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## **Macrobenthos community succession in the northern Gulf of Mexico hypoxic regions: testing the Pearson-Rosenberg model**

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### ABSTRACT

The Pearson and Rosenberg (P-R) conceptual model of macrobenthic succession was used to assess the impact of hypoxia (dissolved oxygen concentration  $\leq 2 \text{ mg L}^{-1}$ ) on the macrobenthic community on the continental shelf of the northern Gulf of Mexico. The stress-response relationship between bottom-water oxygen concentration and the macrobenthic community was used to determine the ecological condition of the benthic habitat. In lieu of testing the effect of decreasing influence of organic enrichment on the macrobenthic community as a function of distance from the source of organic pollution, we tested the effect of bottom-water dissolved oxygen concentration as a proxy for distance from the source. To include the seasonal response in the long-term cumulative effect of recurring hypoxia on the macrobenthic community, samples were collected during early spring (April 2009), late summer (September 2009), and midsummer (August 2010).

The cumulative effect of annual hypoxia on the macrobenthos at each site was apparent from the species diversity, abundance, and biomass. High diversity values at site H7 and low diversity values at site A6 indicated the existence of a westward-diminishing hypoxic gradient on the Louisiana/Texas shelf, interrupted by the Atchafalaya River discharge. Unsurprisingly, the macrobenthos collected during midsummer were generally the most stressed community, with the exception of site E4. Assessment of successional stages of the benthic communities of the four sites with multivariate analyses of nonmetric multidimensional scaling, distance-based redundancy analysis, and principal component analysis identified the influence of bottom-water hypoxia. The study demonstrated the applicability of the P-R model in predicting successional change in the macrobenthic community on the Louisiana continental shelf. Site H7 was in the advanced phase of stage II, sites E4 and D5 were in the intermediate phase of stage II, and site A6 was in the intermediate phase of stage I in the P-R successional continuum.

*Keywords:* Hypoxia, benthic community succession, Pearson-Rosenberg model, multivariate analyses, macrobenthos, Gulf of Mexico

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## 1. Introduction

By definition, a marine system is hypoxic when the dissolved oxygen concentration decreases to  $2 \text{ mg L}^{-1}$  and below. Hypoxia is a growing environmental problem in marine ecosystems around the world. Although hypoxia is a natural phenomenon in some marine systems, its occurrence, frequency, and intensity have increased in marine systems since the onset of industrial production of agricultural fertilizer (Diaz and Rosenberg 1995, 2001, 2008; Zhang et al. 2010). Currently, there are more than 550 marine systems reported to be hypoxic around the world (Diaz and Rosenberg 2008; Conley et al. 2011). The hypoxic zone in the northern Gulf of Mexico is the second largest among those reported systems (Rabalais and Turner 2001; Stachowitsch et al. 2007; Diaz and Rosenberg 2008).

Dissolved oxygen is essential to marine systems because of the high dependency of most marine organisms and its fickle nature to change so drastically within a short span of time (Diaz and Rosenberg 1995). Hypoxia is most catastrophic to sessile benthic organisms because mobile organisms can move horizontally or vertically to an area with higher dissolved oxygen concentrations. The limited mobility of macrobenthic invertebrates, along with relatively longer life spans, makes them good indicators for the environmental health of a marine ecosystem. Macrobenthos react to their surrounding environment by altering the character and size of their community to reflect the environmental quality changes at a particular location (Herman et al. 1999). In addition, macrobenthos play a significant role in ecological functioning by converting the organic detritus to biomass, serve as food for higher trophic organisms, and facilitate the biogeochemical transformation of organic matter through their feeding and burrowing activities (Diaz and Rosenberg 1995; Sanz-Lázaro and Marín 2011).

Hypoxia in the Gulf of Mexico is primarily attributed to the confluence of two major phenomena: the water column stratification induced by the Mississippi River discharge and the microbial decomposition of organic matter in the bottom waters (Rabalais et al. 1991). The hypoxia strongly correlates with the nutrient-rich, freshwater discharge from the Mississippi River (Rabalais and Turner 2001). During the summer months, eutrophication driven by river-supplied nutrients results in an increased flux of fecal material and other organic detritus to lower depths in the water column. The warm, low-salinity river water on top of the cooler, more saline Gulf water creates a density stratification that prevents reoxygenation of the bottom water. Because oxygen consumption at or near the seabed exceeds its production by photosynthesis, the result is depletion of oxygen concentration in the bottom water, or hypoxia (Rabalais and Turner 2001).

The variability in development, maintenance, and expansion of hypoxia in the northern Gulf of Mexico is linked to the dynamics of freshwater discharge from the Mississippi River system (Rabalais and Turner 2001). The general trend in regulated discharge of the Mississippi River system indicates a seasonal pattern, with high discharges during March–May and low discharges in September–November. These seasonal patterns of high and low discharges of the Mississippi River system typically mark the onset and the degradation of water column stratification (Baustian, Craig, and Rabalais 2009). During the early spring

months, hypoxia in the northern Gulf of Mexico is usually inconsistent and transient from late February through May, but it is most extensive, persistent, and depleted in oxygen during the summer months of June, July, and August (Rabalais and Turner 2001). Extension of hypoxia into September and October is rare and is generally dependent on wind patterns.

In a marine benthic ecosystem, the sediment and the animals living within that sediment influence each other to create a unique benthic community structure (Rhoads and Boyer 1982). The response of a benthic community to any environmental variable defines its structure, and the reaction of each species to these variables is a function of their genetic and evolutionary makeup (Pearson and Rosenberg 1978). Each species has its upper and lower limits to any given environmental parameter that are defined by its genetic traits and evolutionary history. Hence, the concept of “niche limits” plays a major role in the inclusion or elimination of a species from a community experiencing a particular environmental stress (Pearson and Rosenberg 1978). Therefore, in a hypoxic environment, where dissolved oxygen is a niche-limiting environmental stress factor, macrobenthos that are sensitive to low dissolved oxygen concentration will be replaced by macrobenthos that are less sensitive to hypoxic conditions in accordance with the  $r/K$  (growth rate/carrying capacity) selection theory of ecology.

The macrobenthos community structure is a good indicator of the condition of the ecosystem because groups of species are better indicators of ecosystem perturbation than just a single species or total species abundance or biomass. Additionally, community degradation and recovery in benthic ecosystems as a response to an environmental stressor provide useful information for the ecological health status. The Pearson-Rosenberg (P-R) model of macrobenthic succession, as it relates to eutrophication and hypoxia, is a classic and well-tested stressor-response relationship that can be used to evaluate the status of a stressed macrobenthic community. In the P-R model, the transition of the benthic community from  $K$ -selected to  $r$ -selected over space and time along an increasing organic matter gradient follows a predictable stressor-response relationship (Pearson and Rosenberg 1978). In essence genetically flexible organisms have a higher survival rate in an unstable environment than genetically rigid organisms. Hence, flexible opportunistic organisms are found in unstable hypoxic environments and should be considered good indicators of environmental disturbance related to hypoxia.

The parameters of the species diversity/abundance/biomass (SAB) model define change not only on a spatial gradient but also on a temporal scale in organic enrichment-induced, low-oxygen or hypoxic systems (Rakocinski et al. 2000). The SAB model defines the species diversity/abundance/biomass where the oxygen is lowest as depauperate of macrobenthos (stage 0) (Pearson and Rosenberg 1978). As bottom-water oxygen concentration decreases, opportunistic species are projected to increase in abundance slowly to reach a peak in response to eutrophication, with a corresponding small and initial peak in the biomass because of high abundances of  $r$ -selected opportunistic species (stage I). This increase is attributed to the increased abundance of one or two opportunistic species that are capable of

colonizing the empty niche with nonexistent competition and abundant food supply. With further increasing oxygen, the macrobenthos community contains many of the organisms of the two overlapping communities, each corresponding to its level of oxygen stress (stage II). The macrobenthos assemblage further along the oxygen stress continuum has high abundances of certain opportunistic species but with decreasing biomass compared with adjacent zones. Beyond that point on the continuum, the biomass reaches the highest peak, where organic matter in the sediment is high enough to provide a rich food source for large *K*-selected species but not high enough to decrease the biological oxygen demand seriously. Past this point, species richness and abundance reach stable values on the SAB curve as the macrobenthos assemblage gradually approaches the characteristics of a normoxic environment (stage III).

Successional stages are shaped by the interaction between environmental stresses, faunal adaptability, and species representation within the ecosystem. In the case of environmental disturbance, the succession of a benthic community is a continuous process, where faunal assemblages change along a stress gradient, where species-rich, stable communities are transformed into species-poor (opportunistic-dominated), transient communities through specific, successional stages (Odum 1971). Conversely, if environmental conditions become favorable, the disturbed benthic community can reverse the succession from any stage and return to the stable, mature community (Rumohr, Bonsdorff, and Pearson 1996; Diaz and Rosenberg 2008).

The impact of seasonal hypoxia on the community structure of the macrobenthos is the focus of this investigation. In this study, we apply the P-R model, which was developed using data from semienclosed, low-energy water bodies with high deposition rates and long bottom-water residence times (i.e., fjords and sea lochs), to the open-water, high-energy marine environment of the northern Gulf of Mexico. High-energy ecosystems may ameliorate some symptoms of eutrophication, such as low diversity and dominance of opportunistic fauna, and may fail to display some successional stages (Puente and Diaz 2015). In this application, bottom-water dissolved oxygen (BWDO) concentration is used as a proxy for organic enrichment because organic enrichment increases as the dissolved oxygen concentration decreases. The SAB parameter used in the P-R model is used to define the successional stages of the macrobenthic community and to assess the ecological condition of the benthic habitat in the northern Gulf of Mexico.

## **2. Methods**

### *a. Study area and experimental design*

Four sites on the Louisiana continental shelf (Fig. 1) from west of the Mississippi River bird-foot delta to just west of Atchafalaya Bay were chosen based on their history of hypoxia exposure as determined by the BWDO values collected during the annual, midsummer, shelf-wide cruises from 2000 to 2008 (Rabalais, Turner, and Scavia 2002; Rabalais et al. 2007). Based on the frequency of bottom-water hypoxia at Louisiana Universities Marine

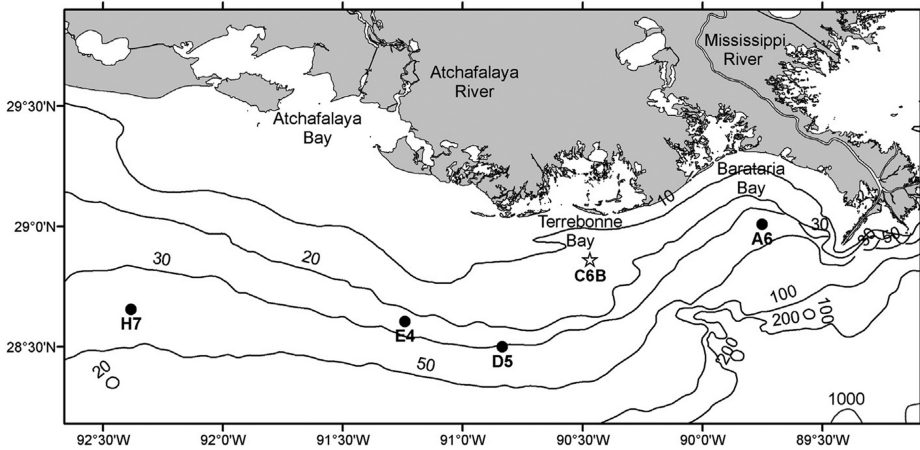


Figure 1. Map of the locations of the four sampled sites. Water depth contours are in meters.

Consortium (LUMCON) survey stations, LUMCON-designated sites H7, D5, E4, and A6 were chosen for sampling.

Site H7, located southwest of Atchafalaya Bay, experienced no documented hypoxia during the 9-year period; site D5, located southwest of Terrebonne Bay, experienced hypoxia in one year (2006); site E4, located south of the Atchafalaya River, experienced hypoxia in three years (2002, 2006, and 2008); and site A6, located west of Southwest Pass of the bird-foot delta, experienced hypoxia in four years (2000, 2002, 2007, and 2008). Because the macrobenthos community likely bears the immediate impacts of existing hypoxic stress and the impact from hypoxic stress from previous years, we characterized the four sites in terms of average BWDO over the span of 2000 to 2010, based on data collected during the annual, midsummer, shelf-wide cruises (N. Rabalais, personal communication). Average BWDO over the 11-year span ranked the sites from highest to lowest BWDO concentration: H7, D5, E4, and A6 (see Table 1).

All sites were arrayed along a 30 to 39 m isobath. Shallower stations were avoided because of their susceptibility to intense, episodic sediment reworking during seasonal storms. Our intention was to reduce the influence of water depth on benthic communities (Briggs Cartwright et al. 2015; Briggs Hartmann et al. 2015; Briggs et al. 2017) and minimize variations in sediment grain-size characteristic of shallower depths. Despite minor differences in proportions of sand and shell fragments among the sites, the sediments of the four sites were predominantly silty clay (Briggs Cartwright et al. 2015; Briggs Hartmann et al. 2015) and characterized as a mud facies, especially in the surficial layer.

#### *b. Sampling procedure*

Three cruises aboard the research vessel R/V *Pelican* were undertaken to collect samples. To sample the stable oxic condition for assessing the impacts of hypoxia in the first

Table 1. Number of species and percentage composition of major taxa abundance according to the 2000–2010 average bottom-water dissolved oxygen (BWDO) at the four sampled sites during spring, late summer, and midsummer samplings.

Site	H7	D5	E4	A6
2000–2010 average BWDO (mg L <sup>-1</sup> )	5.4	3.4	2.6	2.1
Spring 2009				
Number of species	138.0	125.0	95.0	46.0
Opportunists (%)	28.0	37.0	56.0	63.0
Polychaeta (%)	49.9	72.5	71.9	67.5
Bivalvia (%)	20.1	8.1	9.9	26.0
Crustacea (%)	7.7	2.5	3.8	3.7
Echinodermata (%)	1.8	2.3	5.2	—
Other groups (%)	20.5	14.6	9.2	2.8
Late summer 2009				
Number of species	144.0	77.0	94.0	44.0
Opportunists (%)	37.0	26.0	49.0	76.0
Polychaeta (%)	56.7	49.1	65.3	89.4
Bivalvia (%)	21.4	29.4	19.5	4.4
Crustacea (%)	7.9	2.1	1.0	2.0
Echinodermata (%)	0.2	4.5	3.5	0.4
Other groups (%)	13.8	14.9	10.7	3.8
Midsummer 2010				
Number of species	106.0	93.0	82.0	43.0
Opportunists (%)	43.0	36.0	38.0	68.0
Polychaeta (%)	57.8	55.3	72.9	83.9
Bivalvia (%)	20.9	25.5	12.1	13.8
Crustacea (%)	10.9	2.3	0.4	0.1
Echinodermata (%)	1.2	3.4	3.8	—
Other groups (%)	9.2	13.5	10.8	2.2

analyses (and to maximize the recovery time since the last likely hypoxia event), the first set of samples was collected in spring (30 March–6 April 2009), when hypoxia is rare, water column stratification is incipient (Rabalais and Turner 2001), and the macrobenthos community is just emerging from the winter “resting stage” and about to enter the growing season. To capture the potential impacts of a seasonal hypoxia event, the second set of samples was collected in late summer (5–11 September 2009), when the hypoxia season is ending, water column stratification is entering the fall transition (Rabalais and Turner 2001), and the macrobenthos community is concluding its growing season. To capture the effects of hypoxia at the height of the summer hypoxia season, the third set of samples was collected in midsummer (3–9 August 2010), when water column stratification is strongest and hypoxia is typically well developed and widespread (Rabalais and Turner 2001).

Sediment samples were collected at each of the four sites for each of the three cruises using a 0.25 m<sup>2</sup> box corer. From each site six box cores were collected and from each box

core three cylindrical subcores (53.4 cm<sup>2</sup> each) were collected for macrobenthos sampling. Additional cylindrical subcores were collected from the box cores for the measurement of sediment organic carbon and nitrogen and the sediment grain-size distribution.

The sediment samples collected for macrobenthos sampling were processed immediately after coring. These samples were sieved through a 0.3 mm Nitex mesh to collect organisms smaller than the macrofauna usually retained on 0.5 mm mesh because of the potential to capture newly recruited individuals signifying recovery from hypoxia. After sieving the benthic fauna retained on the mesh were immediately fixed in 5% buffered rose Bengal-formalin solution. All the collected fauna were transferred to 70% isopropanol in the laboratory for preservation.

### *c. Laboratory analysis*

Nine subcores from each site per cruise (a total of 108 subcores during all three cruises) were processed and preserved for macrobenthic enumeration. Samples were sorted from the debris, identified to the most specific taxonomic level possible, and counted for total abundance. Macrobenthos abundance was expressed as number of individuals per 100 cm<sup>2</sup>. Macrobenthos biomass for the enumerated individuals was measured as wet weight using a Mettler Toledo UMX2 ultramicrobalance and converted to ash-free dry weight (AFDW) using conversion factors given in Ricciardi and Bourget (1998) and following the approach of Greenstreet et al. (2007). Further details of the weighing protocol are described in Shivarudrappa (2015). Values for macrobenthos biomass were expressed as AFDW per 100 cm<sup>2</sup>. Values of AFDW biomass from each species were divided by the values for abundance to determine the average individual biomass of the macrobenthos specimens.

### *d. Statistical analysis*

Nine replicate samples for each site and for each of the three cruises were assessed for macrobenthos abundance, biomass, estimated average individual biomass, and the diversity indices of inverse Simpson's diversity ( $1/D$ ), Pielou's evenness ( $J'$ ), and species richness ( $SR$ ). Replicate values of the aforementioned parameters were tested for normal distributions using Shapiro-Wilk tests and quantile-quantile plots. For the normally distributed values ( $1/D$ ,  $SR$ ), Tukey's honest significance test for multiple comparisons was used to test the significance difference in mean values; for the values not normally distributed (abundance, biomass, average individual biomass,  $J'$ ), the Mann-Whitney  $U$ -test with the Bonferroni multiple-comparison correction for four sites was used to test the significance difference in median values.

Several multivariate methods were used to examine macrobenthos community structure: analysis of similarity (ANOSIM), nonmetric multidimensional scaling (nMDS), distance-based redundancy analysis (dbRDA), and principal component analysis (PCA). The species abundance data were  $\log_{10}(x + 1)$  transformed to correct the imbalance in significance of abundant and rare species before performing analysis and Bray-Curtis dissimilarity



distances were used in ANOSIM, nMDS, and dbrDA (Anderson 2001). All multivariate analyses were run employing 999 permutations

The random, crossed, one-way ANOSIM with Bonferroni corrections for the pairwise comparisons between sites was used to determine the affinities of the species occurring in the sample cores within and between sites for the three samplings. The nMDS from the vegan package of the R Project for Statistical Computing (R Core Team 2016) was used to extract the strongest patterns in community structure. The dbrDA was performed on the macrobenthos abundance data and measured environmental variables (percent sediment organic carbon, sediment carbon-nitrogen ratio, BWDO concentration, and percent mud [silt + clay]) to identify the impact of the environment on the macrobenthos. These environmental variables were used to assess influence on species distribution during the first and second samplings during April and September 2009. In August 2010 sedimentary organic carbon and the sediment C:N ratio were not measured; hence dbrDA was performed with only two measured environmental variables—BWDO concentration and percent mud. Bray-Curtis dissimilarity distance measure was used to generate the resemblance matrix of macrobenthos abundance. The “capscale” function in the R vegan package was used to run the dbrDA stepwise routine. PCA from the FactoMineR package (R Core Team 2016) was used to visualize the arrangements of sites on the ordination axes to determine the patterns in macrobenthic successional stages. We used the factors of macrobenthos abundance, biomass, species diversity, richness, evenness, percentages of opportunistic species, and BWDO concentration to perform PCA ordination.

The percentage of opportunistic species found at H7, D5, E4, and A6 during April 2009, September 2009 and August 2010 were regressed on the average BWDO values collected from 2000 to 2010 during annual shelf-wide, midsummer cruises (N. Rabalais, personal communication). Average BWDO values from 2000 to 2008, 2000 to 2009, and 2000 to 2010 were employed for regressing the opportunistic species data collected in April 2009, September 2009, and August 2010, respectively. Two regression fits were performed, one including all sites and another excluding the de facto control site (H7).

Values of abundance, AFDW biomass, average individual biomass, diversity, richness, evenness, and percentage of nonopportunistic macrobenthos for each of the four sites were ranked from highest value (= 1) to lowest value (= 4) in each of the three sampling periods. The rankings were then averaged over each of these seven parameters to achieve a mean rank score that was used to signify which stage on the P-R model successional continuum the macrobenthos community at each site resided. High mean rankings signified communities in the later stages of succession (stage II), whereas low mean rankings signified communities in the early stages of succession (stage I).

### **3. Results**

Macrobenthos belonging to 394 different species were identified from the four sites during the three sampling cruises. Polychaete annelids dominated the macrobenthos abundance,

followed by bivalves. There were some spatial and temporal differences in the less abundant groups (Table 1). Echinoderms contributed more to E4 assemblages than to those from H7, and crustaceans decreased in assemblages from spring 2009 compared with the assemblages present in summer 2009 and 2010 at E4. Polychaete annelids also dominated the macrobenthos biomass at all sites.

#### a. Abundance

Multiple comparisons of abundance values for spatiotemporal variations did not indicate significant differences in spatial variation in the three sample collections (Fig. 2a, Table 2). However, D5 and A6 varied seasonally. Abundance values at D5 in September 2009 were significantly less than in April 2009 ( $P$  value = 0.027). Abundance values at A6 from April 2009 were significantly less than those from August 2010 ( $P$  value = 0.032), but neither of the abundance values varied significantly with those from September 2009.

#### b. Biomass

Multiple comparisons of AFDW biomass values for spatiotemporal variations did not show significant differences in temporal variation during the sample collections (Fig. 2b, Table 2). However, A6 was significantly lower in biomass than E4 ( $P$  value = 0.027) or H7 ( $P$  value = 0.038) in September 2009. Biomass values at D5 from September 2009 were significantly less than those from August 2010 ( $P$  value = 0.027).

#### c. Estimated average individual biomass

Multiple comparisons of average individual AFDW biomass values for spatiotemporal variations showed few significant differences (Fig. 2c, Table 2). The size of organisms at D5 significantly increased from September 2009 to August 2010 ( $P$  value = 0.027).

#### d. Diversity indices

Multiple comparisons of inverse Simpson's diversity index (Fig. 3a, Table 2) for spatiotemporal variations showed significant differences spatially and temporally during the three sample collections. Diversity at H7 in August 2010 was significantly less than that in April and September 2009. In April 2009, all sites had significantly different diversity values ( $P$  value < 0.05) from one another, except between E4 and A6. In September 2009, H7 and A6 had the significantly highest and lowest diversity ( $P$  value < 0.05), respectively, but this parameter was not significantly different between sites D5 and E4. In August 2010, H7, D5, and E4 had the highest diversity, but the diversity values were only significantly higher ( $P$  value < 0.05) than those from A6.

Multiple comparisons of species richness (Fig. 3b, Table 2) for spatiotemporal variation showed some significant differences spatially and temporally during the three sample collections. At D5, species richness was significantly greater in April than in September 2009 ( $P$  value = 0.0001). At H7, species richness was significantly greater in September 2009

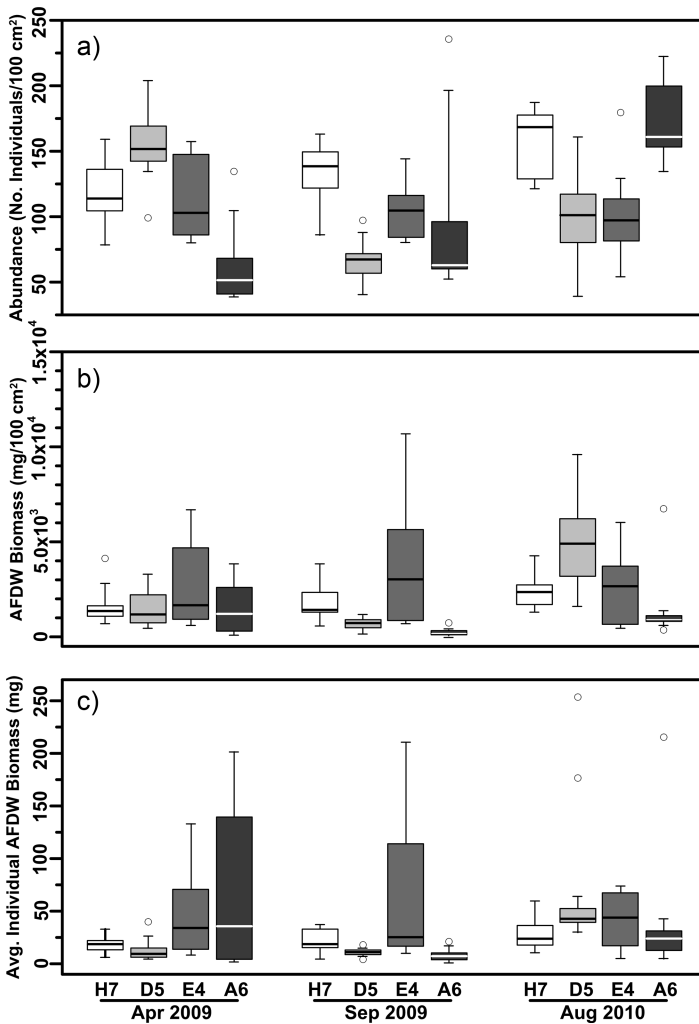


Figure 2. The minimum, maximum, median, lower quartile, upper quartile, and outliers (hollow circles) for macrobenthos abundance (a), AFDW biomass (b), and estimated average individual AFDW biomass (c) ( $n = 9$ ) at sites H7, D5, E4, and A6 during April 2009, September 2009, and August 2010.

than in August 2010 ( $P$  value = 0.034). In April, species richness at E4 was significantly lower than at H7 and D5, but significantly greater than at A6 ( $P$  value < 0.05). In September 2009, species richness at H7 was significantly greater than the other sites ( $P$  values < 0.05). Species richness at A6 was significantly less than the other sites ( $P$  values < 0.05) during all three sample collections.

Table 2. Bonferroni corrected  $P$  values for multiple comparisons of abundance, ash-free dry weight (AFDW) biomass, estimated average individual AFDW biomass, inverse Simpson's diversity index, species richness index, Pielou's evenness index, and pairwise one-way analysis of similarity (ANOSIM; both R-statistic and  $P$ -values) to compare macrobenthos communities from sites H7, D5, E4, and A6 during April 2009 (Apr), September 2009 (Sep), and August 2010 (Aug).

	Abundance		Biomass		Average individual biomass		Diversity		Richness		Evenness		ANOSIM	
	$P$ value	$P$ value	$P$ value	$P$ value	$P$ value	$P$ value	$P$ value	$P$ value	$P$ value	$P$ value	$P$ value	$P$ value	R value	$P$ value
Apr. H7; Apr. D5	1.000	1.000	1.000	1.000	1.000	1.000	0.028*	0.999	1.000	0.909	1.000	0.007*		
Apr. H7; Apr. E4	1.000	1.000	1.000	1.000	1.000	1.000	0.000*	0.000*	0.255	0.799	0.007*			
Apr. H7; Apr. A6	0.352	1.000	1.000	1.000	1.000	1.000	0.000*	0.000*	0.037*	0.959	0.007*			
Apr. D5; Apr. E4	1.000	1.000	1.000	1.000	1.000	1.000	0.032*	0.011*	0.497	0.638	0.007*			
Apr. D5; Apr. A6	0.061	1.000	1.000	1.000	1.000	1.000	0.000*	0.000*	0.069	0.831	0.013*			
Apr. E4; Apr. A6	0.684	1.000	1.000	1.000	1.000	1.000	0.104	0.000*	0.337	0.965	0.007*			
Sep. H7; Sep. D5	0.052	0.236	1.000	1.000	1.000	1.000	0.000*	0.000*	1.000	0.793	0.013*			
Sep. H7; Sep. E4	1.000	1.000	1.000	1.000	1.000	1.000	0.000*	0.000*	1.000	0.930	0.020*			
Sep. H7; Sep. A6	1.000	0.038*	1.000	1.000	1.000	1.000	0.000*	0.000*	0.050	0.985	0.007*			
Sep. D5; Sep. E4	0.175	1.000	1.000	1.000	0.310	1.000	0.969	0.383	1.000	0.445	0.013*			
Sep. D5; Sep. A6	1.000	0.689	1.000	1.000	1.000	1.000	0.015*	0.004*	0.307	0.674	0.020*			
Sep. E4; Sep. A6	1.000	0.027*	1.000	1.000	0.270	1.000	0.000*	0.000*	0.526	0.795	0.007*			
Aug. H7; Aug. D5	0.174	0.886	1.000	1.000	1.000	1.000	0.333	0.789	0.035*	0.907	0.013*			
Aug. H7; Aug. E4	0.599	1.000	1.000	1.000	1.000	1.000	0.999	0.002*	1.000	0.703	0.020*			
Aug. H7; Aug. A6	1.000	0.533	1.000	1.000	1.000	1.000	0.000*	0.000*	0.026*	0.988	0.015*			
Aug. D5; Aug. E4	1.000	1.000	1.000	1.000	1.000	1.000	0.040*	0.330	0.107	0.695	0.007*			
Aug. D5; Aug. A6	0.112	0.177	1.000	1.000	1.000	1.000	0.000*	0.000*	0.026*	0.845	0.007*			
Aug. E4; Aug. A6	0.177	1.000	1.000	1.000	1.000	1.000	0.001*	0.000*	0.026*	0.989	0.007*			
Apr. H7; Aug. H7	1.000	1.000	1.000	1.000	1.000	1.000	0.000*	0.289	1.000	0.639	0.007*			
Apr. H7; Sep. H7	1.000	1.000	1.000	1.000	1.000	1.000	0.996	0.999	0.538	0.817	0.007*			
Aug. H7; Sep. H7	1.000	1.000	1.000	1.000	1.000	1.000	0.000*	0.035*	0.126	0.758	0.007*			
Apr. D5; Aug. D5	0.531	1.000	1.000	1.000	0.052	1.000	0.996	0.031*	0.444	0.592	0.007*			
Apr. D5; Sep. D5	0.027*	0.132	1.000	1.000	1.000	1.000	0.704	0.000	1.000	0.849	0.007*			
Aug. D5; Sep. D5	1.000	0.027*	1.000	1.000	0.027*	1.000	0.114	0.047*	1.000	0.426	0.007*			
Apr. E4; Aug. E4	1.000	1.000	1.000	1.000	1.000	1.000	0.992	0.554	1.000	0.966	0.007*			
Apr. E4; Sep. E4	1.000	1.000	1.000	1.000	1.000	1.000	0.165	10.000	1.000	0.751	0.020*			
Aug. E4; Sep. E4	1.000	1.000	1.000	1.000	1.000	1.000	0.839	0.901	1.000	0.554	0.007*			
Apr. A6; Aug. A6	0.032*	1.000	1.000	1.000	1.000	1.000	10.000	10.000	1.000	0.493	0.007*			
Apr. A6; Sep. A6	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.998	1.000	0.566	0.007*			
Aug. A6; Sep. A6	1.000	0.052	1.000	1.000	1.000	1.000	0.993	10.000	0.596	0.725	0.007*			

\*indicates significant difference ( $P < 0.05$ ) between comparisons.

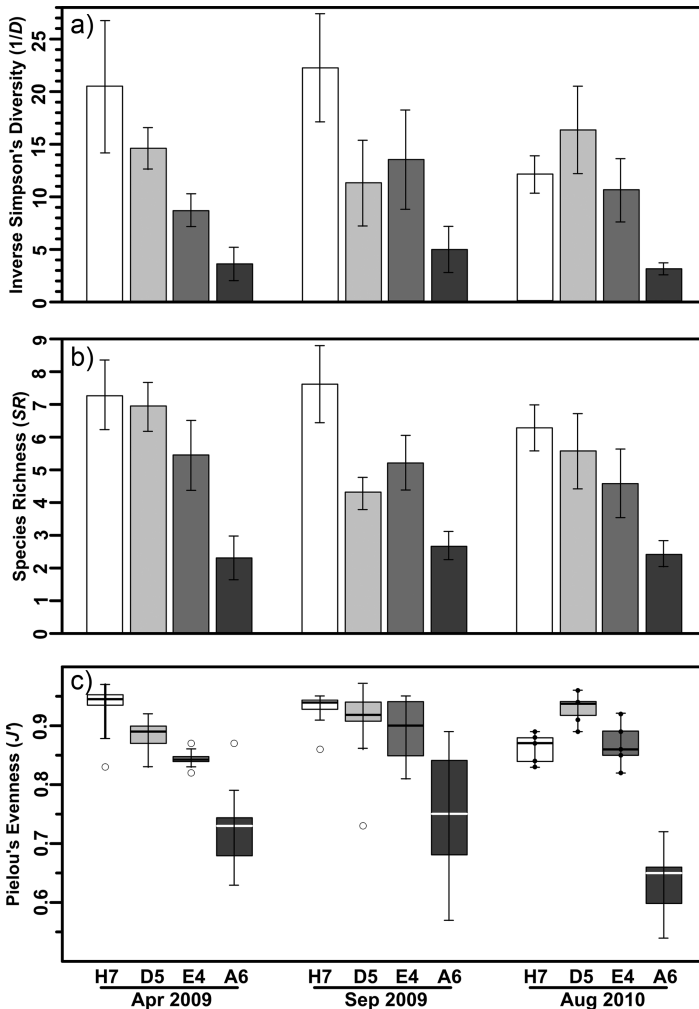


Figure 3. Bar diagrams of mean values (bars)  $\pm$  1 standard deviation (capped lines) of inverse Simpson's diversity index (a) and species richness index (b); box-and-whisker plot of Pielou's evenness index (c) with minimum, maximum, median, lower quartile, upper quartile, and outliers (hollow circles) at sites H7, D5, E4, and A6 during April 2009, September 2009, and August 2010.

Multiple comparisons of Pielou's evenness (Fig. 3c, Table 2) values for spatiotemporal variations showed that evenness at H7 was always significantly greater than that at A6 during all three sample collections ( $P$  values = 0.038, 0.050, and 0.026, respectively). D5 evenness was significantly greater than H7 evenness in August 2010 ( $P$  value = 0.035). Evenness at A6 was significantly lower than all the other sites, but only in April 2009 and August 2010 ( $P$  value < 0.026).

*e. Percentage of opportunistic species*

A6 consistently had the highest percentage of opportunistic species over all three sample collections (Table 1). D5 had nearly the same low percentage of opportunistic species among the sites sampled during all three samplings. The de facto control site (H7) had its lowest average number of opportunistic species during the April 2009 sample collection. After A6, E4 had the next-highest proportion of opportunists over all three sample collections except at H7 in August 2010 but exhibited decreasing percentages of opportunistic species in September 2009 and again in August 2010. H7 and A6 were the only sites to show increases in the proportions of opportunists from spring to late summer and to the following midsummer; in both assemblages, these increases were because of increases in the abundances of the capitellid polychaete *Mediomastus californiensis*.

*f. Multivariate analyses*

The global ANOSIM results indicated that there were no significant differences within sites (between subcores) (Table 2). However, there were significant differences between sites ( $P$  values  $< 0.01$ , corrected for pairwise error). The macrobenthos of site D5 from September 2009 had the highest similarity within the macrobenthos from August 2010 ( $R = 0.426$ ). The macrobenthos from site E4 and D5 during September 2009 were the next most similar set of macrobenthos ( $R = 0.445$ ), whereas the macrobenthos from April 2009 had high similarity with August 2010 macrobenthos at site A6 ( $R = 0.493$ ) (Table 2).

The nMDS ordination of macrobenthos abundance data showed the ecological intersite distance between E4 and D5 to be the shortest during the April 2009 sampling, but becoming greater as the species compositions of these two sites became more dissimilar in September 2009 and August 2010 (Fig. 4). The species compositions of H7 and A6 remained the most dissimilar in April and September 2009 but became more similar—as did the other sites—by August 2010. By the August 2010 sampling, H7 was nearly equally dissimilar to D5, E4, and A6, specieswise (Fig. 4).

Five environmental variables were measured in April and September 2009—sediment carbon-nitrogen ratio (C:N), percent sediment organic carbon (% C), percent sediment organic nitrogen, BWDO concentration, and percent silt + clay (% Mud)—and employed in dbRDA. Sediment organic nitrogen was highly correlated with sediment organic carbon ( $r^2 = 0.97$ ), and because its influence was included in the C:N ratio, this variable was excluded from the dbRDA (Fig. 5). Of the four variables used in the dbRDA model, only the C:N ratio, sediment organic carbon, and BWDO explained the fitted variability (81.3% and 82.0% for April and September 2009, respectively) and total variability (19.6% and 21.6% for April and September 2009, respectively) (Fig. 5a and b). Only the BWDO and percent mud environmental variables were used in the dbRDA model for the August 2010 data, which explained the fitted (100%) and total (18.1%) variability (Fig. 5c).

PCA examined the relationships among the variables of abundance, biomass, species diversity, richness, evenness, percentage opportunistic species, and BWDO concentration

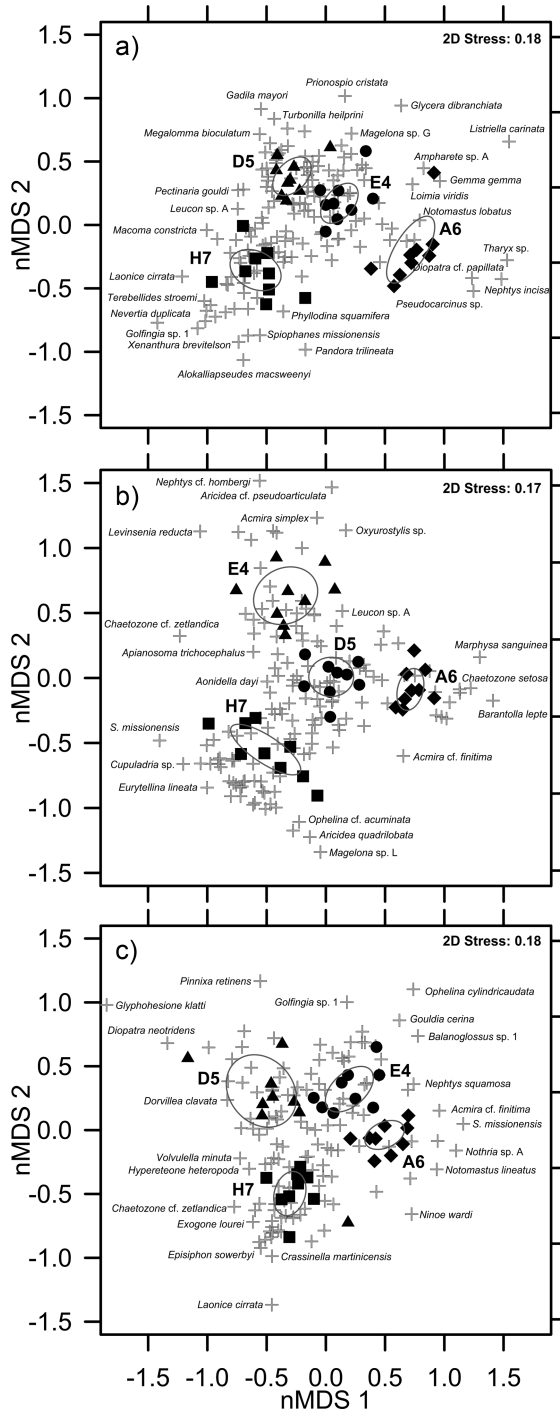


Figure 4. Nonmetric multidimensional scaling (nMDS) ordination of macrobenthos abundance during April 2009 (a), September 2009 (b), and August 2010 (c). Species are marked in crosses (+); subcores are designated as solid squares (H7), solid triangles (D5), solid circles (E4), and solid diamonds (A6). Gray ellipses are drawn around the 95% confidence limit dispersion of site subcores.

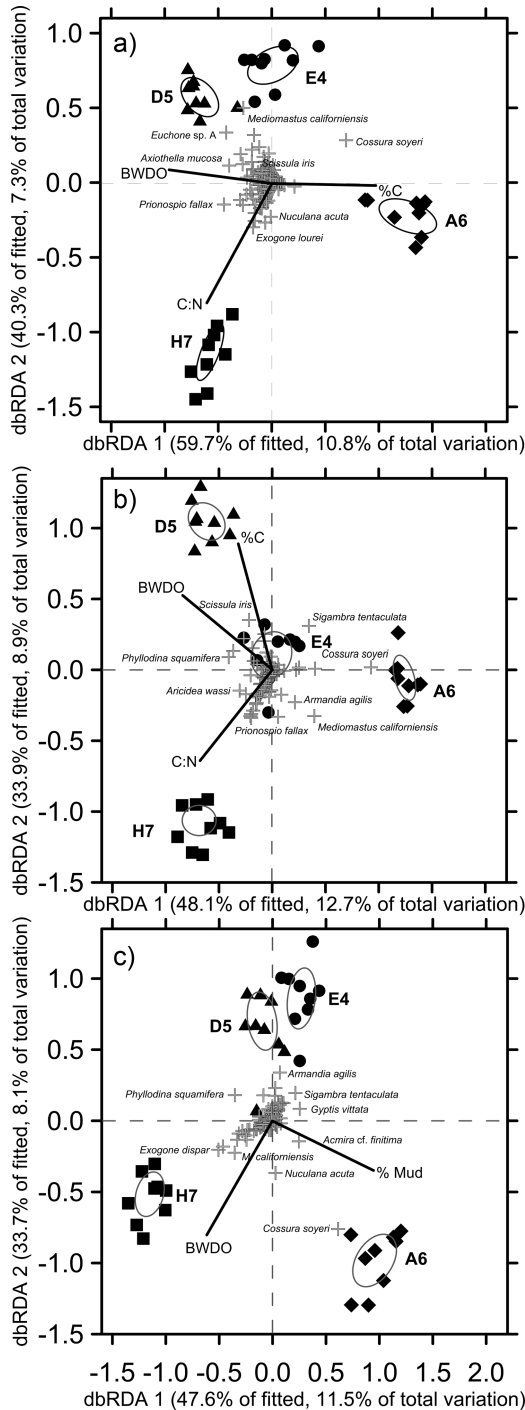


Figure 5. Distance-based redundancy analysis (dbRDA) ordination of macrobenthos abundance during April 2009 (a), September 2009 (b), and August 2010 (c). Designations of plotted data are the same as in Figure 4. BWDO, bottom-water dissolved oxygen.



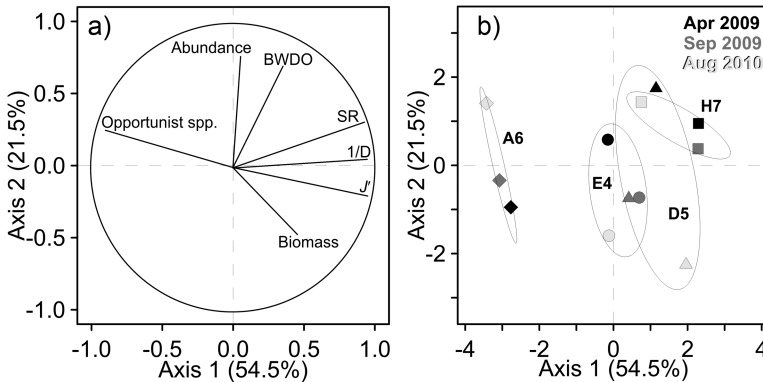


Figure 6. (a) Principal component analysis (PCA) variables factor map for sites H7, D5, E4, and A6 during April 2009, September 2009, and August 2010. The circle has correlation value 1.0, and arrow lengths are proportional to the correlation coefficient for each environmental variable. (b) PCA ordination of sites H7, D5, E4, and A6 during April 2009 (black symbols), September 2009 (50% shaded symbols), and August 2010 (10% shaded symbols). Symbol designations of plotted data are the same as in Figure 4. Ellipses surround the 95% confidence limit for the values at each site. BWDO, bottom-water dissolved oxygen.

at all four sites from all three sample collections (Fig. 6a). This analysis arrayed the sites right to left according to the ranking of their 11-year average BWDO concentration (Table 1) on the first ordination axis (Fig. 6b). H7, D5, and E4, in that order, benefited the most from high values of diversity and BWDO concentration. A6 was separated from these other three sites not only because of lower diversity, but also because of the predominance of opportunistic species. The first PCA axis (PC1) explained 54.5% of the total variance in the sites array, and the first three PCA axes (PC1, PC2, and PC3) cumulatively explained almost 90% of the total variance (Table 3). There are strong correlations among the four sites with species diversity, richness, and evenness (positive) and opportunistic species (negative) along the first PCA axis (Table 3). Macrobenthos abundance and bottom-water oxygen showed relatively high correlations among the four sites on the second PCA axis (Table 3, Fig. 6).

The linear regression fitted to determine if variations in the proportions of opportunistic species could be explained by the recent decadal variations in BWDO concentration had an  $r^2$  value of 0.72 ( $P$  value = 0.0005) (Fig. 7a). The linear regression fitted only for the data from the sites with BWDO concentrations averaging greater than  $2.1 \text{ mg L}^{-1}$  (D5, E4, and A6) improved the  $r^2$  value to 0.92 ( $P$  value = 0.00005) (Fig. 7b).

#### 4. Discussion

The hypoxia in the northern Gulf of Mexico is seasonal, and upon the subsidence of hypoxia stress, the seasonally disturbed benthic community may take a few years to recover

Table 3. Correlation-based principal component (PC) analysis eigenvalues, % variation, and cumulative % variation explained by the first five ordination axes (PC1, PC2, PC3, PC4, and PC5) and linear coefficients (eigenvector) of each PC axis for each variable for sites H7, D5, E4, and A6 during April 2009, September 2009, and August 2010. BWDO, bottom-water dissolved oxygen.

	PC1	PC2	PC3	PC4	PC5
Eigenvalue	3.814	1.505	0.917	0.532	0.157
% Variation	54.481	21.507	13.096	7.601	2.249
Cumulative % variation	54.481	75.988	89.084	96.684	98.933
Eigenvector					
Abundance	0.054	0.768	0.606	-0.155	-0.116
Biomass	0.455	-0.466	0.608	0.451	0.042
Diversity	0.949	0.055	0.022	-0.226	0.115
Richness	0.930	0.312	0.056	-0.078	0.129
Evenness	0.950	-0.199	-0.151	-0.061	0.062
Opportunistic species	-0.900	0.255	0.120	0.029	0.329
Oxygen (BWDO)	0.352	0.702	-0.372	0.493	-0.013

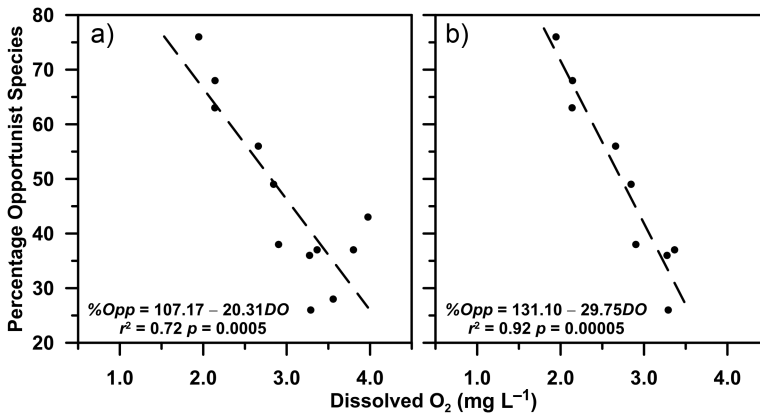


Figure 7. Linear regressions of the percentage of opportunistic species (dependent variable = % *Opp*) on the mean bottom-water dissolved oxygen (independent variable = *DO*) concentration during the April 2009, September 2009, and August 2010 sample collections. (a) Plot includes all four sites. (b) Plot excludes the de facto control site (H7).

completely and become a mature community (Pearson and Rosenberg 1978; Diaz and Rosenberg 2008). If hypoxia is annually recurring, the benthic community has little time to recover between hypoxic periods, and the community may endure a significant loss in diversity because of the stress induced by hypoxia on a recovering community (Pearson and Rosenberg 1978; Diaz and Rosenberg 2008). The benthic community structure will likely reflect its recent encounter with hypoxia as well as the cumulative effects of repeated hypoxic events.

Table 4. Bottom-water oxygen concentration ( $\text{mg L}^{-1}$ ) at sites H7, D5, E4, and A6 during our three sample collections (shaded rows) and the Louisiana Universities Marine Consortium annual midsummer shelf-wide cruise in July 2008, 2009, and 2010. Hypoxic concentrations ( $<2 \text{ mg L}^{-1}$ ) are italicized.

	H7	D5	E4	A6
July 2008	5.64	2.30	<i>0.54</i>	<i>0.07</i>
April 2009	6.90	6.60	6.40	4.30
July 2009	6.00	2.60	4.50	<i>0.20</i>
September 2009	3.90	5.30	4.30	2.70
July 2010	5.70	3.13	3.49	3.39
August 2010	5.70	3.10	<i>1.70</i>	3.90

A6 was close to the Mississippi bird-foot delta and therefore is heavily influenced by the nutrient-rich Mississippi River discharge. By contrast, E4 and D5 were close to the Atchafalaya River and are presumed to be chiefly influenced by the nutrients discharged by the Atchafalaya River (Fig. 1). Although the influence of the Atchafalaya River on the perennial hypoxic zone is unclear (Krug 2007), the sediment and water discharged by the river could help explain the variations in macrobenthos community structure observed at E4 and D5. The nutrient-rich freshwater and sediment discharged from the Atchafalaya River that fuels the localized hypoxia settles east, west, and south of the river mouth, throughout the hypoxic zone, depending on the prevailing winds, currents, and river discharge (Krug 2007; Krug and Merrifield 2007; Hetland and DiMarco 2008; Bianchi et al. 2010). The Atchafalaya discharge could be the chief driver of recent documented hypoxia at E4 (Table 4) during July 2008 and August 2010. D5, though geographically close to E4, might have benefited from organic enrichment as a fringe effect of nearby hypoxia, where primary production is high enough to act as a food source for the benthos but not high enough to prompt hypoxia. H7, situated far west from the bird-foot delta and reasonably far from the Atchafalaya discharge, can be considered to be relatively unaffected by recent hypoxia.

#### a. Benthic community response

Benthic communities in transition or recovery are highly unpredictable. In the successional continuum, only the pioneering and final stages are predictable, and only the final stage is habitat dependent (Pearson and Rosenberg 1978). Nevertheless, scrupulous interpretations of the macrobenthos censuses can provide insight into the dynamics of community structure. Hence, to test the SAB response hypothesis and assess the stress response induced by seasonal hypoxia on the macrobenthic communities, the parameters of abundance, biomass, estimated average individual biomass, species diversity, richness, evenness, and percentage of opportunistic species were interpreted at H7, D5, E4, and A6.

i. *De facto control and westernmost site (H7)*. This site had the highest average BWDO concentration ( $5.4 \text{ mg L}^{-1}$ ) and generally showed the characteristics of a stable community

with high values for macrobenthos abundance, biomass, evenness, and diversity during spring and late summer 2009. In summer 2010, although the abundance values were higher, the diversity values were lower than in 2009. Furthermore, the percentage of opportunistic species also increased because of the dominance of the capitellid polychaete *M. californiensis* during summer 2010. This might be because the areal extent of hypoxia measured in July 2010 was one of the largest off the Louisiana shelf since 1985 (LUMCON 2010). The total area reported was smaller than the actual size of the hypoxic water mass because time constraints precluded the measurement of the full extent of the hypoxic area (LUMCON 2010). Moreover, *M. californiensis* is a second-degree opportunist and a good indicator of an unpredictable environment (Grassle and Grassle 1974; Borja, Franco, and Pérez 2000). This polychaete is usually present in unpredictable environments. When favorable conditions are present, its population size increases and once the recovery process starts, it tends to disappear because of increased competition (Grassle and Grassle 1974). *M. californiensis* produces planktonic larvae during the summer months, and its population attains a large size when the populations of other species are reduced. Its proliferation beyond effective dispersion of planktonic larvae is because of elimination of competition from hypoxia-sensitive species and a consequent increased food supply because of its absence (Grassle and Grassle 1974). Gaston (1985) observed hypoxia-induced elimination of macrobenthos that contribute rarely and moderately to the abundance. He also observed a noticeable decrease in species richness during hypoxia and a regime shift in the macrobenthos community toward juveniles and opportunistic species off the western inner shelf of Louisiana. However, an increase in abundance of *M. californiensis*, a typical opportunist, at H7 during August 2010 might be attributable to degradation of normally favorable conditions because of an unusually large nearby hypoxic zone in the year 2010, or it might have been the result of a random variation in its population.

Based on the observations of high diversity, despite a third of the population being *r*-selected opportunistic species and the population lacking the large-sized macrobenthos characteristic of a mature (climax) stage, we suggest the community at H7 was in the last phase of stage II in the P-R model (Table 5).

*ii. Site intermediate between Atchafalaya and Mississippi Rivers (D5).* D5 had an average BWDO concentration of  $3.4 \text{ mg L}^{-1}$ , which although lower than the de facto control, could have allowed the community sampled in April periods of recovery and growth. The samples collected in spring at this site had a high abundance, moderate biomass, high diversity, high species richness, high evenness, and a low percentage of opportunistic species, all of which indicated a healthy community. The macrobenthos abundance significantly decreased in September 2009. In addition, both the percentage of opportunistic species and the number of all species decreased by late summer. Similar to these results from deeper water, Rabalais, Turner, and Scavia (2002) observed high species richness of macrobenthos in spring and low species richness in summer and late summer months in 1990 and 1991 in <20 m water depth on the Louisiana continental shelf. Furthermore, Baustian, Craig, and Rabalais



(2009) found the macrobenthic communities to be three times less abundant and diverse in September and October 2004 compared with those in March and April 2004 at stations shallower than those of this study.

During September 2009, D5 was on the periphery of the hypoxic zone. Increased predation on benthic invertebrates by demersal fishes moving away from hypoxia-affected areas to surrounding nonhypoxic areas might have been occurring at D5 during late summer 2009 (Baustian 2005), and this might account for the reduced abundance and diversity of the macrobenthos here. The decreased percentage of opportunistic species suggested that D5 was not suffering deleterious effects of hypoxia despite the reduced population size.

In midsummer 2010, however, D5 was far away from the hypoxia-affected area. The macrobenthos assemblage at D5 increased in abundance, biomass, and diversity in August 2010. This profusion of macrobenthos could be a result of D5 being subjected to organic enrichment and reduced predation pressure, in addition to sufficient oxygen for growth. The presence of the large-bodied, *K*-selected macrobenthos (e.g., the bivalves *Angulus versicolor* and *Caryocorbula contracta* and the polychaetes *Ameana trilobata*, *Clymenella torquata*, *Diopatra neotridens*, and *Euclymene* sp. A) provided the largest increases in biomass (Shivarudrappa 2015). Hence, the presence of large-bodied equilibrium species with increased abundance, diversity, and richness in 2010 suggested that the community at D5 had progressed toward recovery from previous hypoxic events. The macrobenthos community at D5 was the only community documented to progress in recovery during the investigation. Although the *r*-selected opportunistic species population was consistent in size with that of H7, the relatively lower diversity of the community at D5 compared with that of H7 indicated a lower stage in the successional continuum. We suggest that the macrobenthos community at D5 was in the intermediate phase of stage II in the P-R model (Table 5).

*iii. Site nearest the Atchafalaya River (E4).* E4 had an average BWDO concentration of 2.6 mg L<sup>-1</sup>, but hypoxia was not present when sampled in 2009. The areal extent of hypoxia in the northern Gulf of Mexico during summer 2009 was one of the smallest, covering only 8,000 km<sup>2</sup> (LUMCON 2009). The macrobenthos abundance, biomass, and individual size at this site remained approximately the same in spring and late summer 2009 (Fig. 2a). An increase in diversity of the macrobenthos assemblage from spring to late summer suggested an improved health of the assemblage (Fig. 3a). The decrease in percentage of opportunistic species from spring to late summer (Table 1) may have been a result of increased competition for food and the filling of previously unoccupied niches following earlier exposures to hypoxia. In essence, the macrobenthos community at E4 was recovering from past exposure to hypoxia in late summer 2009.

The macrobenthos at E4 was sampled 7 days after the 2010 annual midsummer shelf-wide cruise sampling reported the bottom water to be 3.49 mg L<sup>-1</sup> (Table 4). However, at the time the macrobenthos was sampled, the BWDO concentration at E4 was less than 2 mg L<sup>-1</sup> (Table 4). Only the highly sensitive species would have been eliminated at 1.7 mg L<sup>-1</sup>

(Table 4) (Nilsson and Rosenberg 2000). During August 2010, macrobenthos abundance, biomass, and average individual biomass did not change significantly (Fig. 2), nor did the diversity, richness, or species richness (Fig. 3). Because the hypoxia at E4 had just developed or shifted into the area before the macrobenthos sampling, the community was likely unaffected by the incipient hypoxia and instead was exhibiting signs of recovery from previous hypoxic events. The duration of exposure of E4 to hypoxia at the time of sampling was not long enough for opportunists to have responded, which would explain the low percentage of opportunistic species found there (Table 1). Although the opportunists *M. californiensis* and *Cossura soyeri* dominated E4 during spring and late summer 2009 (Shivarudrappa 2015), these two polychaetes were the fourth-most dominant species and insignificant in abundance, respectively, in summer 2010. The relatively high evenness values at E4 were another indication of the scarcity of opportunistic species.

The macrobenthos community at E4 exhibited indications of exposure to stress by recurring hypoxia: lower diversity than either site H7 or D5 and a relatively high percentage of opportunistic species. The macrobenthos community at E4 was at a similar successional stage as H7 and D5 but ranked below them in the successional continuum. We suggest that the E4 macrobenthos community was in the initial phase of stage II (nearest stage I) in the P-R model (Table 5).

*iv. Easternmost site and nearest the Mississippi River (A6).* The 11-year average BWDO concentration at A6 was  $2.1 \text{ mg L}^{-1}$ , and during April 2009, the BWDO was depleted, but not hypoxic. A6 had low macrobenthos abundance, but high estimated mean individual biomass. However, values for estimated mean individual biomass were skewed high by the presence of large epibenthic shrimp, *Alpheus* spp., despite small polychaetes dominating the macrobenthos. During September 2009, the percentage of opportunistic species increased, but macrobenthos abundance, biomass, and estimated average individual biomass did not change significantly. Although the BWDO concentration was above the  $2 \text{ mg L}^{-1}$  hypoxia threshold when the macrobenthos were sampled in September (Table 4), the annual LUMCON cruise in July found the site to be hypoxic. The July hypoxia may have allowed opportunistic species to fill the niche vacated by more stress-sensitive species. Chief among the opportunists at A6 was the cossurid polychaete *C. soyeri*. Cossurid polychaetes are small bodied, grow up to 15 mm, and breed multiple times in a growing season. Bachelet and Laubier (1994) found ovigerous females from February to April of every year, and Ewing (1984) found gravid individuals of *C. soyeri* and *Cossura delta* in the northern Gulf of Mexico during midfall. Zhadan, Vortsepneva and Tzetlin (2012) found a bimodal distribution of cossurid species, indicating the presence of two different cohorts of juveniles and adults in their study in the central basin of the White Sea of the Russian Arctic. In this investigation, two different populations of cossurids were observed at A6 during 2009. In the spring samples before the hypoxic event in July 2009, cossurids made up to 42% of the population, and their average individual body size was  $1.4 \mu\text{g AFDW}$ ; after the hypoxic event in late summer, their contribution to the abundance remained the same (42%), but the

cossurids were smaller, with an estimated average individual biomass of  $0.36 \mu\text{g AFDW}$  (Shivarudrappa 2015). The severe hypoxia ( $0.2 \text{ mg L}^{-1}$ ; Table 4) might have killed the spring cossurid cohort. Cossurids have a semiplanktonic, juvenile dispersal phase, and the cohort found in late summer might have been newly settled recruits. Replacement of earlier recruitment could explain statistically insignificant differences between macrobenthos abundance in spring and late summer samplings and smaller individuals found in the later sampling.

During August 2010, the macrobenthos abundance at A6 increased because of heavy contributions from the opportunist *C. soyeri* (53%, or 827 individuals per site) and other opportunistic polychaetes like *M. californiensis*, *Sigambra tentaculata*, and *Paraprionospio pinnata*. Yet, nonopportunistic species, such as the bivalve *Nuculana acuta* and the paraonid polychaetes *Acmira finitima* and *Aricidea wassi*, occurred in large numbers and contributed to the increased biomass (Shivarudrappa 2015). The increase in cossurid abundance and estimated average individual biomass (from  $0.36 \mu\text{g}$  to  $0.47 \mu\text{g AFDW}$ ) in summer 2010 suggested a likely coexistence of adult cohorts from 2009 with new juvenile recruitment in 2010.

High percentages of opportunistic species, low values of biomass, and low values for species diversity during the three sample collections at A6 were a clear indication of a stressed macrobenthos community composed of a few dominant opportunistic species. The macrobenthos community at this site exhibited the most effects of stress of all the sites and supported the SAB response hypothesis. The macrobenthos community at A6 was far from afaunal (stage 0), but because of its low measures of diversity and near 70% *r*-selected opportunistic species, we suggest that the community was in the intermediate phase of stage I in the P-R model, ranking far behind H7, D5, and E4 on the successional continuum (Table 5).

#### b. Opportunistic species response

Without enough time to recover from previous seasons of hypoxia exposure, sequential oxygen stress events would promote the proliferation of opportunistic species at the affected sites. The empirical relationship in Figure 7 indicated that percentages of opportunistic species were inversely proportional to BWDO concentration. Fewer opportunistic species found in environments with higher oxygen concentrations was in accordance with a community response by equilibrium and opportunistic species.

The lower  $r^2$  value for the linear regression fitted for the data from all sites was because of the increase in species abundance and percentage opportunistic species at H7 during late summer 2009 and summer 2010. The increase in species abundance and percentage of opportunistic species (*Prionospio cristata*, *Prionospio fallax*, *Capitella capitata*, and *M. californiensis*) occurs at all sites during summer in the Gulf of Mexico (Shivarudrappa 2015). Hence, the increase in abundance of opportunistic species in the H7 assemblage during summer 2010 was because of the regular seasonal dominance of *M. californiensis* on



the shelf. Annual factors promoting summer growth might have prompted the proliferation of both *r*- and *K*-selected species at H7. Additionally, the samples collected at H7 during late summer 2009 and summer 2010 were at the end of the hypoxic season, which might have allowed more sensitive *K*-selected species to be eliminated and prompted the growth of opportunists such as spionid and capitellid polychaetes to thrive in unstable environments (Grassle and Grassle 1974; Borja, Franco, and Pérez 2000).

Exclusion of H7 values from the linear regression model in Figure 7 improved the  $r^2$  value and emphasized the effect of hypoxia on the benthic communities in the northern Gulf of Mexico experiencing the greatest stress. At the same time, this regression depended on only a single July measurement of BWDO concentration to represent the entire hypoxic season. Although a single annual measurement of BWDO on the Louisiana shelf is not sufficient to explain short-term and intra-annual variations, collectively the annual measurements may be acceptable to document the long-term and interannual trends in macrobenthic community structure.

### *c. Community succession*

In the 16 months over which the four sites were sampled three times, there was a reduction in number of species, an increase in total abundance, and an increase in shared species among the sites (Shivarudrappa 2015). An aggregate of four sites indicates that they are moving in the same direction on the successional continuum. Multivariate techniques were used in conjunction with community distribution and diversity analyses to evaluate macrobenthos spatiotemporal variability and community succession. ANOSIM and nMDS were used to assess the intra- and interspatial variability, dbRDA was used to assess the impact of environmental variables on the communities, and PCA was used to assess successional stages of the communities.

ANOSIM values indicate that the sites have distinct faunal compositions, despite some sites showing little change over time (comparisons in Table 2 with *R* values < 0.5). Furthermore, the nMDS ordination, grouping all sites separately based on species abundance, revealed that the community composition of the four sites did not see any remarkable changes from spring to late summer in 2009 and late summer 2009 to midsummer 2010 (Fig. 4).

According to the dbRDA, sediment C:N ratio had a high influence on the macrobenthos at site H7. Percent sediment organic carbon had a positive and BWDO had a negative influence on macrobenthos at A6 (Fig. 5a). The influence of sediment organic carbon at A6 can be attributed to the site's vicinity to the river mouth and its associated nutrients and organic matter. This is also an indication of strong benthic-pelagic coupling in the region. The negative influence of BWDO concentration can be attributed to increased biological oxygen demand of decomposing organic matter. By late summer, the influence of the sediment C:N ratio on H7 macrobenthos persisted, but sediment organic carbon was a bigger influence on D5 macrobenthos than on A6 macrobenthos (Fig. 5b). D5 might have been receiving a

higher organic load from the Atchafalaya River in the later part of the summer. Although the percent mud was not a factor in the distribution of the macrobenthos communities in spring or late summer (Fig. 5a and b), in August 2010 its effect influenced A6 macrobenthos and appeared as an important determinant of the macrobenthos community structure (Fig. 5c). The absence of sediment organic matter data may have inflated the significance of the mud content on the A6 macrobenthos.

The PCA results indicated that the community at A6 was the most affected by hypoxic stress and that the community at H7 was in a more advanced stage of succession compared with the other sites when relying on parameters of abundance, biomass, species diversity, number of species, and BWDO concentration (Fig. 6). PCA also showed the community at E4 to be more affected by hypoxia than those at H7 or D5 because of the site's history of exposure to low oxygen, which could be a result of organic enrichment from the discharge from the nearby Atchafalaya River.

The values of species diversity, richness, and evenness generally decreased, and the percentage of opportunistic species increased as the average BWDO decreased (Tables 1 and 5). These results were in accordance with the P-R model when using exposure to seasonal hypoxia as a stressor. The P-R model had been applied in other environments and successfully predicted the increase in opportunistic species abundance in benthic communities, but as a response to increasing total organic carbon rather than increasing incidence of seasonal hypoxia (Magni et al. 2009). In the northern Gulf of Mexico, all sites were in the intermediate stages (I and II) of succession according to the P-R model (Table 5). Despite the absence of a documented hypoxic event for at least 10 years at H7 (N. Rabalais, personal communication), there is no indication of a climax stage (III). These results suggest the existence of a spatial environmental gradient that has a broad effect within the seasonally recurring hypoxic zone in the northern Gulf of Mexico, such that even proximity to hypoxic "dead zones" has a deleterious effect on areas deemed normoxic by systematic sampling.

#### *d. Subsequent community succession*

In the aftermath of this study, the four sampled sites experienced hypoxia infrequently between 2011 and 2015 according to the midsummer annual shelf-wide cruises (E4 in 2013 and 2014; D5 and A6 in 2014; N. Rabalais, personal communication). The persisting absence of a documented hypoxic event at H7 could allow community diversity to increase, but nonhypoxic ( $>2 \text{ mg L}^{-1}$ ), yet depressed BWDO events would still eliminate the highly sensitive species from these relatively mature communities. Because of the recent exposures to hypoxia (as well as low BWDO concentrations) of the three sites to the east, we do not expect their macrobenthic communities to change significantly in terms of ecological succession.

Although it is pertinent to attempt predictions on the community succession based on incidental exposure to hypoxic events, it is not advisable to predict ecological consequences solely based on a "snapshot" from an annual survey without reassessing the macrobenthic

community itself. Furthermore, there is a great need for rigorous, continuous monitoring of hypoxia in the northern Gulf of Mexico, not only to study its impact on community succession of macrobenthos and other trophic levels, but also to understand the temporal dynamics of annually recurring hypoxia.

## 5. Conclusions

The results of this study suggest the existence of a spatial environmental gradient diminishing generally westward from the Mississippi River and created by the seasonally recurring hypoxic zone in the northern Gulf of Mexico; however, the Atchafalaya River influences the development of hypoxia at the site (E4) nearest to its discharge. The macrobenthos community will reach various successional stages according to the frequency (and unknown duration) of exposure to the oxygen-depletion event. The successional stages defined by the ecological model based on an organic enrichment gradient by Pearson and Rosenberg (1978) successfully rank the macrobenthic communities at sites H7, D5, E4, and A6 using species diversity, richness, evenness, BWDO concentration, and incidence of opportunistic species. The communities are ranked consistent with the sites' history of hypoxia exposure as defined by the BWDO concentrations averaged over the years 2000 to 2010, with H7 attaining the most advanced stage of succession and A6 lagging behind all other sites.

The macrobenthos communities were sampled at different periods during the growing seasons, yet the structure of the macrobenthos assemblages indicates a consistent impact of hypoxia. Despite the lack of continuous BWDO observations that would provide the duration of the hypoxia, the long-term response to hypoxic events by the macrobenthos assemblages is apparent. All four sites are in the intermediate stages (I and II) of succession of the P-R model. Furthermore, full recovery from hypoxic stress as defined by the P-R model was never observed at H7, even though bottom-water hypoxia has not been documented for more than 10 years.

Percentages of opportunistic species are inversely proportional to BWDO concentration, which is indicative of the long-term effect of hypoxia on the macrobenthos community. Hypoxia causes both short-term and long-term effects on the macrobenthos community on the Louisiana shelf, but more rigorous monitoring of hypoxia and the macrobenthic population is required to better understand and explain the macrobenthic community variations. More intense sampling would capture spatial and temporal variability that would allow detection of impacts of and recovery from transient hypoxia.

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