.

$$Fullness = \left(\frac{Weight_{gut\ contents}}{Weight_{specimen}}\right) \times 100$$

DISCUSSION

Comparisons with Other Diet Studies

The prevalence of polychaetes in the diets of croaker and spot at both hypoxic and normoxic sites is not surprising given that polychaetes have been found to be the most prevalent group of macrofauna in GOMEX sediments [39] and that croaker [38,67,86] and spot [18] are known to prey upon polychaetes. A study of croaker diet in the GOMEX found that polychaetes made up 54-100 percent of the identifiable prey in croaker stomachs consequently finding that the electivity of polychaetes, and crustaceans, was high while selectivity for mollusks was extremely low [67], findings that are supported by the prevalence of polychaetes and crustaceans and lack of mollusks in croaker stomachs in this study and previous work on spot and croaker [93].

Diet studies of the banded drum suggest that this species preys heavily on large zooplankton [86,94] and that banded drum at hypoxic sites feed almost exclusively on large zooplankton [86]. While the type of large zooplankton on which banded drum prey upon the most frequently differs between this study and previous studies, the diet of banded drum from hypoxic sites in this study was composed almost entirely of sergestid shrimps, which are larger zooplankton [95]. The presence of sergestid shrimps, larval teleosts, and lucifer shrimps, which are known to co-occur with sergestid shrimps [96], in banded drum diets further reinforces the idea that banded drum feed heavily on planktonic crustaceans, especially under hypoxic conditions.

Seatrout in this study were found to prey primarily on fishes and crustaceans, a diet which is supported by previous diet studies [71-74]. Previous studies also indicate that the diet of seatrout shifts from smaller planktonic crustaceans to larger, likely more benthic, crustaceans and fishes [71-74] which is a trend that was also observed in this study, although not reported. It should be noted that the majority of the previously cited diet studies focused on *Cynoscion arenarius* [72-74] with *Cynoscion nothus* only being examined in one of the studies in which the diets of the two species were found to be similar [71].

Research into the ecology of *Prionotus longispinosus* is sparse. Some items in the diet of *P. longispinosus* observed in this study are congruent with the diets of other species of searobins such as the blackwing searobin , *Prionotus rubio*, feeding heavily on decapod crustaceans [76], which were found in stomachs of *P. longispinosus* from both normoxic and hypoxic sites. Leopard searobin, *Prionotus scitulus*, were found to feed more on infaunal prey items than decapods [77], which is similar to the frequent occurrence of amphipod prey in the stomachs of *P. longispinosus* in this study. In a study that compared the diets of *Bellator militaris*, *Prionotus alatus*, *Prionotus martis*, *Prionotis roseus*, and *Prionotus stearnsi*, the diets of the 5 species varied, but contained decapod crustaceans, some planktonic crustaceans such as mysids, and amphipods [97] which is congruent with the searobin diet observed in this study.

The predominance of larger crustaceans as well as the importance of sergestid shrimps in the diet of the rock seabass from this study are similar to previous work on the diet of the rock seabass [93,98], although the prevalence of fish in the stomachs of rock seabass from this study far exceeds what has been reported previously. This difference in the importance of fish in the diet of rock seabass could be due to the lower sample size of this study, however geographic and temporal differences between studies could also be contributing factors.

In some respects, my findings differ from recently published work comparing the diets of GOMEX fishes from hypoxic sites with diets of fishes from normoxic sites. In this study polychaetes accounted for a larger average percent of croaker diets at hypoxic sites compared to normoxic sites. Glaspie et al. (2019) found that the percent contribution of polychaetes in croaker diets at hypoxic sites was lower than what was observed at normoxic sites with polychaetes making up less than 10 percent of the diet of croaker from hypoxic sites [86]. This study found that while the contribution of polychaetes to the overall prey biomass of croaker was lower at hypoxic sites compared to normoxic sites, polychaetes still accounted for more than 30 percent of the overall prey biomass in croaker at hypoxic sites. This difference could be explained by differences in sampling sites, particularly how hypoxic sites were defined.

Hypoxic sites in this study were defined as less than or equal to 2 mg/L DO whereas Glaspie et al. (2019) defined hypoxic stations as being below 1.71mg/L DO [86]. Additionally, of the croaker diets most associated with polychaetes (Figure 17), most of these individuals were from two stations, 199 (2016) and 32 (2016). Both stations were located on the edge of hypoxia and the DO of station 199 was 2.04 (rounded down) making it possible that infaunal organisms at these stations were in the process of being exploited by fish from less hypoxic waters. Being at the edge of hypoxia could allow for quick forays into hypoxic areas in order to exploit stressed or migrating infaunal invertebrates, similar to what has been observed in the Chesapeake Bay [18].

Diet Differences at Hypoxic Sites

Branchyurans and stomatopods accounted for a larger percentage of croaker and seatrout diet in hypoxic sites compared to normoxic sites with branchyurans making up a higher percentage of searobin diets at hypoxic sites compared to normoxic sites. Increased predation on these larger crustaceans could be a result of opportunistic feeding. Hypoxic conditions are known to cause lethargy and reduced movement in marine organisms [11-13] and fishes are known to take advantage of hypoxia stressed macroinvertebrate prey [18]. Given that branchyurans and stomatopods are among the most well armored groups of large crustaceans in the GOMEX; predation on these groups likely comes with behavioral or physiological costs, especially for smaller fishes. If hypoxic conditions impair the ability of branchyurans and stomatopods to flee or defend themselves, it would not be surprising to find predation of these crustaceans increasing in hypoxic conditions. An alternative explanation for the increased abundances of branchyurans and stomatopods in the diets of fishes in hypoxic sites could be that these branchyurans and stomatopods are more vulnerable to predation in hypoxic areas.

Stomatopods in the genus *Squilla* are known to dwell in burrows [62], including *Squilla empusa* [61], one of the most common stomatopods in the northern GOMEX. If hypoxic conditions forced *Squilla empusa* to emerge from their burrows, this would increase the ability of groundfish to encounter and subsequently prey upon them. Increased predation upon previously inaccessible infaunal prey resulting from hypoxic conditions has been documented in the Chesapeake Bay [18] and could explain the increase in stomadopods in fish diets from hypoxic sites.

Diet Overlap

All study species, except for the rock seabass, had significantly higher overall average diet overlap with the banded drum at normoxic sites compared to hypoxic sites (Kruskal Wallis, $p<0.05$). This is likely due to the fact that banded drum, which had diverse diets at normoxic sites, preyed almost exclusively on sergestid shrimps at hypoxic sites. Despite the small sample size of banded drum from hypoxic sites in this study, it is similar to what has been reported in the literature. Glaspie et al. (2019) reported that the diet of banded drum from hypoxic sites consisted almost entirely of shrimp larvae [86], which, while not sergestid shrimps, still shows a near complete shift to feeding upon zooplankton in hypoxic sites.

Fullness

Both Atlantic croaker and searobin had higher proportions of empty stomachs at hypoxic sites compared to normoxic sites (chi square, $p<0.05$), whereas seatrout had significantly higher proportions of empty stomachs at normoxic sites compared to hypoxic sites, although most of these empty stomachs for seatrout came from a single station. Additionally, when fullness (stomach content weight/fish weight x100) for pooled individuals of all species is plotted as a function of DO, the resulting second order polynomial model was significant for all variables. This overall trend suggests that fishes at sites with DO values intermediate between hypoxia and normoxia tend to have lower stomach fullness values. This trend of more successful feeding under both hypoxic and normoxic conditions is at least partially supported by the known phenomenon of organisms aggregating at the edges of hypoxic zones in the GOMEX [40] as well as the documented cases of fishes taking advantage of episodic hypoxia in the Chesapeake Bay to feed on stressed invertebrates [18]. As discussed above, the higher occurrences of stomatopods and branchyurans in the stomachs of multiple species of fish from hypoxic sites compared to fish from normoxic sites suggests that these decapods are more readily encountered and/or easier to prey upon under hypoxic conditions. Successful feeding under normoxic conditions is expected; increased fish stomach fullness under hypoxic conditions is further confirmation of amplified foraging opportunities on oxygen-stressed benthic prey.

Prey items more associated with the water column rather than the benthos could also be contributing to the abovementioned trend of higher fullness at hypoxic sites compared to intermediate DO sites. Zooplankton, such as copepods, have been found to occur in higher numbers within hypoxic bottom waters in stratified estuaries, with their predators, anchovies, sometimes making brief forays into the hypoxic zone to forage [44,99]. Additionally, in a study investigating diet shifts in GOMEX fishes relative to hypoxia, it was observed that benthic prey are consumed less in hypoxic waters while predation on zooplankton increased [86]. This was evident in the diets of banded drum in this study which shifted from a mix of planktonic sergestid shrimps and benthic invertebrates at normoxic sites, to entirely sergestid shrimps at hypoxic sites, a pattern that was also observed by Glaspie et al (2019) [86].

While fishes in hypoxic zones exhibiting relatively higher fullness compared to areas with higher DO could be a result of fishes taking advantage of stressed or more available prey, it does not adequately explain why intermediate levels of DO would have lower fullness than both hypoxic areas and areas with higher values of DO. Increased foraging opportunities at hypoxic sites would not deplete the available food at sites with intermediate DO values, however the migration of mobile organisms could potentially deplete the available food or increase competition at intermediate DO sites. Both fishes and crustaceans are known to have the ability to detect and respond to hypoxic conditions [12,17] by migrating away from these areas [12]. Hypoxic conditions in the GOMEX have been shown to alter the spatial distribution of both croaker and brown shrimp [36] and have been shown to impact the spatial distribution and habitat preferences of several species of flatfish [37]. If hypoxia-induced migrations result in an increase in the overlap and food competition of GOMEX fishes then a subsequent decrease in the amount of food per individual fish would not be unlikely. The contribution of hypoxia-induced habitat shifts to food competition in GOMEX fishes should be further explored, as this study was not designed to address this idea.

Potential Food Competition

The aim of the study was to increase understanding of how GOMEX groundfish interact with their prey under hypoxia, and more specifically, whether and how hypoxia impacts groundfish food competition. Utilizing niche overlap to address questions about competition between two species, Wiens (1977) argued that the resources/niche dimensions that are most important to how the two species interact must be well understood [100] but the natural history of organisms has often been overlooked [101]. Resource selectivity is an important aspect of natural history that is missing from this study and previous diet studies.

One of the most important factors for determining whether there is competition for a resource is how limiting said resource is, as a resource must be limiting for competition over it to occur. Consequently, organisms utilizing the same resource does not necessarily imply that these organisms are in competition for that resource [102]. In order to establish that GOMEX groundfish are competing over food resources more studies similar to Baustian et al. (2009), which examined both diet and prey availability, need to be conducted [67]. This is not to say that inferences cannot be made using detailed community composition studies like Briggs et al. (2015) and Briggs et al. (2017), in which detailed accounts of both macro and megafaunal abundances relative to hypoxia were conducted. However, temporal fluctuations of environmental conditions can also alter the results of food competition between species [103] and the environmental conditions in the GOMEX, especially hypoxia, are known to be extremely variable across both time and space.

It has been observed that both exploitation [102] and interference [104] competition can often be stronger between conspecifics than between different co-occurring species. In this study, there was an overall trend of having higher stomach fullness at low and high DO sites than intermediate sites, which had lower fullness values. Atlantic croaker also had a higher proportion of empty stomachs at hypoxic sites compared to normoxic sites which could suggest increased competition among croaker for limiting food resources, however a patchier distribution of prey items in hypoxic sites would also explain this observation, further highlighting the need for future research.

Diet as a Potential Explanation of Fish Abundance Trends in Hypoxic Zones

While deleterious physiological effects of hypoxia exposure, like reproductive impairment and ovarian masculinization [105,106] likely contribute to the distribution and abundance of groundfish relative to hypoxia, diet could also be playing an important role in how hypoxia influences the abundance and distribution of fishes in the GOMEX. Spot, for example, are one of the most common groundfish species in the GOMEX, however they have been shown to have a lower average abundance at hypoxic sites compared to normoxic sites (Cyrana and Bart in prep). The polychaete based diet of spot coupled with the manner in which this species forages could explain why it is less common at hypoxic sites.

Spot have been shown to be more associated with areas with high numbers of macrobenthic prey as opposed to areas of high macrobenthic biomass [38]. Additionally, feeding in spot has been described as random sediment ingestion coupled with the sorting of prey items from the sediment in the mouth [18]. Spot specimens from this study frequently had lower digestive tracts that were filled with sediment, and the stomachs of several specimens were not able to be analyzed due to most of the stomach containing sediment. Additionally, most of the stomach contents for spot in this study could not be identified, which could be a consequence of the fish ingesting such high amounts of inorganic material. This feeding strategy, combined with differences in infaunal prey density and abundance could partially explain, why the abundance of spot is significantly lower at hypoxic sites.

Briggs et al. (2017) described how the lower biomass and individual size of the dominant polychaete at hypoxic sites coupled with the majority of the macrobenthic biomass residing deeper in the sediment at hypoxic sites could make foraging in these areas not energetically viable [38]. Since spot are known to randomly sort through sediments in search of prey, the lower energy return per prey item, because of the prey being smaller, could make foraging in hypoxic areas untenable. Additionally if this random sorting of the sediment is confined to the uppermost layers of the sediment, as has been found to be the case for spot foraging in the Chesapeake Bay [18], then spot would be unable to access the majority of prey biomass in hypoxic areas.

It should be noted that the environmental conditions and subsequent invertebrate community being described by Briggs et al. (2015) and (2017), and being discussed above, are markedly different than what was described by Pihl et al. (1994) in which spot were found to be feeding upon stressed invertebrates in the upper layers of the sediments. Briggs et al. (2017) sampled the benthic community in late summer after hypoxic conditions had persisted for months [38,39], while the hypoxic conditions described in Pihl et al. (1994) were more episodic in nature and the feeding by spot occurred soon after hypoxia formation [24]. Conditions at the edge of the GOMEX hypoxic zone, or early in the summer when hypoxia is first forming, are likely more similar to the conditions in Pihl et al (1994).

Further research into the foraging patterns, including potential changes to foraging strategy in hypoxia, as well as work into the energy balance of hypoxia foraging and hypoxia exposure are needed to better understand what roles diet, foraging, and available prey play in explaining the distribution and abundance of species relative to hypoxia. This line of inquiry is especially relevant considering the known reproductive consequences associated with hypoxia exposure in croaker [105,106] and spot (Cyrana at al. in review, Chapter 3). Research into the potential benefits and physiological costs of residing in hypoxic waters would increase understanding of the overall consequences of marine hypoxic zones.

Summary

This study supports previous work detailing changes in groundfish diets under hypoxic conditions, finding relatively low diet overlap among groundfish species. Additionally, I found evidence that diet overlap among some groundfish species differs between hypoxic and normoxic areas, but this was not the case for the majority of my study species. This study also shows that the amount of food in the stomachs of groundfish is affected by hypoxic conditions. However, the effect is complex, as the ratio of empty stomachs was significantly higher in species like croaker and searobin (it is significantly lower in species like seatrout) but the amount of food in the stomachs relative to body size was also lowest in areas with intermediate levels of DO. As a whole, these results highlight the importance of continued research into how hypoxia affects species interactions in the GOMEX.

CHAPTER 3

INTRODUCTION

Endocrine disruption and reproductive impairment are two of the most well-documented physiological impacts of hypoxia exposure on marine organisms, specifically fishes [107]. Fishes exposed to hypoxic conditions tend to have lower average gonadosomatic index scores (GSI – an indicator of reproductive condition) than fishes exposed to normoxic conditions, as observed in *Fundulus grandis* [108], *Cyrpinus carpio* [107], and *Micropogonias undulatus* [41,106,107]. Both episodic (diel) and chronic hypoxia have been shown to reduce the reproductive capacity of estuarine fishes [108,109]. Chronic exposure to hypoxic conditions has been further linked to masculinization in females [106] and reduced sperm production and viability in males of the species *M. undulatus* [105]. Masculinization of female *M. undulatus* resulting from chronic exposure to hypoxia has been documented in both field and laboratory studies [41,106].

Other environmental stressors have been shown to have differential effects on fish reproduction, depending upon the taxa in question. One such stressor, estrogen-mimicking tree extracts present in paper mill effluent, has been shown to induce masculinization in fishes. This phenomenon is particularly well-studied in the mosquitofish, *Gambusia affinis*. Despite the clear link between exposure to phytosterols in paper mill effluent and masculinization in *G. affinis* [110-112], no such effect was observed in the guppy, *Poecilia reticulata* [113], or Longear Sunfish, *Lepomis megalotis*, also exposed to papermill effluent [114]. *G. affinis* and *P. reticulata* are members of the same family (Poeciliidae), while *L. megalotis* is in Family Centrarchidae of Order

Perciformes, demonstrating that the wide variability in response to a hormone-like substance is not consistent even within taxonomic families.

The above studies highlight that masculinization can result from a variety of environmental stressors in marine and freshwater fish species from different families, emphasizing the importance of exploring the breadth of the phenomenon across multiple species and higher taxonomic groups. In this chapter, I assess whether the well-established reproductive effects of seasonal hypoxia on *M. undulatus* in the GOMEX [41,105,106,115] extend to other co-inhabiting species of fishes, specifically *Leiostomus xanthurus* and *Prionotus longispinosus*. Both *M. undulatus* and *L. xanthurus* are members of the Family Sciaenidae (Order Perciformes). *Prionotus longispinosus* is within the Family Triglidae (Order Scorpaeniformes). Finding evidence of reproductive abnormalities in additional species from the same family and from different orders, as in the case of *P. longispinosus,* would demonstrate that the effects of seasonal hypoxia in the GOMEX are more widespread taxonomically, with broader implications for fisheries management, than previously thought.

METHODS

Sample Collection and Location

Fish specimens were collected aboard the NOAA research vessel RV Oregon II during legs I and II of the Southeast Area Monitoring and Assessment Program (SEAMAP) summer groundfish survey in 2016, 2017, and 2019 (Figure 22). Sampling sites were scattered throughout the area in which GOMEX hypoxia seasonally develops,

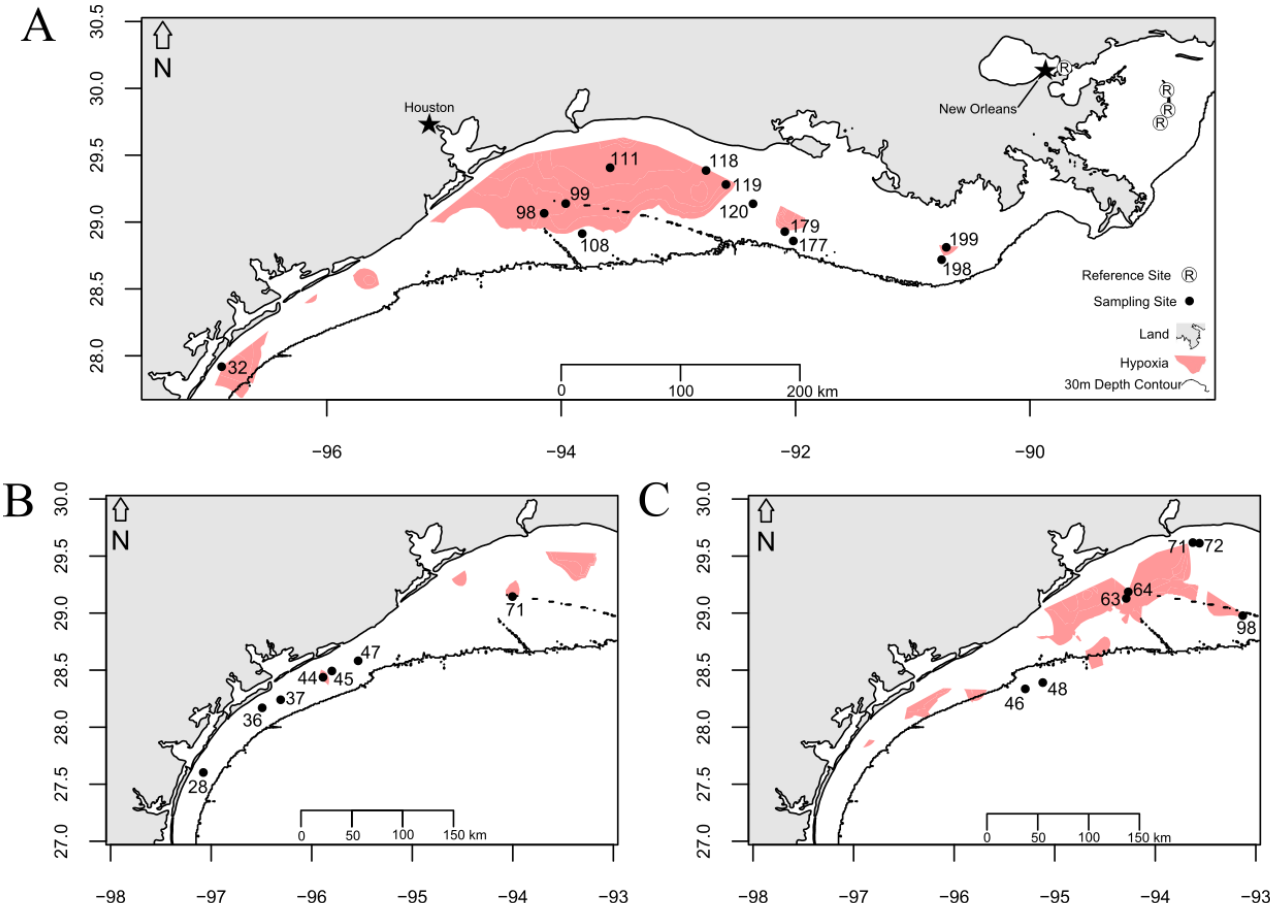


Figure 22. 2016 sampling locations (A), 2017 sampling locations (B), and 2019 sampling (C) locations. All sampling for the study was conducted during the SEAMAP Summer Groundfish Survey aboard the RV Oregon II. Note that the shoreward boundary of the hypoxia extent polygons for each year does not necessarily represent the extent of hypoxia in that year; the boundary reflects the hydrographic survey used to generate the polygons.

ranging from southern Texas through Louisiana. Samples were collected as described by the SEAMAP sampling protocol at trawling stations less than 30 meters in depth. The samples were fixed in a 10% formalin solution and transferred to ethanol for storage. Dissolved oxygen (DO) values were collected immediately prior to trawling, according to SEAMAP water quality data protocol. A station was defined as hypoxic if the DO value was 2.0 mg/L or lower, which is consistent with the recent hypoxia literature [2].

We obtained reference samples for *M. undulatus* and *L. xanthurus* from the Royal D. Suttkus Fish Collection at the Tulane University Biodiversity Research Institute (TUBRI). We selected reference samples collected during the summer (June-September) at either the Chandeleur Islands (LA) or the Rigolets (LA). We selected samples collected between 1955 and 1969. Neither the Chandeleur Islands nor the Rigolets are known to be hypoxic during the summer and the timeframe of 1955-1969 represents a time when hypoxia was not as widespread or common. The location and time of collection of our reference samples makes it unlikely that reference fishes were exposed to hypoxic conditions prior to sampling.

Sample Processing

Whole fish total length (nearest mm) and wet weight (nearest 0.001g) were measured and recorded. Preserved specimens of the three species were dissected, their gonads were removed, and wet weights of the gonads were taken (only reported for *M. undulatus* and *L. xanthurus*). Gonadal tissue from sub-samples of male and female *M. undulatus*, *L. xanthurus*, and *P. longispinosus* collected in 2016 from both normoxic and hypoxic sites were mounted, sectioned, and stained with hematoxylin and eosin (HE).

Gonadosomatic Index (GSI)

GSI was calculated using the formula $\frac{Weight_{gonads}}{(Weight_{fish} - Weight_{gonads})} \times 100$. In order to compare the GSI of fishes of varying sizes, a residual index of the GSI values was computed. The residual index has been shown to be an effective way to reduce the influence of body size in analyses of organismal condition [116] and has been demonstrated to be effective in the analysis of fish morphometric data [116,117]. The residual index is the difference between observed GSI value and the predicted GSI value determined by a trendline generated using pooled data. We plotted GSI as a function of body weight for both sexes of *M. undulatus* and *L. xanthurus*, and trendlines were calculated using pooled fish from hypoxic and normoxic sites. Positive values for the residual index indicate that the observed GSI is greater than the predicted (from the trendline) GSI, whereas negative values indicate that the observed GSI value is less than the predicted value. Overall body condition (total length/weight) was calculated and compared between hypoxic and normoxic sites using the residual index of the pooled male and female length/weight relationship. In order to compare both sexes simultaneously, the weight used for the overall condition analysis represents the weight of each fish minus the weight of its gonads.

Statistical Treatment

Mean residual index values were compared between fishes from hypoxic sites and fishes from normoxic sites using sites as replicates. The residual indices of both overall body condition and GSI for *L. xanthurus* are normally distributed (Shapiro-Wilk, p>0.05) and have statistically indistinguishable variances (Bartlett’s test, p>0.05), thus we utilized a two-way ANOVA (α=0.05) to compare the residual indices of female *L. xanthurus* collected from hypoxic and normoxic sites (♀$n_{hypoxic}$= 36, ♀$n_{normoxic}$= 31, ♂$n_{hypoxic}$= 31, ♂$n_{normoxic}$= 28). The residual index values for female *M. undulatus* GSI, and the overall condition for both sexes are also normally distributed and have statistically indistinguishable variances so we analyzed these data in the same manner as described above for *L. xanthurus*. The residual index for male *M. undulatus* GSI is not normally distributed, thus we utilized a Kruskal Wallis test (α=0.05) for this data (♀$n_{hypoxic}$= 37, ♀$n_{normoxic}$= 44, ♂$n_{hypoxic}$= 48, ♂$n_{normoxic}$= 26).

RESULTS

Ovarian Masculinization

Micropogonias undulatus ovaries exhibited testicular tissue primarily in connective tissue along the periphery of the ovary (Figure 23). The testicular tissue observed consists of clusters of spermatids within connective tissue, similar to determinations made in previous studies [106] (Figure 23). Six of the 17 *M. undulatus* ovaries exhibited evidence of masculinization, with masculinized ovaries observed only at hypoxic sites. The spermatogenic tissue observed within ovarian tissue resembled spermatogenic cysts of lobular type testes [118]. One specimen, the gonads of which externally resembled testes, contained gonads that would best be described as transitional, i.e. an ovary that was transitioning to a testis. The gonads of this specimen organizationally resemble testes, however degenerated oocytes are present surrounding

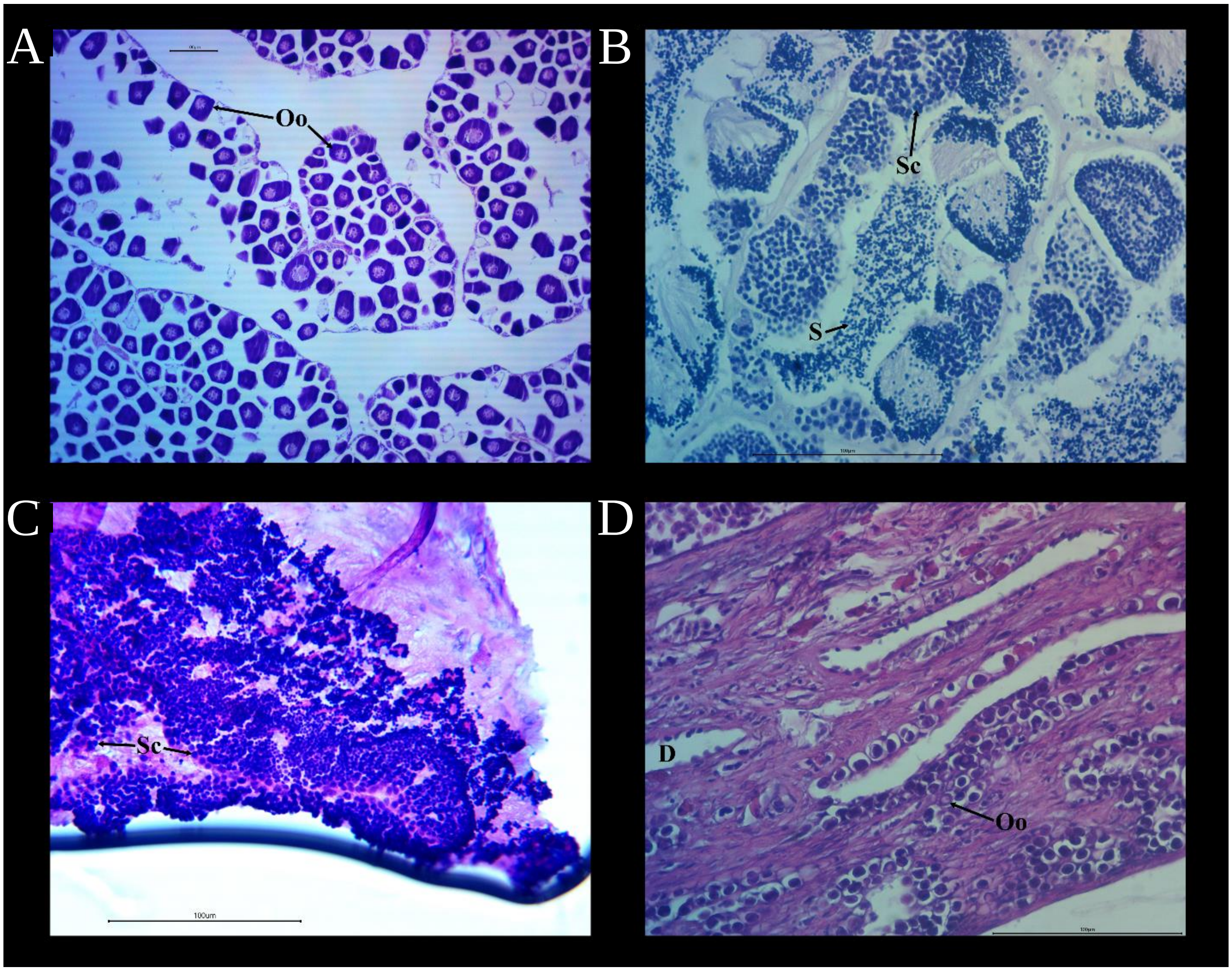


Figure 23. A. Ovary of a reference specimen showing Pre-Vitellogenic Oocytes (Oo) with no signs of spermatogenic tissue or any evidence of Oocyte degradation.

B. Mature testis of a male *M. undulatus* (caught in the GOMEX) from a control treatment of a laboratory study (Cyrana et al. in preparation). Spermatocytes (Sc) in various stages of development are seen along with fully developmed Spermatids (S). No cells in this mature testis resemble Oocytes.

C. Section of an ovary of a specimen from station 99 in 2016 (DO=1.1 mg/L) showing both Spermatocytes (Sc) in various stages of development.

D. Transitional gonad from a specimen collected at station 98 in 2016 (DO=1.4 mg/L), externally classified as male, showing degrading or degenerating Oocytes (Oo) surrounding clear ducts (D). The structure clearly resembles a testis with reproductive cells surrounding duct like structures. Degenerating oocytes observed in this gonad are larger and visually distinct from spermatocytes observed in mature testes (*c.*).

duct like structures. Spermatogenic tissue was not present. The ovaries of reference specimens contained no evidence of spermatogenic tissue or ovarian anomalies.

Masculinized *L. xanthurus* ovaries (Figure 24) exhibited a similar masculinization profile to *M. undulatus*. Three of seven examined ovaries from *L. xanthursus* exhibited masculinization in peripheral ovarian connective tissue with one of the masculinized specimens coming from a normoxic site. Two of the specimens of *L. xanthurus* we examined contained ovaries that would be best described as transitional (Figure 24). These transitional gonads have structures more like a typical testis with ducts surrounded by spermatogenic tissue interspersed in connective tissue. One of these transitional gonads contained numerous heavily degenerated oocytes in addition to a large duct/lumen filled with byproducts of oocyte degeneration and large melano-macrophage centers. The other transitional gonad contained heavily degenerated oocytes and testicular like structure while lacking the egg debris and melano-macrophage centers. The ovaries of reference specimens appeared more organized compared to the gonads of specimens from 2016 and showed no evidence of spermatogenic tissue within the ovaries.

Masculinization in *Prionotus longispinosus* manifested as spermatogenic cells in various stages of development interspersed among oocytes as well as peripheral tissue (Figure 25). One of seven ovaries examined *P. longispinosus* showed evidence of masculinization.

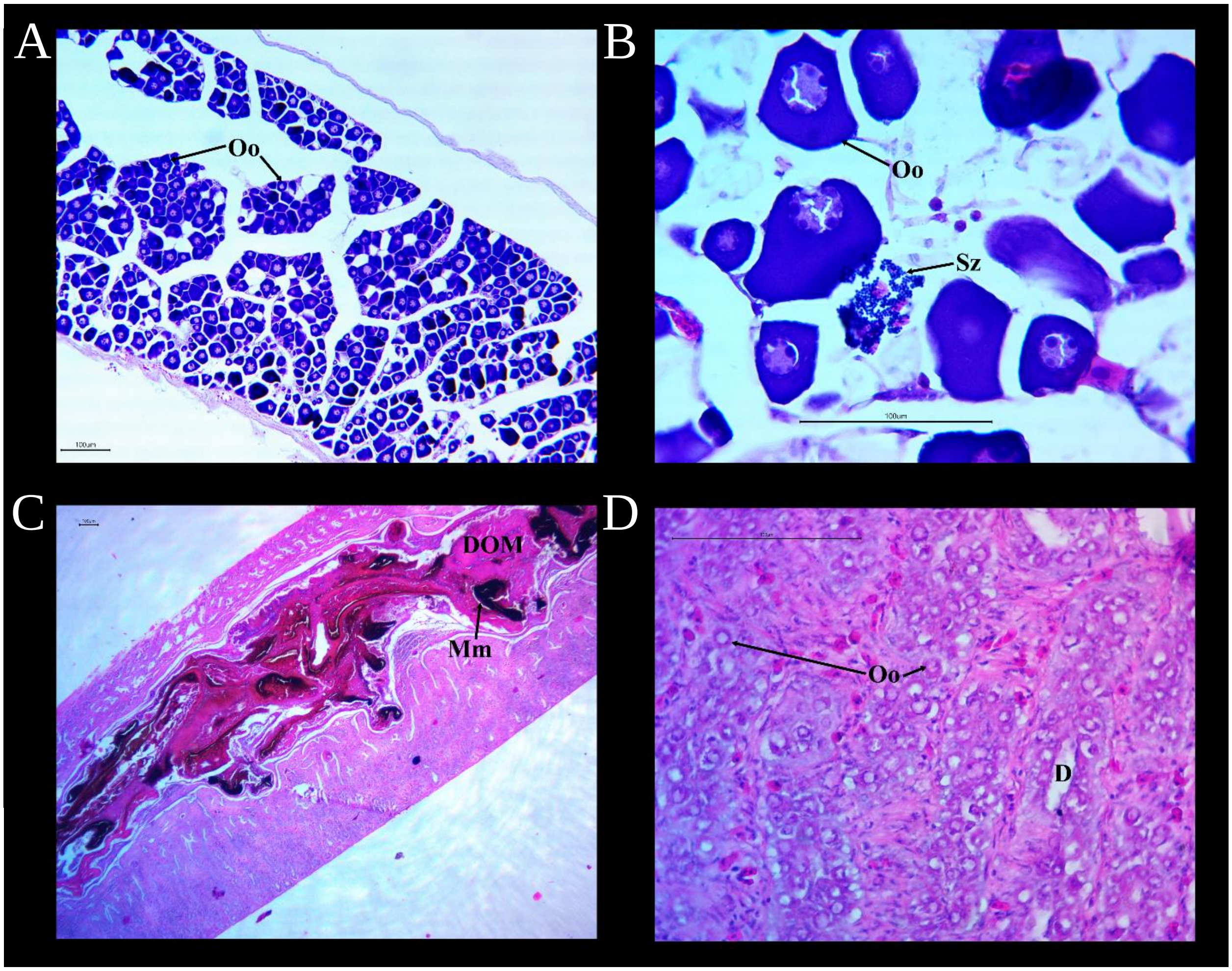


Figure 24. A. Ovary of a reference specimen showing Pre-Vitellogenic Oocytes (Oo) with no evidence of spermatogenic tissue or Oocyte degradation.

B. Ovary of a specimen collected at station 177 in 2016 (DO= 4.1 mg/L) which was adjacent to an area of hypoxia. Oocytes (Oo) are clearly visible, as are clustered Spermatozoa (Sz).

C. Transitional gonad from a specimen sampled from station 198 in 2016 (DO=2.0 mg/L). Specimen was originally classified as a testis through external examination, showing testicular features like ducts and a preponderance of connective tissue while also exhibiting evidence of previous Oocyte degradation such as degraded oocyte material (DOM) and melanophore macrophage aggregations (Mm).

D. Same specimen as in *c.)* at higher magnification, showing degraded or degenerating Oocytes (Oo) embedded in the connective tissue. Testicular like ducts (D) are also visible, supporting the idea that this gonad was in the process of transitioning to a testis from an ovary at the time of sampling.

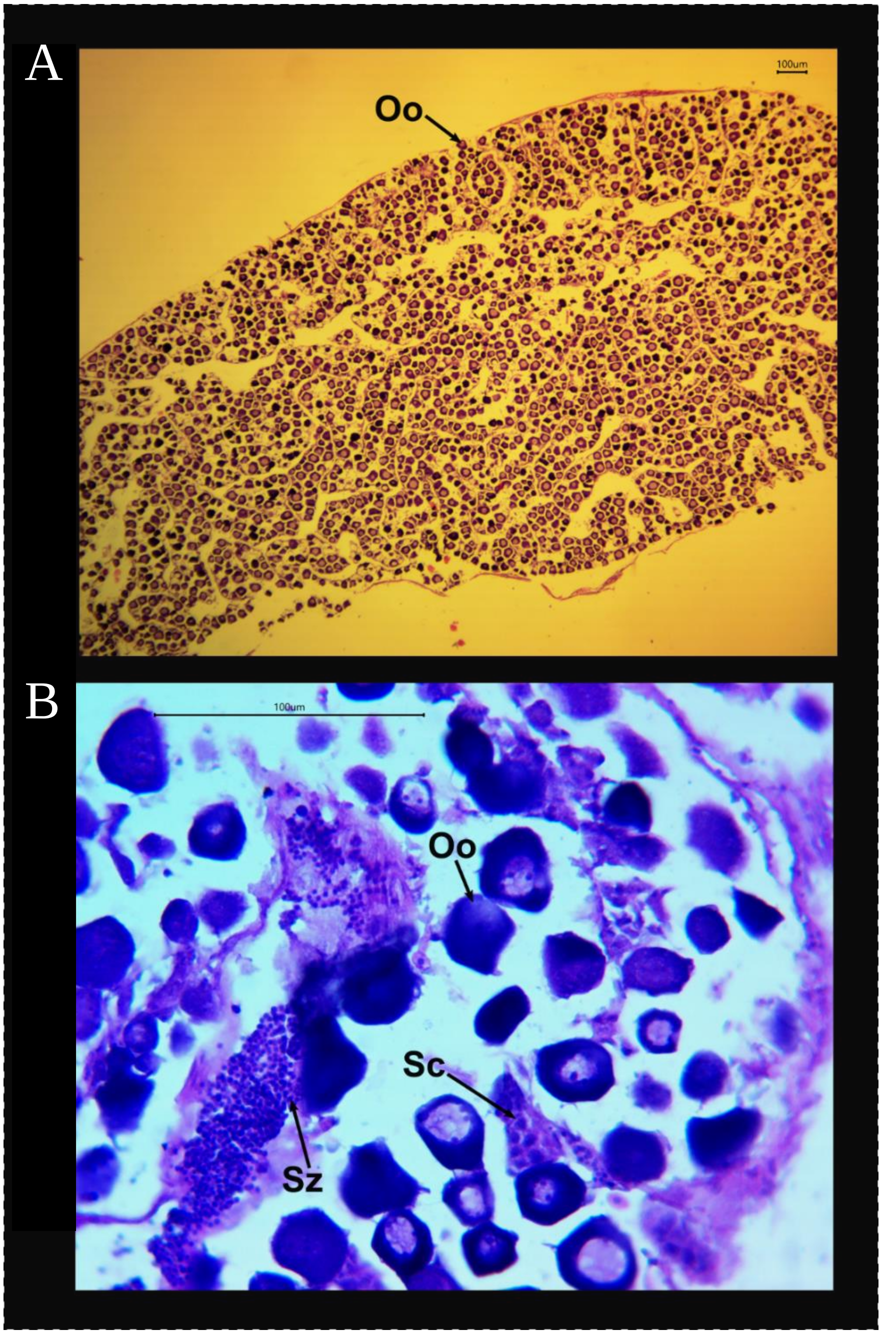


Figure 25. A. Ovary specimen collected at station 177 in 2016 (DO= 4.1 mg/L) showing no signs of masculinization or Oocyte degradation. Pre-Vitellogenic Oocytes (Oo) are visible throughout the ovary.

B. Ovary from a specimen caught at Station 110 in 2016 (DO=0.56mg/L) showing Oocytes (Oo) as well as Spermatocytes (Sc) in various stages of development and spermatozoa (Sz).

GSI and Body Condition

The mean residual index of overall body condition of pooled female and male *M. undulatus* was significantly higher in fish from hypoxic sites compared to fish from normoxic sites (Kruskal-Wallis, p<0.05) (Figures 26 and 27). The residual GSI of both male and female *M. undulatus* from hypoxic sites did not significantly differ between hypoxic sites and normoxic sites (ANOVA, p>0.05) (Figures 26 and 27).

The mean residual GSI of female *L. xanthurus* from hypoxic sites was significantly lower (ANOVA, p<0.05) than that of females from normoxic sites (Figures 28 and 29) and male *L. xanthurus* from hypoxic sites also exhibited significantly lower GSI values than males from normoxic sites (ANOVA, p<0.05). The mean residual index of overall body condition for pooled female and male *L. xanthurus* did not significantly differ between specimens from hypoxic sites and specimens from normoxic sites (ANOVA p>0.05) (Figures 28 and 29).

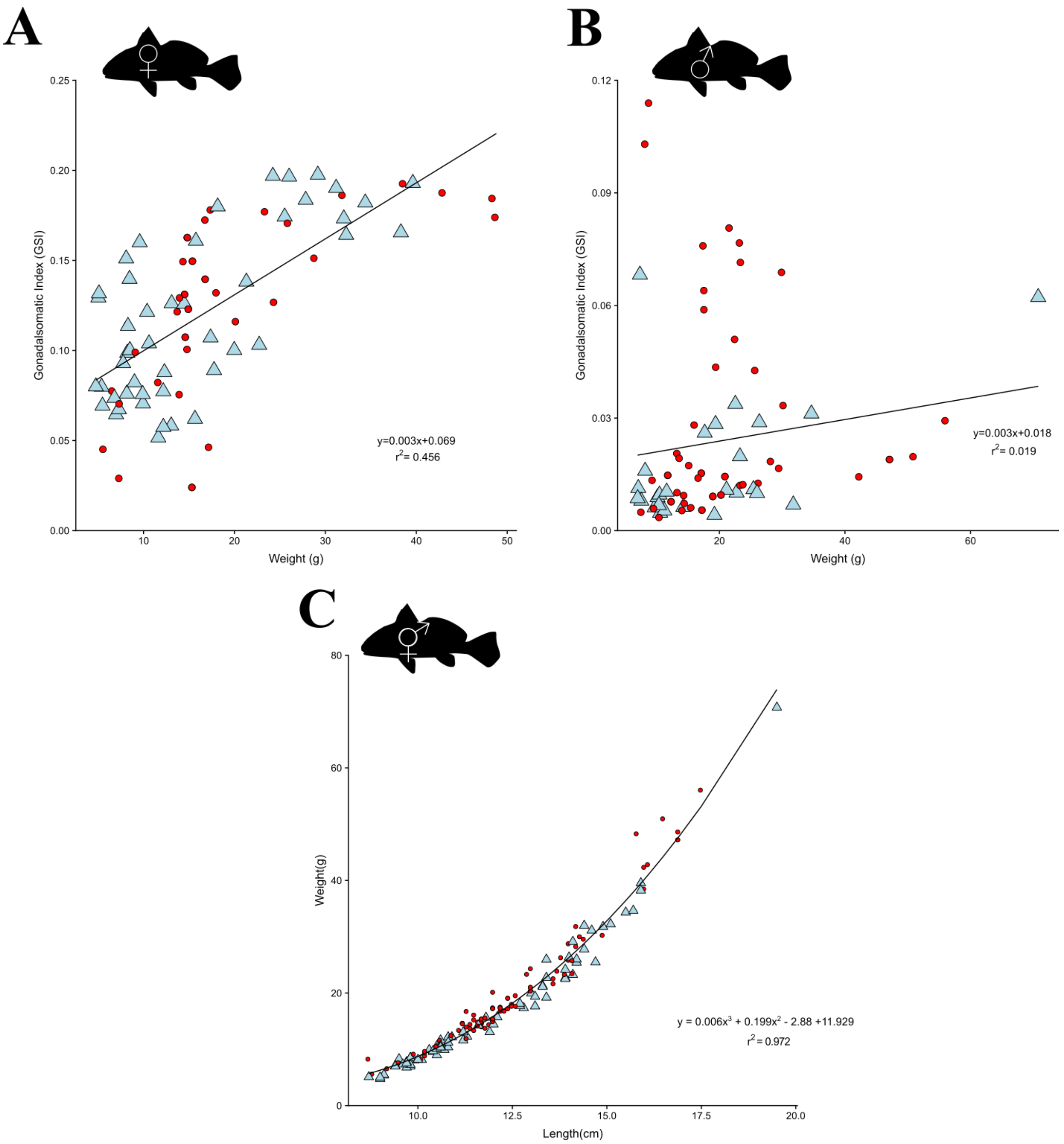


Figure 26. The weight(g)/GSI relationship for female (A) and male (B) *M. undulatus* caught in hypoxic (red circles) or normoxic (blue triangles) and the length(cm)/weight(g) relationship for pooled female and male (C) *M. undulatus* from sites in the northern GOMEX from Texas through Louisiana. Trendlines were calculated using both hypoxic and normoxic fish.

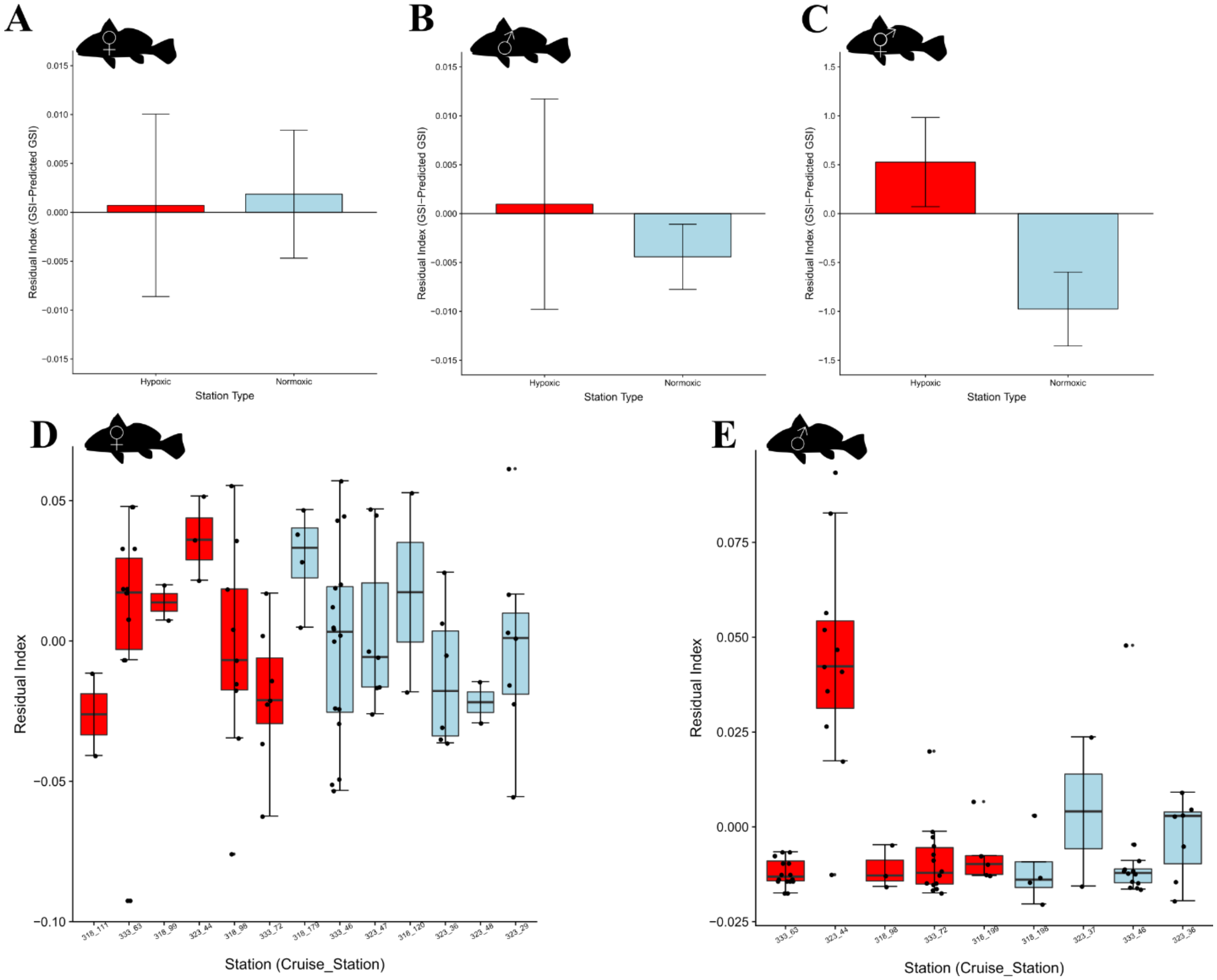


Figure 27. Average residual index of the GSI for female (A) and male (B) *M. undulatus* with stations serving as replicates. Average residual index of overall body condition between pooled male and female fish from hypoxic sites and normoxic sites for *M. undulatus (*ANOVA, $p<0.05$, $n_{hypoxic}=9$, $n_{normoxic}=11$) with stations serving as replicates (C). Boxplots of the individual GSI values by station for female (D) *M. undulatus* and male (E) *M. undulatus*.

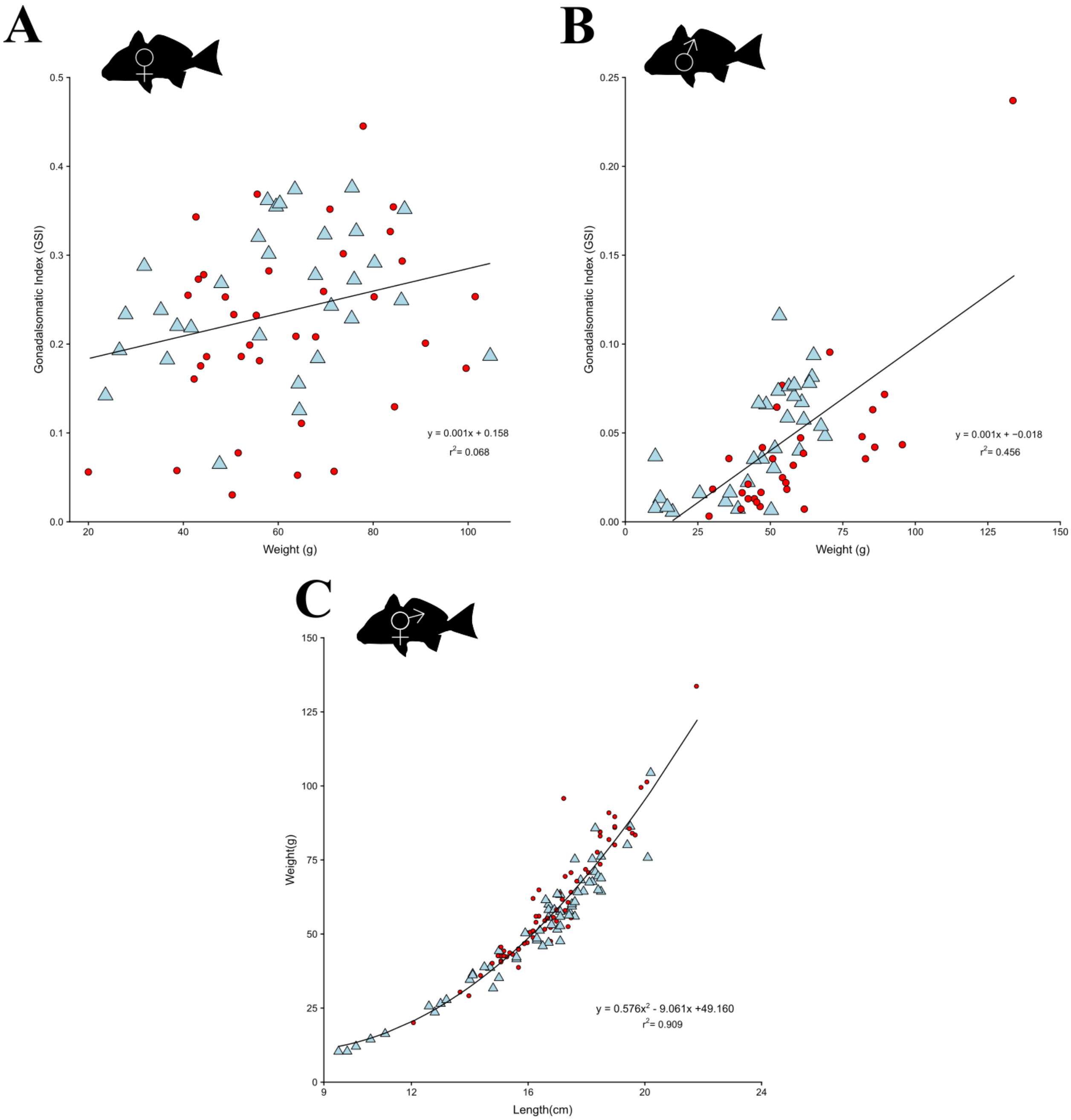


Figure 28. The weight(g)/GSI relationship for female (A) and male (B) *L. xanthurus* caught in hypoxic (red circles) or normoxic (blue triangles) and the length(cm)/weight(g) relationship for pooled female and male (C) *L. xanthurus* from sites in the northern GOMEX from Texas through Louisiana. Trendlines were calculated using both hypoxic and normoxic fish.

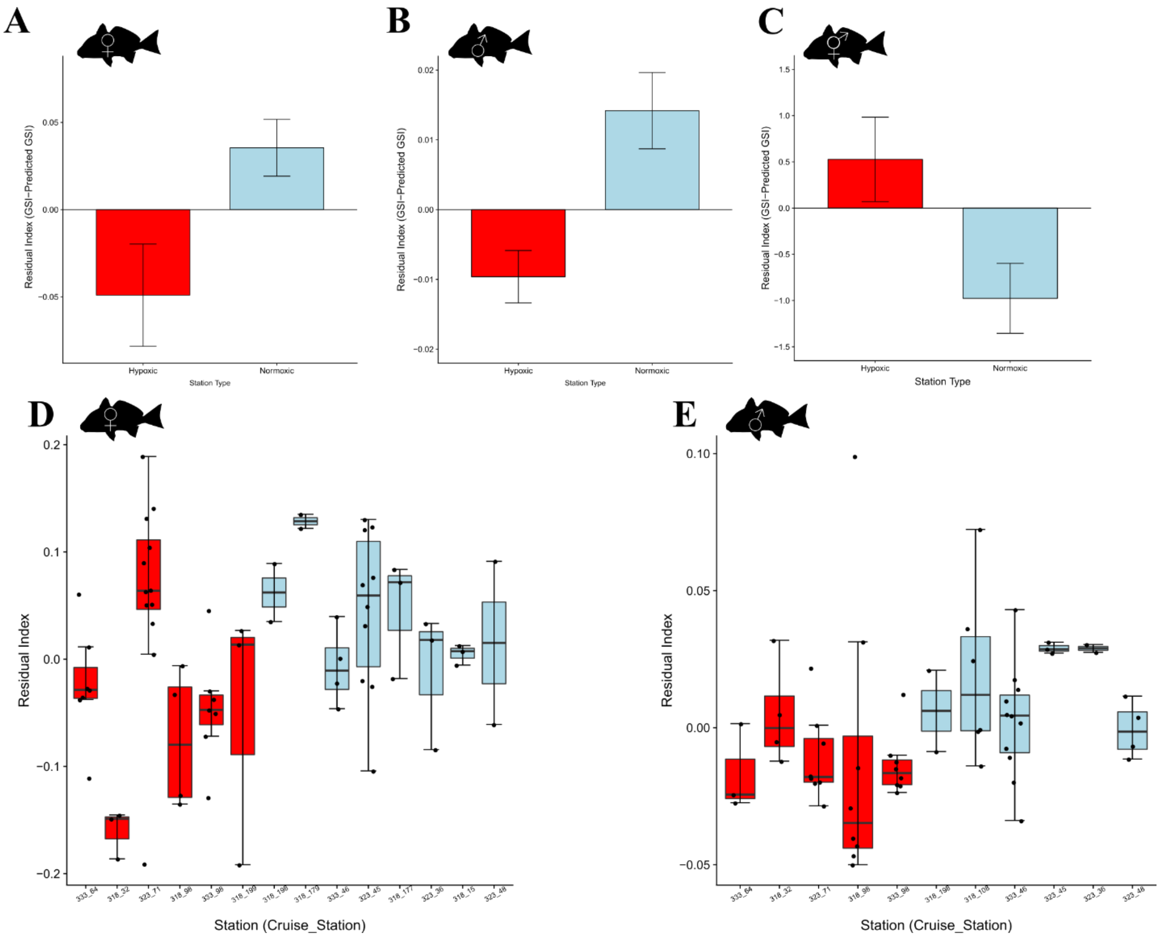


Figure 29. Average residual index of the GSI for female (A) and male (B) *L. xanthurus* with stations serving as replicates (ANOVA, $p < 0.05$, $n_{hypoxic}=5$, $n_{normoxic}=6$). Average residual index of overall body condition between pooled male and female fish from hypoxic sites and normoxic sites for *L. xanthurus* (ANOVA, $p < 0.05$, $n_{hypoxic}=5$, $n_{normoxic}=6$) with stations serving as replicates (C). Boxplots of the individual GSI values by station for female (D) *L. xanthurus* and male (E) *L. xanthurus*.

DISCUSSION

Scope and Possible Causes of Ovarian Masculinization

Prior to this study, the only species from the GOMEX known to show evidence of ovarian masculinization was *M. undulatus*. Six of 17 ovaries of *M. undulatus* examined in this study exhibited evidence of masculinization. Moreover, this study is the first to report ovarian masculinization in two additional GOMEX species, *L. xanthurus* and *P. longispinosus*. Three of seven sub-sampled ovaries of *L. xanthurus* exhibited masculinization, while one of seven sub-sampled *P. longispinosus* ovaries exhibited masculinization.

The cause of the ovarian masculinization in this study cannot be determined definitively, however hypoxia induced masculinization is the most likely possibility. It is well known that estrogen, the synthesis of which is regulated by aromatase, is a primary factor in the sex determination of fishes. Guiguen et al. (2010) discusses a number of experiments that show that suppression of estrogen synthesis (Aromatase inhibition) in fishes can result in the formation of testicular tissue [119]. Aromatase inhibitors have been demonstrated to induce masculinization in several species of fish including the naturally hermaphroditic honeycomb grouper, red grouper, and European seabass [120-122], and in the naturally gonochoristic common carp [122]. Long term aromatase suppression has even been shown to convert functional ovaries into functional testes in zebrafish [123]. Hypoxic conditions have been shown to function as an aromatase inhibitor in Atlantic croaker, decreasing mRNA expression and aromatase activity in fish exposed to hypoxia both in the wild and in the lab [106]. It is likely that the widespread ovarian masculinization observed in fish from hypoxic sites in the GOMEX in the present study is also a consequence of hypoxia-induced inhibition of aromatase and concomitant suppression of estrogen synthesis. The well documented capacity of aromatase suppression to cause masculinization in fishes, coupled with the broad geographic extent of hypoxia encountered during the sampling for this study, strongly suggests that the observed ovarian masculinization is hypoxia related.

Some of the sample sites in our study were normoxic, but these sites were in close proximity to areas of hypoxia, suggesting that even specimens from normoxic sites could have been exposed to hypoxia. Both *M. undulatus* and *L. xanthurus* reside in coastal marshes as juveniles and move offshore as they mature. The broad extent of hypoxia in 2016 would have exposed many of these fishes to hypoxic conditions as they moved offshore. It has been suggested that continual exposure to estrogen is necessary to maintain ovarian differentiation in some fishes [124], thus fishes caught in normoxic conditions could still contain male tissue that was a result of aromatase suppression while they were moving offshore. Additionally, ovaries dissected from specimens from the Royal D. Suttkus Fish Collection, collected from sites east of the Mississippi River that are not known to experience seasonal hypoxia, showed no evidence of masculinization. This further supports the idea that hypoxia is driving the ovarian masculinization we observed.

Our observation of ovarian masculinization in *M. undulatus* and two other GOMEX species extend both the geographic scope and taxonomic breadth of ovarian masculinization first reported by Thomas and Rahman (2011) [106]. If our observations of testicular tissue in the ovaries of wild caught specimens is not hypoxia related, then the phenomenon is either due to some other environmental factor or it represents naturally occurring background masculinization. Bahamonde et al. (2013) reviews this phenomenon in great detail, including studies of gonochoristic species that have been shown to exhibit intersex individuals naturally [125]. One such study involving the freshwater species, *Micropterus dolomieu*, showed significantly more occurrences of intersex fish during the pre-spawn period compared to the reproductive period [126]. The spawning period of *M. undulatus* in the GOMEX begins as early as October and ends as late as June [127], suggesting a protracted spawning period. Research on *P. longispinosus* in the GOMEX suggests that it also has a protracted spawning period, from January to June [75]. Spot, *L. xanthurus*, is reported to be a winter spawner in the GOMEX, with the exact timing of spawning contingent on longitude [127]. Even though our ovarian samples were taken from fishes collected primarily in the pre-spawning period, we consider it unlikely that evidence of masculinization we observed is the result of inherent intersex characteristics described by Bahamonde et al. (2013).

The vast majority of examples of naturally occurring intersex individuals in gonochoristic fishes outlined by Bahamonde et al. (2013) are examples of male fishes with testicular tissue that contains oocytes [125], as the paper is primarily focused on the process of male feminization. The feminization of testicular tissue would imply the opposite hormonal mechanism - aromatization of androgens into estrogens - from that presumed to occur in ovarian masculinization, making it incompatible with our interpretation. Additionally, our observation of heavily masculinized specimens, including specimens of *L. xanthurus* and *M. undulatus* best described as transitional (in the process of undergoing sex change), with degenerating oocytes, suggests that these specimens are transitioning from female to male, similar to the transition of zebrafish ovaries to functional testes as a result of aromatase suppression [123]. Thus, while the occurrence of naturally intersexed individuals in otherwise gonochoristic fishes requires serious consideration, the few examples of naturally masculinized individuals in the literature, the high degree of masculinization in some of our specimens, and the lack of any observable masculinization in our reference samples makes an argument of naturally occurring intersexuality as an explanation for our observations unparsimonious.

Manifestation of Masculinization

Previous reports of masculinization in *M. undulatus* describe the masculinization as “spermatogenic cyst like structures” [106], and this description fits the majority of masculinized *M. undulatus* ovaries and the masculinized *L. xanthurus* specimen from a normoxic site, albeit adjacent to hypoxia. In addition to this more typical masculinization manifestation, we also encountered three specimens undergoing masculinization to a degree that has yet to be described in wild specimens from the GOMEX.

Two specimens of *L. xanthurus* and one specimen of *M. undulatus* displayed masculinization to such a large extent that their gonads are best described as transitional, as these specimens show degenerating/regressing female reproductive cells while having a testicular like overall structure. These specimens’ gonads contained well defined ducts surrounded by highly degraded oocytes. One of the *L. xanthurus* specimens also contained a large duct/lumen space filled with material consistent with follicular atresia and melano-macrophage material. An additional *L. xanthurus* specimen with similar degraded oocytes and well-defined male tissue was observed; however, this individual lacked a large lumen and melano-macrophage aggregations.

The evidence of oocyte degradation combined with the well-defined testicular structure and spermatogenic tissue suggests masculinization beyond what has been observed previously in GOMEX fishes. The characterization of these individuals as females undergoing masculinization, as opposed to males undergoing feminization, is supported by work on zebrafish by Sun et al. (2014) in which aromatase suppression led to a complete sex change in females characterized by a degradation of oocytes and replacement of the oocytes by spermatogenic cells [123]. Histological observations in naturally hermaphroditic fishes transitioning from female to male report similar evidence of oocyte breakdown prior to the transition from female to male [128,129]. The zebrafish ovotestis also contained large heavily pigmented regions consistent with melano-macrophage aggregates, which have been used as pathological signs of environmental degradation and hypoxia in fishes [130] including GOMEX fishes such as *M. undulatus* [131], albeit in the spleen.

The more structurally complex nature of the masculinization we observed in this study coupled with the discovery of transitional individuals highlights the need for further research into this phenomenon. Fish specimens examined in previous work were sampled later in the season and were more reproductively developed than fishes examined in this study [106]. How this difference in season and maturation might impact the manifestation and cause of masculinization is unclear.

Ovarian Masculinization and Reproductive Impairment

Neither female nor male *M. undulatus* showed statistically significant differences in the residual index of the GSI between hypoxic and normoxic sites, although a trend of increased GSI in specimens from normoxic sites was observed. This finding contradicts previous studies that have found statistically significant decreases in GSI values in *M. undulatus* from hypoxic sites [106]. While unexpected, the results of the present study could be explained by differing severities of hypoxic conditions between the investigations (hypoxia in 2016 was less fragmented and more extensive) or differences in sexual maturity of sampled specimens, highlighting the importance of continued research into hypoxia and its effects.

Both female and male *L. xanthurus* exhibited significantly lower ($p<0.05$) residual index values of the GSI in hypoxic sites compared to normoxic sites. This finding supports previous observations in *M. undulatus* [106] and further highlights the potential of population-and ecosystem-level impacts of an environmental stressor such as hypoxia on fish reproduction.

For both female and male *M. undulatus*, and female *L. xanthurus*, hypoxic stations from 2017 (cruise 323) exhibited some of the highest GSI values observed for both species and sexes (Figures 27 and 29). The patches of hypoxia in which these 2017 stations were sampled were a fraction of the size of the hypoxic zone sampled in 2016 (mostly one large, uninterrupted area) and 2019 (patchier than in 2016 but larger patches than in 2017) (Figure 22) and likely resulted in less severe exposure to hypoxia compared to 2016 and 2019. Smaller areas of hypoxia could provide benthic fishes opportunities to feed on stressed infaunal invertebrates, similar to what has been observed in the more episodic zones of hypoxia that develop in the Chesapeake bay [18]. This could also explain why fish from hypoxic stations in 2017 exhibit such high GSI values. We cannot, however, make any assumptions related to feeding based on the data presented here. Balancing potential increased foraging opportunities associated with hypoxic conditions with the physiological costs associated with hypoxia exposure has been cited as one explanation for the context-dependent nature of hypoxia avoidance thresholds in GOMEX groundfish [40], further highlighting the need for more research in order to place the reproductive costs associated with hypoxia in the broader context of GOMEX ecology.

Conclusions

Our study demonstrates that reproductive impairment, specifically the presence of inter-sexed individuals associated with hypoxic conditions, impacts more species of fish than previously documented. We also have evidence of reproductive anomalies such as the presence of egg debris in early stages of ovarian development, from fish in and around the 2016 GOMEX hypoxic zone. These coupled with the lack of ovarian masculinization and reproductive anomalies in reference fishes, and the significantly lower GSI values exhibited by *L. xanthurus* from hypoxic sites strongly supports the idea that hypoxic conditions alter the reproductive health of fishes.

Research into the extent that ovarian masculinization impacts reproductive potential and output in these fishes would make the population-level impacts of masculinization clearer. This is particularly important considering the finding that fishes collected at normoxic stations show evidence of masculinization. Another potential implication for this finding is that ovarian masculinization can develop in DO values above 2.0 mg/L, which would require scientists and policy makers to rethink management of hypoxia-prone areas. The potential lingering effects of hypoxia exposure have broad and particularly relevant implications for the commercial fishing industry, considering that all fish included in this study are important forage fish for commercially-fished species. The potential population-level disruption to the GOMEX ecosystem warrants further, more in-depth investigations, especially given the large scale of GOMEX seasonal hypoxia. The results of the present study open avenues for further exploration into the relationship between hypoxia exposure and reproductive impairment, the duration of observed effects, and the impacts of hypoxia exposure at different developmental stages.

MAJOR FINDINGS

In this dissertation I showed that, when examined at a GOMEX wide scale and across at least eleven years, hypoxic sites have a significantly lower community biodiversity compared to normoxic sites. I also found that 21.9 percent of examined species had significantly lower average yearly CPUE values at hypoxic sites compared to normoxic sites with the average yearly CPUE at hypoxic sites only being significantly greater than the average yearly CPUE at normoxic sites for one species, *S. lanceolatus*. Additionally, I found evidence that the effects of hypoxia on species occurrence is not uniform with depth underscoring the continued need for further research on the subject.

I have found that there is a significant interaction between the proportion of fishes with empty stomachs and whether the station was hypoxic or normoxic for *M. undulatus, P. longispinosus*, and *Cynoscion spp* with *M. undulatus* and *P. longispinosus* having a higher proportion of empty stomachs at hypoxic sites and *Cynoscion spp.* having a lower proportion of empty stomachs at hypoxic sites. I also found that the fullness of fishes tended to be lower at sites with intermediate levels of dissolved oxygen and that the consumption of crabs and mantis shrimp tended to increase at hypoxic sites compared to normoxic sites.

I found evidence of ovarian masculinization in *M. undulatus, L.* xanthurus, and *P. longispinosus* including evidence of oocyte degradation and degrees of ovarian masculinization that has yet to be reported. I have found that the average residual index of the GSI for *L. xanthurus* was significantly lower for males and females from hypoxic sites with *L. xanthurus* from hypoxic sites also having a lower average residual overall condition (length/weight ratio).

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BIOGRAPHY

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