

**Macrobenthos and megabenthos responses to long-term, large-scale hypoxia on the Louisiana  
continental shelf**

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## **Abstract**

The macrobenthos and megabenthos responses to long-term, recurring hypoxia on the Louisiana continental shelf were compared at four locations with different historical (2000 – 2010) episodes of annual exposure to bottom-water hypoxia. Measurements of abundance, biomass, species diversity, and community composition of the two size classes of benthos suggested that the macrobenthic response is driven chiefly by tolerance to hypoxia, whereas the megabenthic response was affected by the ability to migrate and the availability/unavailability of macrobenthos prey at the sediment surface. The site exposed to the historically lowest average bottom-water dissolved oxygen (BWDO) concentration exhibited the lowest species diversity for macrobenthos and the highest species diversity for megabenthos, exemplifying the differential effects of hypoxia on different size classes. The high diversity and smaller average size of the megabenthos at the lowest DO site was due to high abundance of invertebrates and a preponderance of small, less vagile fishes that appeared to remain in the area after larger dominant sciaenids had presumably emigrated. The average size and the depth of habitation in the sediment of macrobenthos prey may have also influenced the abundance and biomass of megabenthos foragers.

*Keywords:* Hypoxia; Macrobenthos; Megabenthos; Assemblage composition; Cluster analysis; Diversity; Gulf of Mexico; Louisiana

## **1. Introduction**

Seasonal hypoxia has become a common problem in coastal ecosystems over the last 60 years (Lim et al., 2006; Diaz, 2008; Conley et al., 2011). Seasonal, summertime hypoxic ( $\leq 2.0 \text{ mg O}_2 \cdot \text{L}^{-1}$ ) bottom-waters recur on the northern Gulf of Mexico inner continental shelf (29-60 m water depth) on a yearly basis. Stratification of water masses and high inputs of nutrients and organic matter from the Mississippi-Atchafalaya river system promote the development of the second-largest zone of human-caused coastal hypoxia in the world (Rabalais et al., 2002b; Justić et al., 2005, Bianchi et al., 2010). The hypoxic area varies annually and has covered up to 22,000 km<sup>2</sup> of the Louisiana shelf according to yearly systematic

BWDO surveys (Rabalais et al., 2007). Periodic exposure to bottom-water hypoxia has been linked to alteration of sediment properties by benthos (Briggs et al., 2015a); declines in benthic species richness, abundance, biomass and biomass-size spectra (Diaz and Rosenberg, 1995; Rabalais et al., 2001; Baustian et al., 2009; Seitz et al., 2009; Shivarudrappa et al., 2011; Shivarudrappa, 2015; Qu et al., 2015; Rakocinski and Menke, 2016); reduction in movement and feeding activity of benthos (Tyson and Pearson, 1991; Diaz et al., 1992; Weissberger et al., 2009; Briggs et al., 2015b); movements and spatial distributions of large invertebrates and demersal fish (Pihl et al., 1991; Craig and Crowder, 2005; McAllen et al., 2009; Kodama et al., 2010; Craig et al., 2010; Craig, 2012; Craig and Bosman, 2013); and effects on the spatial distribution and landings of commercial fisheries (e.g., penaeid shrimp; Purcell et al., 2016; Zimmerman and Nance, 2001; O'Connor and Whitall, 2007).

The structure of benthic communities exposed to hypoxia may be controlled by a combination of recruitment, migration, predation, and hypoxia tolerance (Gaston, 1985; Harper et al., 1991; Powers et al., 2001; Rabalais et al., 2001; Powers et al., 2005; Rakocinski and Menke, 2016). Post-hypoxia recruitment of individuals can come from dispersal of larvae settling from the overlying normoxic water column (Powers et al., 2001) or migration from nearby areas unaffected by localized hypoxia (Pihl et al., 1991; Tyler and Targett, 2007). Some macrobenthos may enter resting states or find refuge by burrowing deeper into the sediment (Llansó, 1992; Sagasti et al., 2001; Baustian et al., 2009; Montagna and Froeschke, 2009). Other macrobenthos may leave their burrows and move to the sediment surface during hypoxic events. This vertical migration may subject the animals to increased predation by megabenthos (Diaz et al., 1992), though Montagna and Ritter (2006) found no evidence of increased predation during hypoxic episodes in Corpus Christi Bay, presumably due to avoidance of the area by hypoxia-intolerant megabenthos. Nevertheless, low-oxygen conditions can change predator-prey relationships and result in major alterations in trophic pathways and energy flow (Breitburg et al., 1997; Baird et al. 2004).

Studies of shelf and estuarine macrobenthos indicate that the most tolerant species to low BWDO concentrations are the polychaete annelids, typically members of the families Spionidae, Nereididae,

Magelonidae, Pilargiidae, Lumbrineridae and Cossuridae (Harper et al., 1981; Van Colen et al., 2010; Kodama et al., 2012; Rakocinski and Menke, 2016). Hydroid cnidarians, burrowing anemones, and some epibenthic muricid gastropods have also been observed to be tolerant to hypoxic conditions (Sagasti et al., 2001; Riedel et al., 2008). Among demersal fishes, a number of species appear to tolerate conditions at or slightly below  $2 \text{ mg O}_2 \cdot \text{L}^{-1}$  (Tallqvist et al., 1999; Eby and Crowder 2005; Switzer et al., 2009; Craig 2012). Typically, these tolerant species are the first to return in great abundance following the return of normoxic conditions at the seabed. Rapid recovery from a hypoxic event has been documented in some studies (Niermann et al., 1990; Boesch and Rabalais, 1991; Lu and Wu, 2000; Lim et al., 2006), but effects of hypoxia on benthic community structure has also been shown to linger for years (Josefson and Widbom, 1988; Harper et al., 1991; Gray et al., 2002; Van Colen, et al. 2010).

The frequency and duration of hypoxia exposure and the duration of the interim recovery between successive hypoxic events can have important effects on benthic community structure. For instance, the structure of benthic communities is different for environments experiencing frequent hypoxic episodes or severe hypoxia than those environments experiencing brief episodes of hypoxia separated by months (Rabalais et al., 2001). Those areas subjected to sustained severe or frequent hypoxia have the greatest reduction in species richness and abundance and typically become dominated by a few short-lived species (Llansó, 1992). Even short-duration hypoxia (*i.e.*, days) with long intervening periods of normoxia, however, can result in increased predation on macrobenthos that have migrated to the sediment surface by crustaceans and demersal fishes, causing changes in macrobenthos assemblages (Long and Seitz, 2008).

In general, severe hypoxia leads to mass mortality of non-mobile and weakly mobile benthos, which would reduce the transfer of secondary production to higher trophic levels. With sufficient resources (*i.e.*, organic matter and small-size-class prey) to support the production of macrobenthos following a hypoxic episode, the growth and development of macrobenthos assemblages in post-hypoxic environments becomes an important linkage between hypoxia and the size structure and species composition of megabenthos assemblages. Of interest, therefore, is not only the response of the macrobenthos community

to oxygen stress, but the response of the megabenthos community to oxygen stress and to the state of the macrobenthos community. System-level linkages between benthic production and demersal fisheries yields under the influence of eutrophication indicate a coincident fluctuation between the two standing crops (Caddy, 1993; Diaz, 2001), but the complexity of the interactions between these two size classes is not well understood. We characterized macrobenthos assemblages at four sites in 2009 with differing 10-year average dissolved oxygen concentrations (Briggs et al., 2015a,b; Shivarudrappa, 2015). Results indicated that macrobenthos assemblage structure differed among sites differing in historical bottom-water dissolved oxygen (BWDO) concentrations and these effects could not be explained by regional differences in sediment type (Briggs et al., 2015b). Our objective in this paper was to revisit the four sites one year later (i.e., summer 2010) and re-sample the macrobenthos as well as sample the megabenthos to address the following questions:

- Do macrobenthos and megabenthos assemblage structure (species and size composition) differ among stations with different histories of annual bottom-water hypoxia?
- Does vertical distribution of macrobenthos within the sediment differ among regions differing in long-term, summer dissolved oxygen concentrations and how does this potentially influence prey availability to megabenthos?
- How do the combined macrobenthos and megabenthos abundance, biomass and assemblage structure differ according to long-term, summer dissolved oxygen concentrations?

## **2. Methods**

### *2.1. Site descriptions and experimental design*

Four sites were chosen based on historical BWDO data from mid-summer (late July) shelf-wide surveys conducted by the Louisiana Universities Marine Consortium (LUMCON) (Fig. 1; Rabalais et al., 2002a; N. Rabalais, personal communication). The site designations H7, E4, D5, and A6 were derived from the nearest LUMCON survey station. Each site had a different history of dissolved  $O_2 \leq 2 \text{ mg}\cdot\text{L}^{-1}$

over the period 2000 – 2010 as indicated by different average BWDO concentrations (Briggs et al., 2015a, b) (Table 1). H7 experienced no documented hypoxia during the eleven-year period; D5 experienced hypoxia in one year (2006); E4 experienced hypoxia in three years (2002, 2006, and 2008); and A6 experienced hypoxia in five years (2000, 2002, 2007, 2008, and 2009). All sites were located along the 30-m depth contour to avoid confounding effects due to variation in water depth that would present different hydrodynamic regimes that control distribution of sedimentary organic matter, occurrence of feeding types, and larval supply, and thus, affect benthic assemblage composition (Snelgrove and Butman, 1994). Critical to our sampling design was the occurrence of a normoxic site (H7) as well as sites exposed to various documented episodes of oxygen stress (E4, D5, and A6). Based on the occurrence of hypoxia-tolerant benthic forams (Osterman, 2003), the effects of long-term annual exposure to hypoxia at our sites appeared consistent with the BWDO concentrations from the LUMCON annual survey data (Briggs et al., 2015b). Notably, the site with the lowest average BWDO concentration (A6) had the greatest proportion of hypoxia-tolerant forams and was most similar in its foram assemblage to that of the LUMCON site C6B, which experiences near-annual hypoxia (Rabalais et al., 2001). Based on measurements made in 2009 (Briggs et al., 2015b), the sediments of the four sites are predominately silty clay, though the sediment at H7 contained more gravel-size material deeper than 12 cm. Because the coarsest sediments (sand and gravel) occur in thin storm laminations or lag layers below the sediment-water interface, the surficial 10 cm of interest for all four sites can be characterized as a mud facies. From 2009 measurements the sites are also relatively similar in terms of organic matter content (Table 1). Multivariate analysis of macrobenthos abundance and biomass at the four selected sites in 2009 indicated that sediment grain size had an insignificant effect compared to BWDO concentration, sediment organic carbon, and C:N ratio (Briggs et al., 2015b).

The 2010 annual LUMCON shelf-wide survey was conducted eight days prior to our macrobenthos and megabenthos sampling. Results from CTD (Sea-bird Electronics SBE-9+ underwater unit) casts with the SBE 43 dissolved oxygen sensor (calibrated by Winkler titration from the water samples collected by

the SBE 32 rosette with 5L Niskin bottles) made at the time of our benthos collections were mostly consistent with results from this prior cruise (Shivarudrappa, 2015). The one anomaly occurred at site E4, where the bottom water sampling by N. Rabalais (personal communication) did not detect an BWDO concentration below  $2 \text{ mg}\cdot\text{L}^{-1}$ , but our measurements indicated that the bottom water at the E4 site was  $1.8 \text{ mg}\cdot\text{L}^{-1}$  (Table 1).

## 2.2. *Macrobenthos sampling*

Collections were made during 4 to 10 August 2010 aboard the R/V *Pelican*. Six  $0.25\text{-m}^2$  GOMEX box corer samples were collected at each site for characterizing the macrobenthos assemblages. One or two subcores (30-cm-long, 8.2-cm i.d.) were taken from each of the six box cores for determination of abundance and biomass of macrobenthos, resulting in a total of nine samples from each of the four sites. With one exception (see section 2.4), the subcore data were treated as independent samples. Each subcore was sectioned at 1-cm (0-2 cm depth), 2-cm (2-10 cm depth), and 5-cm (>10 cm depth) intervals before sieving in saltwater with 0.3-mm screens (Briggs et al., 2015b). The screens with the sieved material were fixed in 5% formalin that was buffered with sodium borate and stained with rose Bengal vital stain. Upon arrival at the laboratory, samples were transferred to 70% isopropanol. Samples from the individual sections of the nine subcores devoted to the macrobenthos census were sorted to major taxa, identified to species, and weighed. Preserved specimens were removed from the alcohol, patted dry with a paper towel, and weighed to the nearest microgram with a Mettler Toledo UMX ultra microbalance to determine the wet weight for consideration of their size distribution.

## 2.3. *Megabenthos sampling*

Large invertebrates and demersal fishes were collected with a 12.5-m semi-balloon shrimp trawl fabricated of 3.7-cm stretch mesh with a 1.27-cm stretch mesh heavy knotted liner in the cod end, with steel doors, weighted head rope, and floats on the top of the net opening. The net was deployed from the stern and towed for 10-20 min at a vessel speed of  $5 \text{ km}\cdot\text{h}^{-1}$ . All organisms were identified to species,

measured (nearest millimeter), and counted. Three tows were conducted at each of the four sites between 0400-0830 local time. Only the calculation of the rarefaction species diversity used pooled data from the three replicate tows (see section 2.4). The invertebrates were fixed in 5% formalin buffered with sodium borate and transferred to 70% isopropanol at the laboratory. The preserved invertebrate specimens were patted dry and weighed to the nearest milligram with an Acculab V-3mg electronic balance for consideration of their size distribution. Wet weights of invertebrates were recorded after allowing specimens to air dry for a specific time interval based on the size and species of the specimens according to an *a priori* protocol. Wet weights penaeid shrimps were estimated by using average weights of representatives of the various size classes and the numerical densities within each size class. Wet weights of demersal fishes were estimated from published length-weight relationships (FishBase, 2013). Length-weight relationships of congeners were used for species not available in FishBase.

#### 2.4. Data analysis

The macrobenthos was quantified as numbers of individuals per m<sup>2</sup>, µg wet weight, and mg wet weight per m<sup>2</sup>. The vertical distribution of macrobenthos abundance and biomass was determined at sediment depths of 0 to 2 cm, 2 to 4 cm, 4 to 8 cm, and 8 to 15 cm. Wet weight biomass was converted to ash-free dry weight (AFDW) biomass using the conversion factors from Ricciardi and Bourget (1998) and the protocol followed by Greenstreet et al. (2007) to estimate the average individual size of the surficial macrobenthos. The megabenthos was quantified as numbers of individuals and kg wet weight biomass captured per hour of bottom trawl time (catch rate). Area swept by the trawls was determined from the net width and the distance between the beginning and ending location of the trawls. Abundance and biomass of macrobenthos and megabenthos were normalized to numbers of individuals per km<sup>2</sup> and grams wet weight per km<sup>2</sup> to make spatial comparisons between the two size classes of benthos.

Abundance and wet-weight biomass of macrobenthos and megabenthos were tested for differences among sites with the Kruskal-Wallis non-parametric ANOVA. Tukey's pair-wise post-hoc comparisons



between sites were performed to control the familywise error rate. The biomass was divided by the abundance for each species of macrobenthos and megabenthos to estimate the average size of individuals. Macrobenthos and megabenthos individuals were grouped according to taxa to determine their respective size distributions at the four sites.

Hurlbert's species diversity by rarefaction (expected species:  $E[S_n]$ ) was calculated using the routine available in the PAST software package (Hammer et al., 2001).  $E(S_n)$  diversity was based on the numerical density of the species in the replicates at each of the four sites, but plotted as the pooled assemblages for each site for clarity. Heterogeneity  $\chi^2$  analysis (Jumars, 1975) performed on replicate and pooled samples indicated that the curves depicting the pooled samples were not biased high. The jackknife estimator of Heltshe and Forrester (1983) calculated pseudo-values for the mean and variance of  $E[S_n]$  diversity measures, which were used to calculate t-statistics for Welch's t-test. Comparisons of rarefaction diversity among the sites used the minimum  $n$  value from all samples at all sites. The Bonferroni method was used to correct the critical  $\alpha$ -value to 0.008 for multiple comparison testing.

Only species that occurred four times or more at a site (regardless of frequency within hauls or cores) were included in the multivariate analysis. Abundance and biomass per unit area and catch rates were log  $(x+1)$  transformed to achieve balance among contributions from abundant and rare species. Dissimilarity matrices composed of Bray-Curtis coefficients were clustered based on species composition and abundance (or biomass) using the group-average linkage hierarchical agglomerative clustering technique and presented in the form of dendrograms (Boesch, 1977). Individual groups within assemblages were identified by visual inspection of the dendrograms and separated at a dissimilarity level of at most 60% (Craig and Bosman, 2013). Canonical Correspondence Analysis (CCA) was used to analyze the relationship between the megabenthos site by species matrix and two variables, BWDO concentration and macrobenthos prey (abundance and wet-weight biomass). Due to the transient nature of annual hypoxia and evidence of long-term (cumulative) effects on benthic communities (Briggs et al., 2015a, b), we used

the averages of the BWDO concentration values measured at each of the four sites by the LUMCON annual shelf-wide survey from 2000 to 2010 (Table 1, N. Rabalais, personal communication).

### 3. Results

#### 3.1. Abundance, biomass and diversity of macrobenthos

The macrobenthos assemblages at sites H7 and A6 exhibited the highest abundance (11,591.5 and 17,189.6 individuals·m<sup>-2</sup>, respectively) and were significantly greater than the assemblages at sites D5 (9,801.8) and E4 (10,551.0) (Fig.2A; H7: Tukey's Q=5.64,  $p < 0.003$ ; Q=5.23,  $p < 0.005$ ; A6: Q=6.19,  $p < 0.0008$ ; Q=5.78,  $p < 0.002$ ). The macrobenthos assemblage at D5 exhibited the highest wet weight biomass (6,710 mg·m<sup>-2</sup>) of the four sites, however, which was significantly different than the biomass of the macrobenthos assemblages at sites H7, E4, or A6 (2,300, 1,840, and 880 mg·m<sup>-2</sup>, respectively) (Fig.2B; H7: Tukey's Q = 4.89,  $p < 0.008$ ; E4: Q =5.41,  $p < 0.004$ ; A6: Q =6.47,  $p < 0.0006$ ). There were no significant differences in biomass among the macrobenthos assemblages at sites H7, E4, or A6.

The expected species rarefaction curve ( $E[S_{21}]$ ) for the assemblage at A6 indicated significantly lower diversity compared to the other three sites (Fig.3; H7:  $t = 50.6$ ,  $p \ll 0.0001$ ; D5:  $t = 46.2$ ,  $p \ll 0.0001$ ; E4:  $t = 28.8$ ,  $p \ll 0.0001$ ). The assemblages at D5 were significantly more diverse than the assemblages at E4 ( $t = 6.42$ ,  $p < 0.0002$ ) and H7 ( $t = 9.76$ ,  $p \ll 0.0001$ ), though the magnitude of these differences were small compared to those at A6 (Fig. 3).

Between 53% and 84% of the macrobenthos individuals at the four sites were found in the top two centimeters of the sediment (Fig. 4A). At sites H7 and A6 macrobenthos wet weight biomass appeared to be similarly concentrated in the top two centimeters (52% and 62%, respectively), though biomass was more evenly distributed with sediment depth than was numbers (Fig. 4B). For example, macrobenthos biomass reached maxima below four centimeters sediment depth at two sites (D5 and E4). Sequestering of larger fauna deeper in the sediment appears to be occurring at D5, where 47% of macrobenthos by abundance and 85% by wet weight biomass (88% by AFDW) reside below the uppermost 2 cm of the

seabed. However, the average sizes of individual macrobenthos, (based on AFDW) indicated that the largest macrobenthos occurred at the sediment surface (0-2 cm) at sites H7 (8.5  $\mu\text{g}$  AFDW) and D5 (16.7  $\mu\text{g}$  AFDW), which had average BWDO concentrations between 5.4 and 3.4  $\text{mg}\cdot\text{L}^{-1}$  (Table 2).

### 3.2. Abundance, biomass and diversity of megabenthos

The megabenthos assemblage showed similar patterns in numbers and biomass across the four sites (Fig. 2). The easternmost site (A6) showed about four-fold lower catch rates in both numbers ( $740.0\cdot\text{h}^{-1}$ ) and biomass ( $3.7\text{ kg}\cdot\text{h}^{-1}$ ) compared to the other three sites, but differences were not always statistically significant due to the high variability among trawls. For example, the abundance catch rate of the megabenthos assemblage at E4 ( $5,947.1\cdot\text{h}^{-1}$ ) was about seven-fold higher than the lowest catch rate at A6, but was not statistically significant (Tukey's  $Q = 3.93$ ,  $p = 0.091$ ). The lowest wet weight biomass catch rate at A6 ( $3.7\text{ kg}\cdot\text{h}^{-1}$ ) was only significantly different from the assemblage at E4 ( $130.1\text{ kg}\cdot\text{h}^{-1}$ ; Tukey's  $Q = 5.02$ ,  $p = 0.031$ ), which had the highest measured biomass catch (Fig. 2B).

The abundance and biomass of macrobenthos and megabenthos normalized to quantities collected per area show only subtle differences and no significant statistical differences from those depicted in Fig. 2. Numerical density of megabenthos was lowest at A6, where the numerical density of macrobenthos was highest (Fig. 2A). In fact, median macrobenthos abundance was negatively correlated with median megabenthos abundance ( $r = -0.95$ ). Median macrobenthos biomass, however, was not correlated ( $r = 0.25$ ) with median megabenthos biomass (Fig. 2B).

In contrast to the macrobenthos, the expected species rarefaction curves for the megabenthos assemblage at A6 was higher than the curves from the other assemblages, indicating higher megabenthos diversity at the site with lowest macrobenthos diversity (Fig. 5). There were 10 species that occurred only once and five species that occurred twice in the pooled megabenthos assemblages, producing a pronounced "tail" in the A6 species distribution, which resulted in a rarefaction curve that did not approach an asymptote (Fig. 5). The  $E[S_{339}]$  rarefaction diversity at A6 was much greater than those at the

other three sites (H7:  $t=14.5$ ,  $p<0.0002$ ; D5:  $t=14.9$ ,  $p<0.0002$ ; E4:  $t=13.2$ ,  $p<0.0002$ ) and diversity at E4 was slightly greater than at H7 ( $t=5.44$ ,  $p<.006$ ) or D5 ( $t=5.98$ ,  $p<.004$ ). However, rarefaction diversity was not significantly different between sites H7 and D5.

Average individual size of megabenthos was estimated separately for demersal fishes, crustaceans, and lesser-occurring taxa (Table 3). Site A6 had the smallest megabenthos, with an average individual wet weight that was 53–77% of that at the other sites. For crustaceans, average wet weight was two-fold lower at the easternmost site (A6) compared to the other sites. Site A6 also had the smallest average size demersal fishes, whereas the intermediate site (D5) had the largest demersal fishes. Overall, site D5 had the largest average individual size of megabenthos.

### 3.3. Assemblage composition

#### 3.3.1. Macrobenthos

At the westernmost site, which had the highest average BWDO (H7), assemblages were comprised mostly of the capitellid polychaete *Mediomastus californiensis*, but with significant contributions from bivalves, other polychaetes, and an isaeid amphipod (Fig. 6A). However, in terms of wet weight biomass the westernmost assemblage was dominated by the maldanid polychaete *Clymenella torquata*, the buccinid gastropod *Gemophos tinctus*, and the bivalve *A. versicolor*. At the easternmost site, which had the lowest average BWDO (A6), macrobenthos assemblages were numerically dominated by the small cossurid polychaete *Cossura soyeri* and the protobranch bivalve *N. acuta* (Fig. 6D). The sites that had an average BWDO concentration between 3.4 and 2.6  $\text{mg}\cdot\text{L}^{-1}$  exhibited different numerically dominant species (bivalves at D5 and polychaetes at E4) (Fig. 6B, 6C). In terms of wet weight biomass, the assemblage at D5 was dominated by the bivalves and large polychaetes. In terms of wet weight biomass, the assemblage at E4 was also dominated by large polychaetes.

#### 3.3.2. Megabenthos

At the westernmost site with the highest average BWDO concentration (H7) the megabenthos assemblage was dominated by Atlantic croaker (*Micropogonius undulatus*), Atlantic cutlassfish (*Trichiurus lepturus*), and brown shrimp (*Farfantepenaeus aztecus*) (Fig. 7A). In terms of wet weight biomass per hour hauled, *T. lepturus*, *M. undulatus*, and *F. aztecus* accounted for 96% of the catch at H7. In contrast, the site with the lowest average BWDO concentration (A6) was numerically dominated by the brown shrimp *F. aztecus* and the antenna codlet *Bregmaceros* sp. (Fig. 7D). However, in terms of wet weight biomass the megabenthos assemblage at A6 was dominated by *F. aztecus*, *T. lepturus*, and the white shrimp *Litopenaeus setiferus*. The megabenthos assemblage at D5, which had relatively low diversity, was dominated by *M. undulatus* and *F. aztecus* in terms of both abundance and wet weight biomass (Fig. 7B). The megabenthos assemblage at E4 was comprised mostly of *M. undulatus* in terms of both abundance and biomass (Fig. 7C).

#### 3.4. Macrobenthos assemblage structure

Cluster analysis identified a number of groups within the macrobenthos assemblages. Clustering macrobenthos by abundance identified three major groups, 16 minor groups, and numerous outlier groups clustered at dissimilarity values greater than 60% (Fig. 8). The two most speciose groups were abundance group 1 with a spionid/capitellid polychaete-bivalve mollusk clade comprised of the dominant and cosmopolitan macrobenthos species, and abundance group 3 with an amphipod-mollusk clade comprised of macrobenthos species that were only dominant at site H7. The macrobenthos species that were dominant at the sites that averaged between 5.4 and 2.6 mg·L<sup>-1</sup> (H7, D5, and E4) occurred in abundance group 2. Species common at the low BWDO site (A6) were comprised mostly of species from abundance group 1.

#### 3.5. Megabenthos assemblage structure

Clustering megabenthos by abundance identified five major groups, eight minor groups, and several outlier groups at dissimilarity values greater than 60% (Fig. 9). There were two speciose major groups

clustered by abundance: group 3 with 5 species comprising a sciaenid-sparid fish clade that were found primarily at the sites with BWDO from 5.4 to 2.6 mg·L<sup>-1</sup> (H7, D5, and E4), and group 5 with 5 species comprising a fish-echinoderm clade that were found primarily at site E4. The dominant and cosmopolitan megabenthos species were clustered in assemblage group 4. The megabenthos that occurred at the easternmost site with the lowest BWDO (A6) comprised the species in assemblage group 1.

### 3.6. Relationships among megabenthos, macrobenthos standing crop, and BWDO

Taking the 2010 megabenthos species composition into account with the variables of the 11-year average BWDO concentration and 2010 standing crop of potential prey (macrobenthos abundance and biomass) in a canonical correspondence analysis (CCA) allows some discrimination among the megabenthos assemblages at the four sites according to environmental stress and trophic considerations (Figs. 10A, B).

In the case of megabenthos abundance distribution (Fig. 10A), the H7, D5, and E4 sites are arrayed toward the higher values of BWDO and in the opposite direction of site A6, which is depicted to the lower right quadrant. There is not a strong effect from BWDO, but certain species (the codlet *Bregmaceros* sp. and the brown shrimp *F. aztecus*) seem to be aligned with low BWDO concentrations. Macrobenthos abundance, a proxy for prey availability, affects the populations of the tonguefish (*S. plagiusa*) and the cutlass fish (*T. lepturus*) that occur in large numbers, especially at site H7. The macrobenthos biomass, in terms of wet weight prey, had a small effect on sites E4 and D5 and abundance of the croaker *M. undulatus*. The megabenthos biomass ordination follows that of the abundance ordination, with low BWDO concentration being the province of site A6, along with biomass of the catfish *Arius felis*, the brown shrimp *F. aztecus*, and most of the megabenthos (Fig. 10B). The macrobenthos prey abundance and prey biomass eigenvectors respectively indicate the separation between sites H7 and A6 (high prey abundance and low prey biomass) and sites E4 and D5 (low prey abundance and high prey biomass). Biomass of Spot (*Leiostomus xanthurus*) seems aligned with high

numbers of macrobenthos prey and biomass of croaker (*M. undulatus*) seems aligned with high biomass of macrobenthos prey.

#### **4. Discussion**

##### *4.1. Macrobenthos response to hypoxia*

From the macrobenthos abundance and diversity data collected in 2010, the easternmost site nearest the Mississippi River delta (A6) showed the strongest effects of low BWDO, compared to the westernmost site that experienced the highest average BWDO concentration (H7) and the two intermediate sites (D5 and E4). Although there was considerable variability at each site, the macrobenthos community within each site was relatively similar in abundance and biomass over two consecutive years (Shivarudrappa 2015; Briggs et al. 2015b). Diversity indices at the same sites in the previous year generally follow the same trends apparent in 2010, with site A6 having the fewest number of macrobenthos species and the lowest diversity of the four sites in the spring and late summer of 2009. Similar to the 2010 results presented here, the westernmost site (H7) was the most speciose and diverse of the sites in 2009, whereas sites D5 and E4 had similar diversity that was intermediate to that at sites H7 and A6 (Shivarudrappa, 2015).

An effect of long-term hypoxia on the structure of macrobenthos assemblages is supported by differences in abundance, diversity, and assemblage composition among sites with different BWDO conditions. Macrobenthos were structured primarily as three groups: a cosmopolitan group dominated by a capitellid polychaete (*M. californiensis*) with contributions from several mostly opportunist polychaetes, a diverse group of species found nearly exclusively at the westernmost site (H7), and species common to both intermediate sites (D5 and E4). Although we cannot rule out other spatially structured environmental factors, the high dissimilarity among the groups (> 60%) is consistent with the hypothesis that differential exposure to hypoxia over the long term has resulted in different macrobenthos assemblages across the Louisiana shelf.

Based on the 2010 macrobenthos data, site A6, which experiences recurring, severe, and perhaps chronic hypoxia, was more affected in terms of abundance, biomass, and diversity than the other three sites. The large numbers of small individuals and low diversity of the assemblage at A6 was due primarily to the numerical dominance of the small, subsurface deposit-feeding polychaete *C. soyeri* and secondarily to the surface deposit-feeding protobranch bivalve *Nuculana acuta*. Early colonizers of post-hypoxic environments such as the spionid polychaete *Paraprionospio pinnata*, the pilargid polychaete *Sigambra tentaculata*, and the semelid bivalve *Abra aequalis* (Boesch and Rabalais, 1991; Powers et al., 2001; Lim et al., 2006) were present at all four sites. Despite the ubiquity of these opportunistic macrobenthos, differences in their relative abundance in 2010 may represent different successional stages of their respective assemblage's response to hypoxia. In the region of the shelf where low BWDO typically occurs (A6, E4, and D5), these three species were present at high aggregate densities ( $1144.6 - 1935.4 \cdot \text{m}^{-2}$ , Briggs et al., 2015a), whereas densities at the westernmost site (H7), which did not experience hypoxia over the period of record, were much lower ( $645.1 \cdot \text{m}^{-2}$ ), perhaps due to interspecific competitive interactions or predation effects that were more intense compared to hypoxic areas. *P. pinnata* were among the larger macrobenthos at all sites, suggesting that emigration of adult individuals, perhaps in addition to larval recruitment, may play a role in seasonal recolonization, as has been shown for spionid polychaetes in other regions such as Chinhae Bay, South Korea (Lim et al., 2006).

#### 4.2. Megabenthos response to hypoxia

The high diversity observed for megabenthos and the low diversity observed for macrobenthos at the most severe hypoxia site (A6), suggests these two provincial size assemblages respond differently to hypoxia exposure. Whereas the macrobenthos response appears to be largely based on the mortality of hypoxia-sensitive species and the thriving of hypoxia-tolerant species, the low catch rates of megabenthos at A6 are consistent with avoidance of severe hypoxia by mobile fishes and crustaceans (Zhang et al. 2009; Craig 2012). The larger sciaenids (e.g., croaker, spot) and crustaceans (e.g., brown shrimp), which were dominant species at the other three sites, were not common at A6, probably due to emigration from



the area. Westward emigration of mobile fishes in the Gulf has been reported for other species, supporting this possibility (Switzer et al. 2009; Langseth et al. 2014). As a result, a number of less common, less vagile, and smaller species (e.g., gobies, sea robins, pancake batfish, and flatfishes) remained. The one exception was sand seatrout *C. arenarius* which was common at site A6 but is also highly tolerant of hypoxia (avoidance threshold = 1.06 mg L<sup>-1</sup>; Craig 2012). The oxygen tolerance of the other common finfish at site A6, the codlet *Bregmaceros* is unknown, though based on its abundance we would predict a relatively high tolerance to low DO (Gallo and Levin, 2016). Emigration of the numerically dominant species from site A6 combined with the presence of several, less common species resulted in higher megabenthos diversity in this low-BWDO habitat compared to the other sites.

In addition to lower abundance, megabenthos at A6 had a smaller average body size (i.e., mean individual wet weight) compared with that at the other sites. Smaller average body size at A6 was due to a comparatively higher numerical density of invertebrates as well as a preponderance of small fishes. Hence, in addition to emigration of larger more vagile species, depressed feeding and growth rates in response to annual hypoxia (Baden et al., 1990; Petersen and Pihl, 1995; Breitburg, 2002) may also have contributed to the lower biomass at A6, which historically experienced the lowest average BWDO concentrations.

The unusually high catch rates of megabenthos at E4 compared to the other sites (Figs. 2) were anomalous and perhaps the result of an aggregating effect of recently developed hypoxia. The BWDO at the time the megabenthos was sampled was 1.8 mg L<sup>-1</sup> but the same site was 3.49 mg·L<sup>-1</sup> during sampling only eight days earlier (N. Rabalais, pers. comm.). In this region of the Gulf at typical summertime bottom water temperatures eight days is enough time for oxygen to decline from normoxic to hypoxic levels, supporting this possibility. Previous studies in the Gulf have also shown that organisms evading hypoxia aggregate at relatively short distances (< 5 km) from the edge of the hypoxic zone (Craig and Crowder, 2005; Craig, 2012; Zhang et al. 2009). Because megabenthos assemblages were only sampled in August 2010, it is unclear how often aggregating events occur over the course of annual hypoxia or how

long aggregations persist. That D5 and E4, sites having average BWDO concentrations between 3.4 and 2.6 mg·L<sup>-1</sup>, have similar values of abundance, biomass, and diversity is consistent with rapid recolonization of hypoxia exposed areas upon dissipation of hypoxia (Pihl et al., 1991; Tyler and Targett, 2007; Kodama et al., 2010). However, a prior study indicated several flatfish species moved westward away from severe hypoxia and had not recolonized previously hypoxic sites by the following fall (Switzer et al. 2009). This suggests the behavioral response of highly mobile species to hypoxia may be a combination of fairly large-scale (10s to 100s km) emigration away from hypoxic areas and small-scale (5-10 km) aggregation in response to acute hypoxic events. This combination of behavioral responses may be the primary factor governing spatial patterns in the structure of the megabenthos community (Baustian et al., 2009; Kodama et al., 2010; Craig and Bosman, 2013).

#### 4.3. *Macrobenthos-megabenthos trophic relationships*

The dominant trawl species were croaker (*M. undulatus*), brown shrimp (*F. aztecus*), big eye sea robin (*Prionotus longispinosus*), and spot (*L. xanthurus*), all bottom feeders that forage on polychaete and crustacean macrobenthos (Jones, 1973; Ross, 1989; Pihl et al., 1992; Baustian et al., 2009). Furthermore, polychaetes and mollusks, the two most abundant macrobenthos taxa at the four sites, have been identified as macrobenthos prey for demersal predators (Tallqvist et al., 1999; Powers et al., 2005; Baustian et al., 2009), and these two taxa congregate in the upper two cm of the sediment at each of the four sites (Shivarudrappa 2015). If the megabenthos were exhibiting a numerical response to the abundance of macrobenthos prey, we would expect the highest megabenthos densities at site A6, given the highest density of macrobenthos occurred there; instead the lowest megabenthos density occurred where the macrobenthos was most abundant. This suggests that both the size distribution of the potential macrobenthos prey and their vertical distribution within the sediment affect the abundance of the megabenthos. Despite the high numerical density of macrobenthos at site A6, biomass density was the lowest of the four sites suggesting the low abundance of megabenthos at A6 may be related to the lack of appropriately sized macrobenthos prey. Data previously published indicated the macrobenthos at A6 had

the smallest average size, with the small-sized polychaete *C. soyeri* the dominant species (Briggs et al., 2015a). Foraging for numerous small prey in the upper layer of the sediment may be energetically inefficient due to high search and handling costs which would influence trophic transfer from macrobenthos prey to the megabenthos, perhaps contributing to lower production in the larger megabenthos size classes. Alternatively, larger, more energetically profitable prey were found deeper in the sediment and may be unavailable or require large energetic expenditures (e.g., search and handling costs) in order to consume. For example, the larger macrobenthos prey at site D5 may be less available to megabenthos predators due to their occurrence at depth in the sediment, perhaps contributing to the lack of positive correlation between the catch rates of megabenthos and the abundance and biomass of macrobenthos. This apparent size selection by megabenthos predators of the macrobenthos prey is a possible explanation for the opposing macrobenthos abundance and biomass eigenvectors of the canonical correspondence analysis of megabenthos abundance (Figs. 10A, B). Evaluating these possibilities requires detailed knowledge of the predation process, in particular the search time, handling costs, and energetic returns of different species and sizes of macrobenthos prey and megabenthos predators across a range of sediment depths and BWDO concentrations.

Another potential explanation for the lack of correlation between standing crops of macrobenthos and megabenthos is differences in hypoxia tolerance. Macrobenthos are typically more tolerant to hypoxia than larger megabenthos, and hypoxia-tolerant macrobenthos can reach high abundance if low-BWDO environments function as a refuge from predation (Altieri, 2008). The compositions of the assemblages at hypoxia-exposed sites are a function of mortality of fauna sensitive to hypoxia, survival of fauna tolerant of hypoxia, and successional change among opportunist and equilibrium fauna (Shivarudrappa, 2015). Because the macrobenthos was dominated by relatively small species with low vagility, the alteration of macrobenthos assemblages toward a diverse equilibrium fauna appears primarily a function of larval recruitment, perhaps with a minor effect from migration of larger mobile benthos. Other potential explanations for the lack of correlation between standing crops of macrobenthos and megabenthos include

the exclusion of megabenthos from sites due to other environmental factors (e.g., temperature, salinity), insufficient time for re-colonization, and/or differences in the spatial scale at which megabenthos respond to the distribution and abundance of prey resources.

The severity and areal extent of hypoxia varied extensively over the period 2000 – 2010 (5,000 to 22,000 km<sup>2</sup>; Turner et al., 2012). Moreover, the seasonal dynamics of the annually recurring hypoxia (small expanses or a series of short-lived events vs. an extensive, persistent event) are also uncertain. Given the limited information on the spatial and temporal dynamics of hypoxia in this region and the variability in macrobenthic abundance and biomass it is difficult to unequivocally determine the causal relationship between long-term, low BWDO and macrobenthos community structure. Higher resolution sampling of BWDO and the macrofaunal community in both space and time, and perhaps an experimental approach would further elucidate the causal linkages between recurring hypoxia and the macrobenthic community on the Louisiana shelf.

## **5. Conclusions**

Several generalizations emerge from the macrobenthos and megabenthos sampling on the Louisiana shelf in August 2010:

(1) At the site that experienced the lowest average BWDO concentration (A6), the macrobenthos assemblage exhibited the lowest diversity, whereas the megabenthos assemblage exhibited the highest diversity, indicating a differential response to annually recurring hypoxia between these two size groups.

(2) Lack of correlation between standing stocks of the two size groups suggests that the respective assemblages are structured by different demographic responses to oxygen stress: the macrobenthos response is driven by mortality and successional change, whereas the megabenthos response is primarily driven by emigration and immigration.

(3) High biomass of megabenthos at the only site experiencing hypoxia at the time of sampling (E4) are probably the result of mobile megabenthos aggregating along a boundary of recently developed low-oxygen bottom water. The macrobenthos were relatively unaffected by this hypoxic event because the macrobenthos

are less mobile and more hypoxia tolerant than the megabenthos and thus their dynamics are representative of the long-term (years) and seasonal change in BWDO conditions.

(4) There was large variation among sites in the distribution of macrobenthos with depth in the sediment that may influence foraging efficiency of megabenthos predators and contribute to the lack of correlation between the abundance and diversity of these two groups.

(5) This and previous work demonstrates that the cumulative effects of annually recurring hypoxia have a dominant influence over ecological succession of the macrobenthos community on the Louisiana shelf that may supersede the effects of short-term hypoxic episodes (Briggs et al., 2015a, b), whereas the reverse may be the case for megabenthos. The dynamic nature of the formation and dissipation of annual hypoxia and the complexity of the macrobenthos and megabenthos response to oxygen stress make linking the effects of hypoxia on these two interacting size groups difficult. A highly synoptic approach to monitoring BWDO, macrobenthos, and megabenthos combined with laboratory and field efforts on trophic interactions under different BWDO conditions can provide a more rigorous understanding of these interactions.

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## Table captions

**Table 1** Measurements for the top 2 cm of sediment (type, organic C, C-N ratio) and the bottom-water oxygen (BWDO) concentration (measured during the August 2010 cruise and the average over July 2000 – 2010 collected by LUMCON, with 1 standard deviation [N. Rabalais, personal communication]).

**Table 2** Average sizes ( $\mu\text{g AFDW}$ ) of potential macrobenthos prey in the 0-2 cm layer of the sediment sampled in the Gulf of Mexico in August 2010 at the four sites with differing hypoxia exposure. BWDO = Bottom-Water Dissolved Oxygen ( $\text{mg L}^{-1}$ ).

**Table 3** Average sizes (g wet wt.) of megabenthos sampled in the Gulf of Mexico in August 2010 at the four sites with differing hypoxia exposure. Other megabenthos consist of bivalves, echiurans, and echinoderms. BWDO = Bottom-Water Dissolved Oxygen ( $\text{mg L}^{-1}$ ).

## Figure captions

Figure 1 Locations of the four sites on the Louisiana shelf. [2-column]

Figure 2 (A) Median abundance of macrobenthos ( $\text{individuals}\cdot\text{m}^{-2}$ ) and megabenthos ( $\text{individuals}\cdot\text{h}^{-1}$ ) assemblages and (B) median biomass of macrobenthos ( $\text{mg}\cdot\text{m}^{-2}$ ) and megabenthos ( $\text{kg}\cdot\text{h}^{-1}$ ) assemblages collected from sites H7, D5, E4, and A6 in August 2010. Subscripted letters indicate significant differences based on Tukey's pair-wise comparisons. The 'C' subscript refers to macrobenthos; the 'G' subscript refers to megabenthos. [1-column]

Figure 3 Rarefaction curves of expected species based on pooled macrobenthos abundance from nine cores collected from sites H7, D5, E4, and A6 in August 2010. Pooled species curves are biased toward higher  $E(S_n)$  values but depict proper ranking in diversity values calculated for individual samples. [1-column]

Figure 4 Depth distributions of (A) macrobenthos individuals and (B) wet weight biomass collected from nine cores at sites H7, E4, D5, and A6. [1-column]

Figure 5 Rarefaction curves of expected species based on pooled megabenthos abundance catch rates from three trawls collected from sites H7, D5, E4, and A6 in August 2010. Pooled species curves are biased toward higher  $E(S_n)$  values but depict proper ranking in diversity values calculated for individual samples. [1-column]

Figure 6 Dominant species of macrobenthos by abundance (black bars) and by wet weight biomass (shaded bars). [1.5-column]

Figure 7 Dominant species of megabenthos by abundance (black bars) and by wet weight biomass (shaded bars). [1.5-column]

Figure 8 Dendrogram of species grouped by Bray-Curtis dissimilarity values based on macrobenthos abundance at the four sites. [1.5-column]

Figure 9 Dendrogram of species grouped by Bray-Curtis dissimilarity values based on megabenthos abundance catch rates at the four sites. [1.5-column]

Figure 10 Canonical Correspondence Analysis (CCA) ordination of megabenthos species (filled circles) abundance (A) and wet weight biomass (B) from the four sites (open triangles) with environmental variables (eigenvectors) of BWDO concentration, macrobenthos prey abundance (Macro), and macrobenthos prey wet weight biomass ( $\text{mg Macro}$ ). Dominant megabenthos species are identified. [2-column]

**Table 1** Measurements for the top 2 cm of sediment (type, organic C, C-N ratio) and the bottom-water oxygen (BWDO) concentration (measured during the August 2010 cruise and the average over July 2000 – 2010 collected by LUMCON, with 1 standard deviation [N. Rabalais, personal communication]).

Site	Sediment type	Sediment organic C (%)	Sediment C-N ratio	Bottom-water O <sub>2</sub> concentration	
				2010 (mg L <sup>-1</sup> )	2000 – 2010 (mg L <sup>-1</sup> )
H7	clayey sand	0.035	9.3	5.8	5.4 ± 1.2
D5	sand-silt-clay	0.095	10.6	3.1	3.4 ± 1.1
E4	sand-silt-clay	0.063	9.2	1.8	2.6 ± 1.6
A6	clay	0.109	9.2	3.9	2.1 ± 1.4

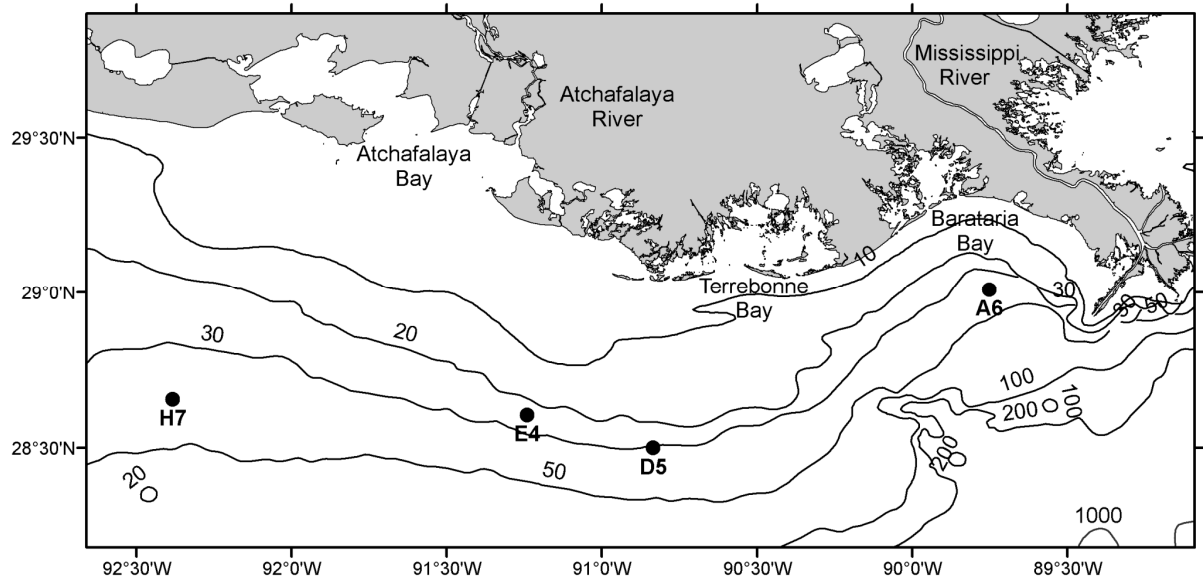
**Table 2** Average sizes ( $\mu\text{g}$  AFDW) of potential macrobenthos prey in the 0-2 cm layer of the sediment sampled in the Gulf of Mexico in August 2010 at the four sites with differing hypoxia exposure. BWDO = Bottom-Water Dissolved Oxygen ( $\text{mg L}^{-1}$ ).

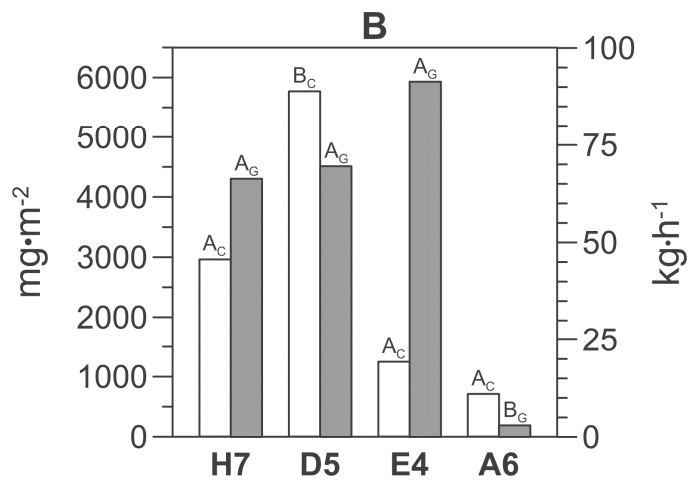
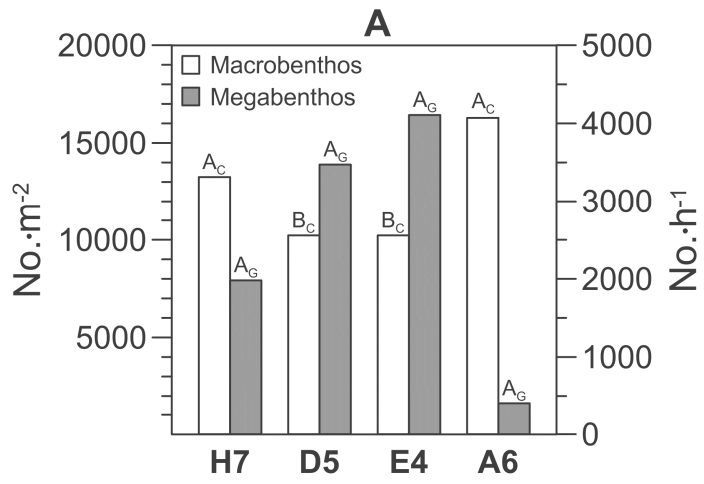
	<b>H7</b>	<b>D5</b>	<b>E4</b>	<b>A6</b>
BWDO Average (2001–2010)	<b>5.4</b>	<b>3.3</b>	<b>2.6</b>	<b>2.2</b>
Polychaetes	6.3	10.3	4.0	1.4
Mollusks	14.3	28.7	12.7	5.3
Crustaceans	3.1	30.3	3.2	0.5
Ophiuroids	0.6	0.9	0.7	—
Nemertean	19.2	9.7	3.5	6.2
Weighted Average	8.5	16.7	5.4	2.1

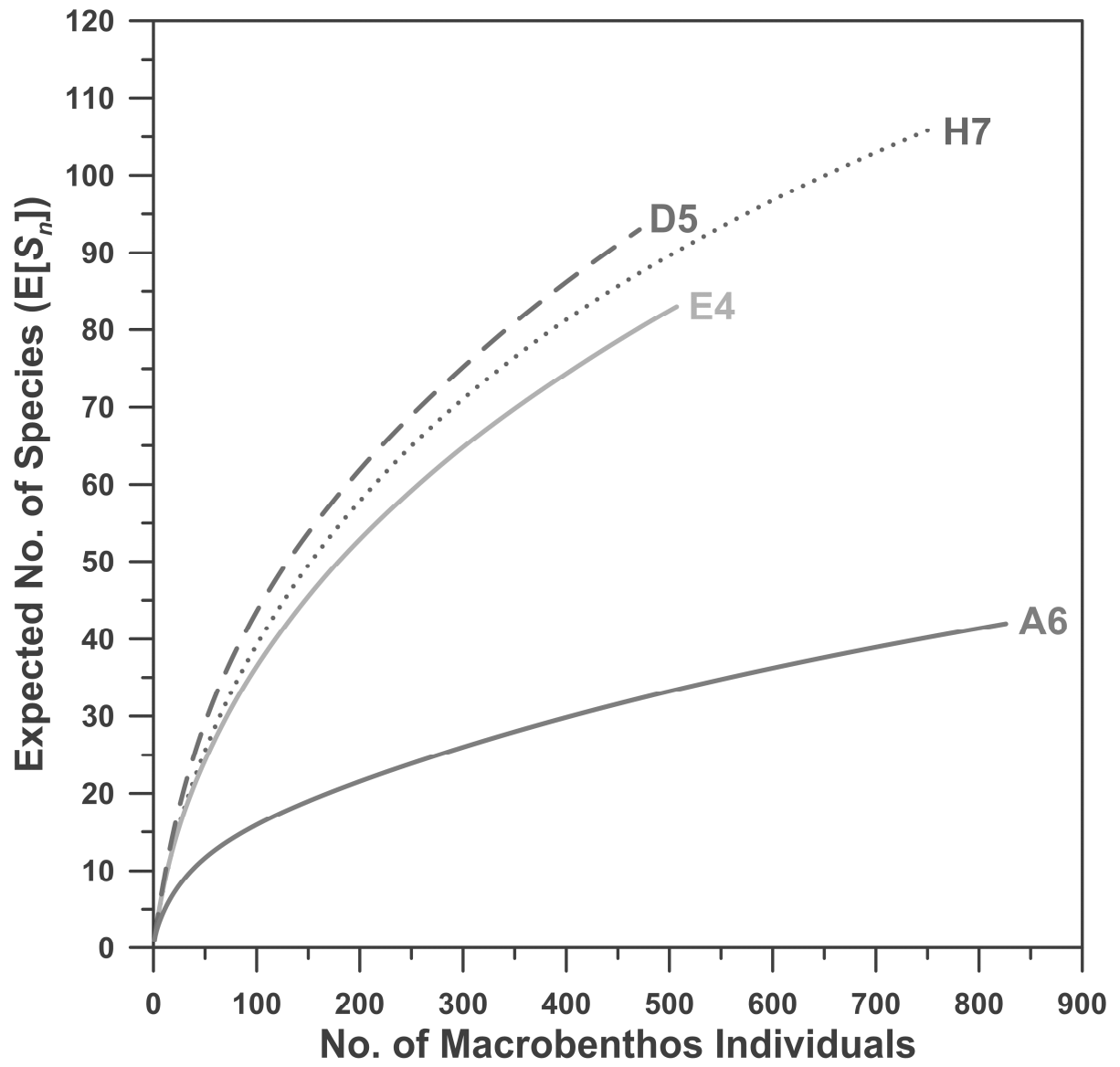


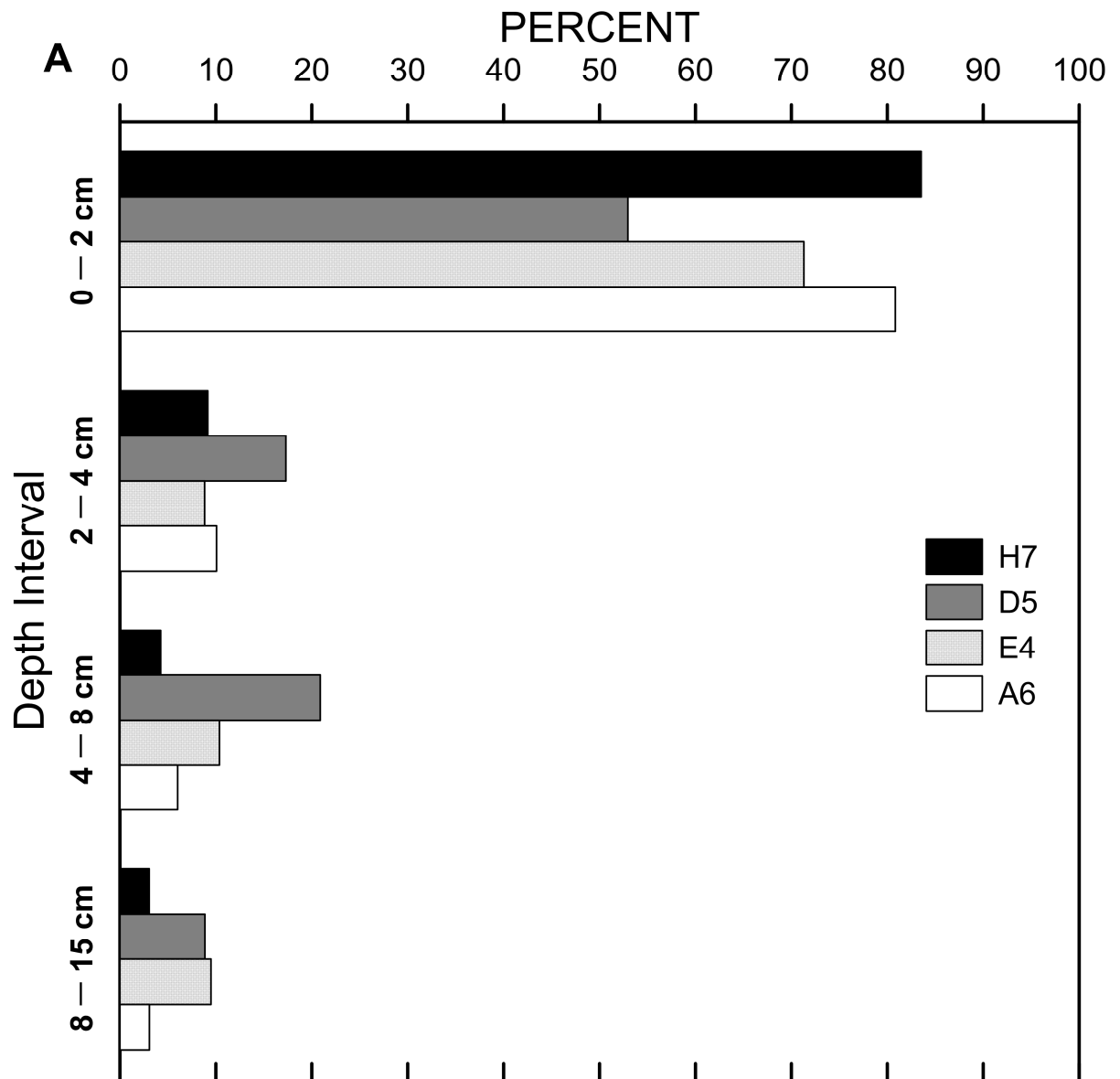
**Table 3** Average sizes (g wet wt.) of megabenthos sampled in the Gulf of Mexico in August 2010 at the four sites with differing hypoxia exposure. Other megabenthos consist of bivalves, echiurans, and echinoderms. BWDO = Bottom-Water Dissolved Oxygen ( $\text{mg L}^{-1}$ ).

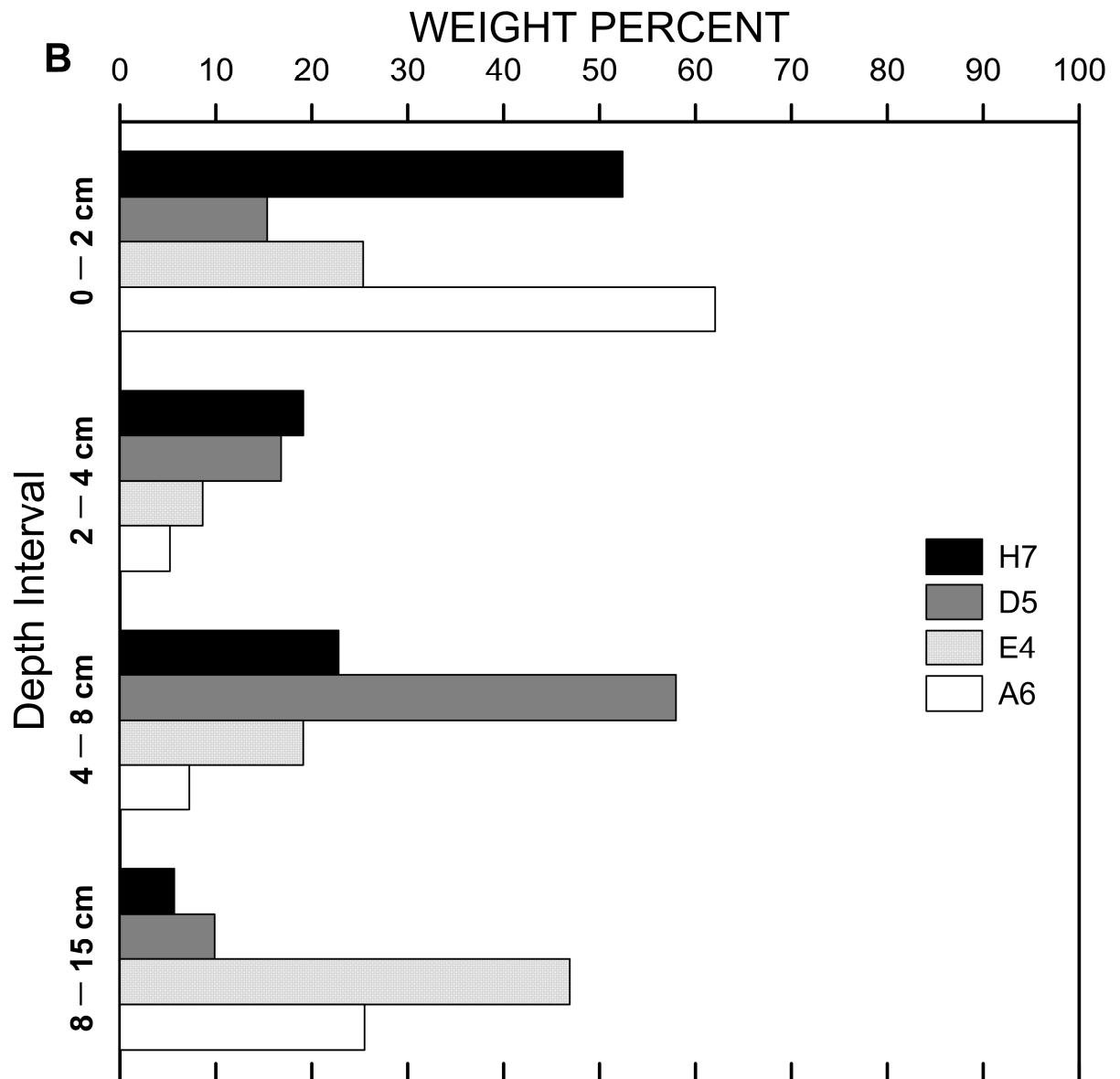
	<b>H7</b>	<b>D5</b>	<b>E4</b>	<b>A6</b>
BWDO Average (2001-2010)	<b>5.4</b>	<b>3.3</b>	<b>2.6</b>	<b>2.2</b>
Fishes	34.81	49.53	29.72	22.85
Crustaceans	9.12	9.82	9.13	4.76
Other	1.23	–	2.26	3.84

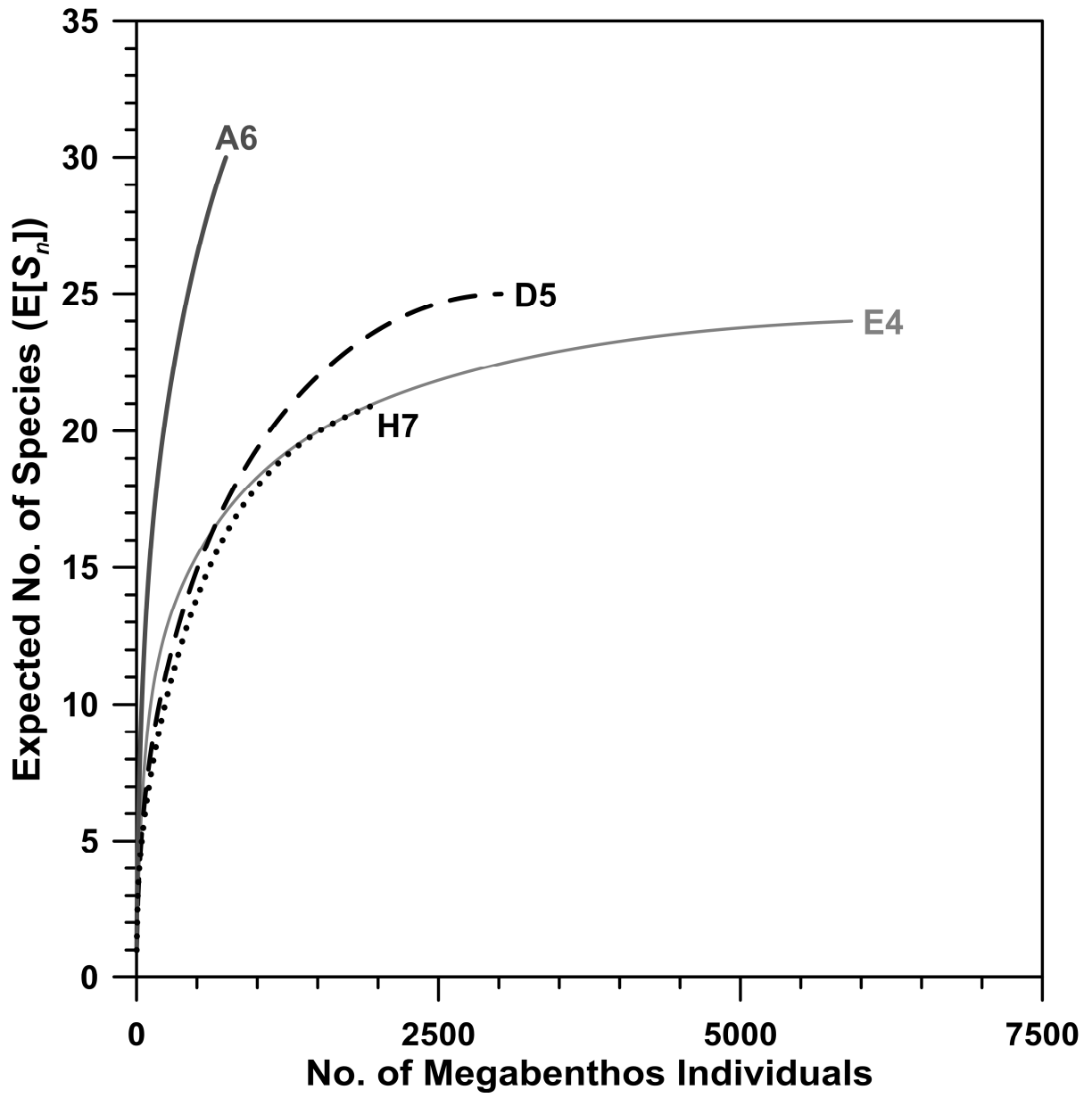


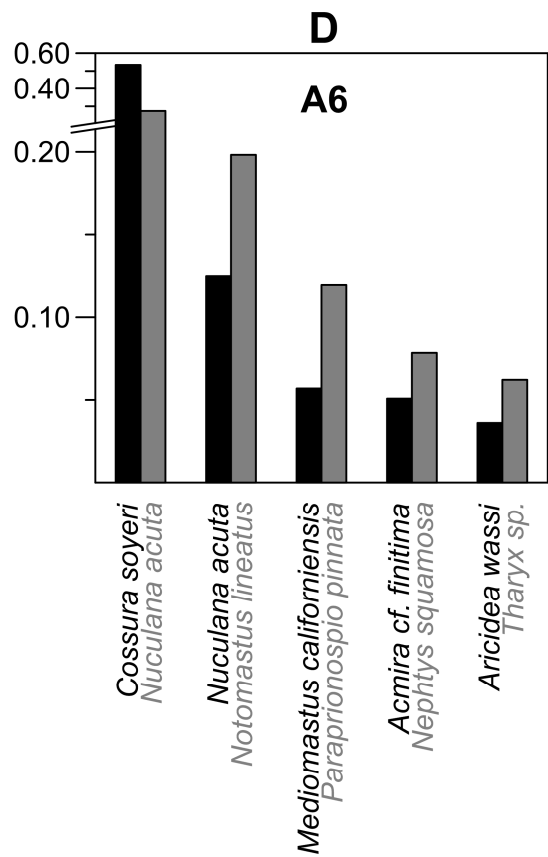
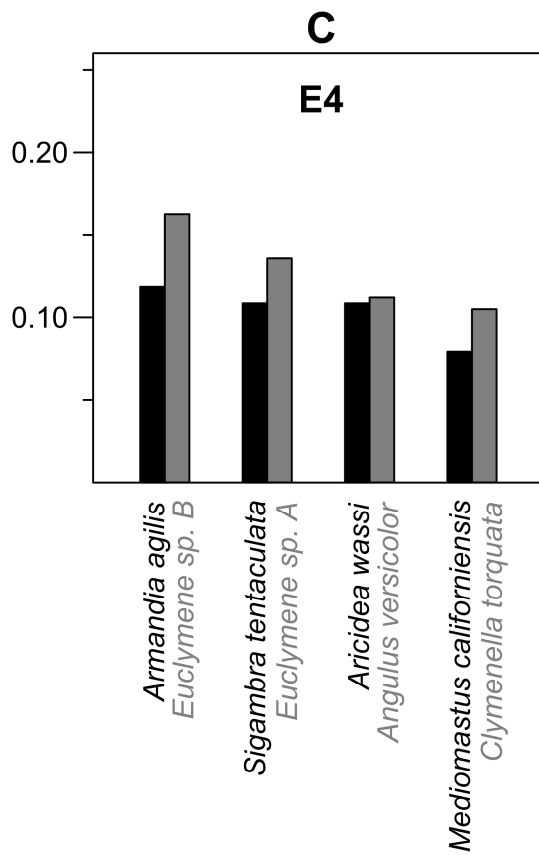
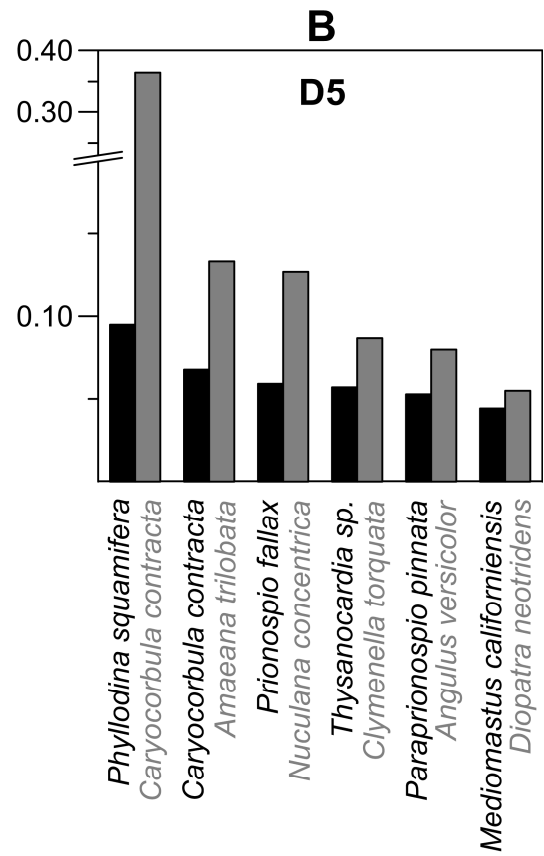
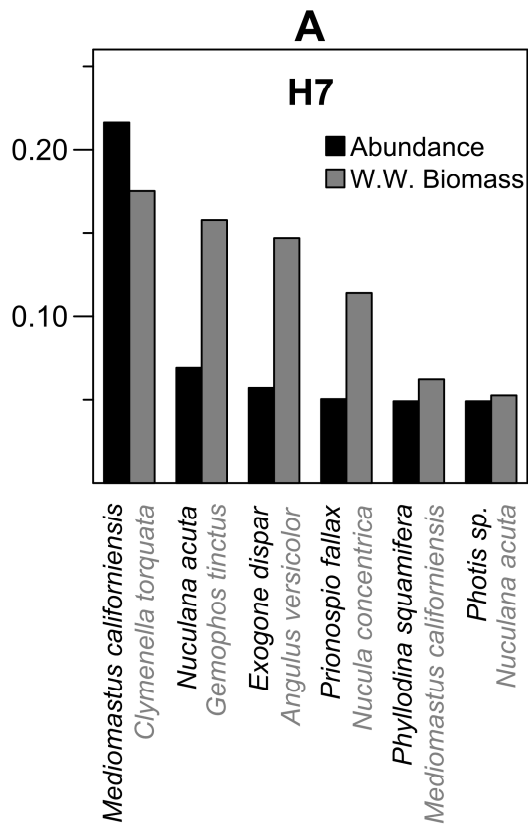




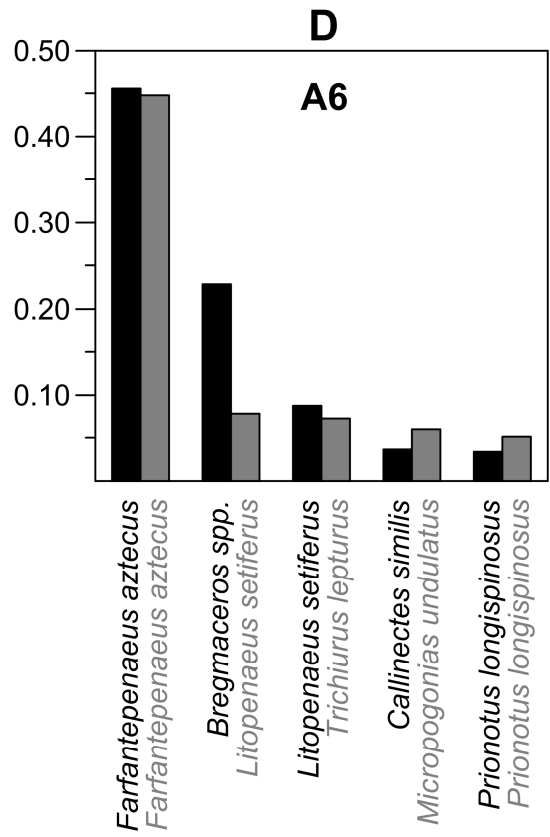
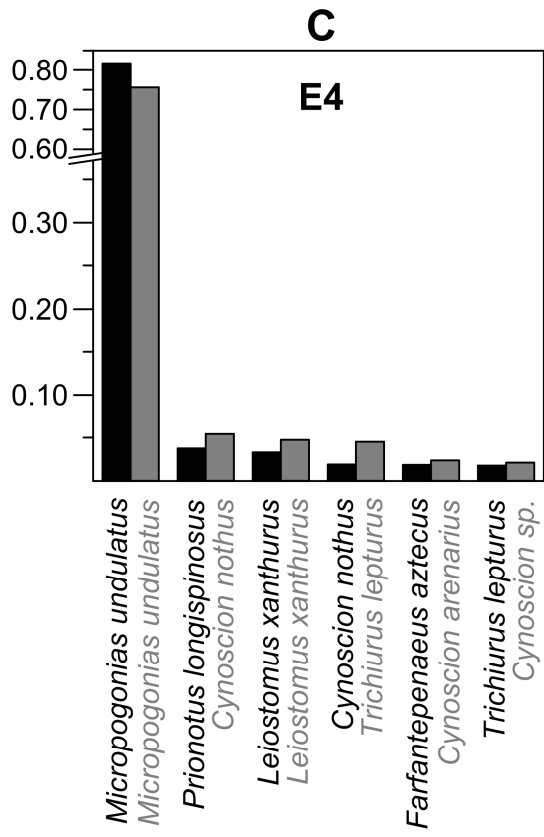
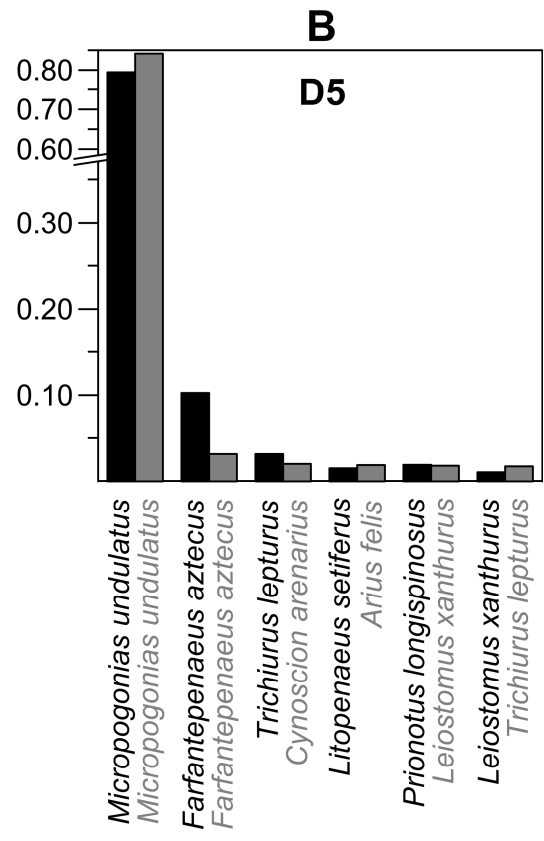
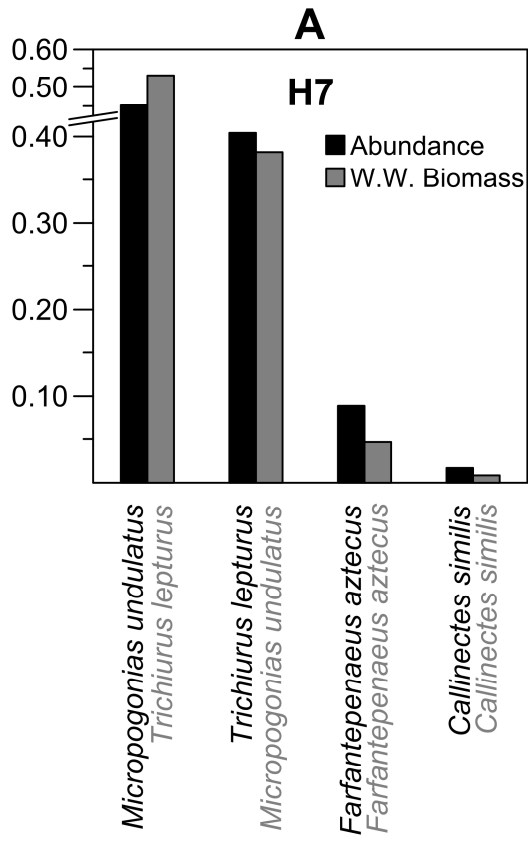




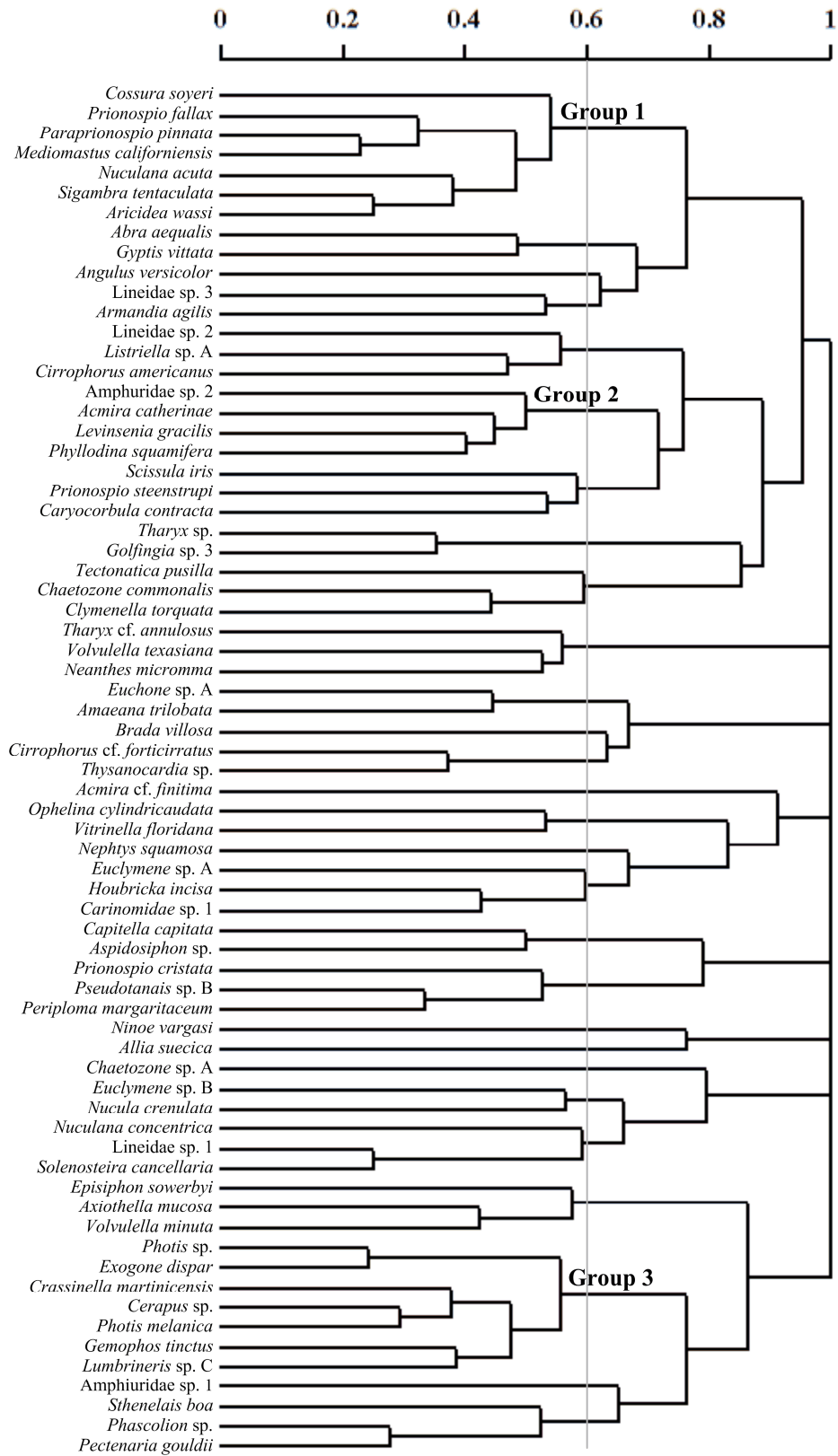


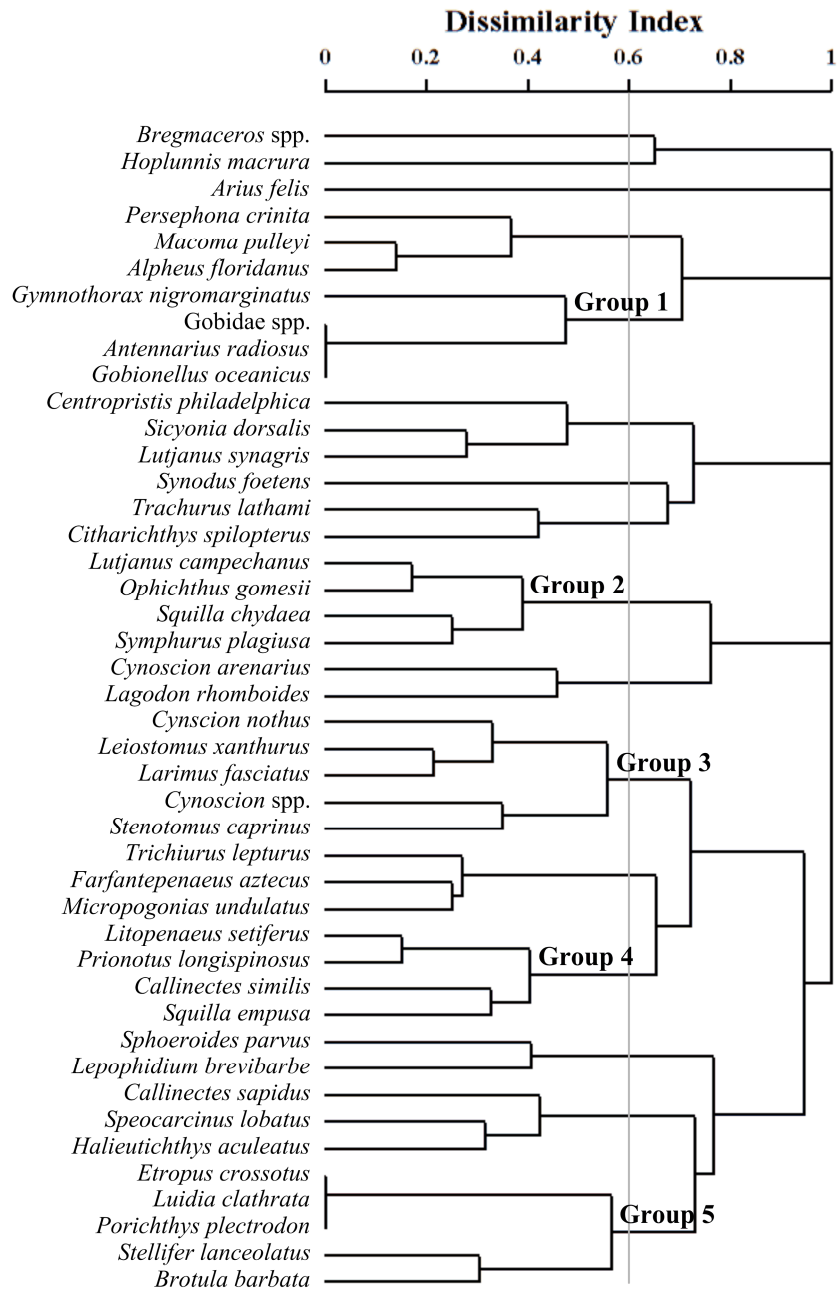


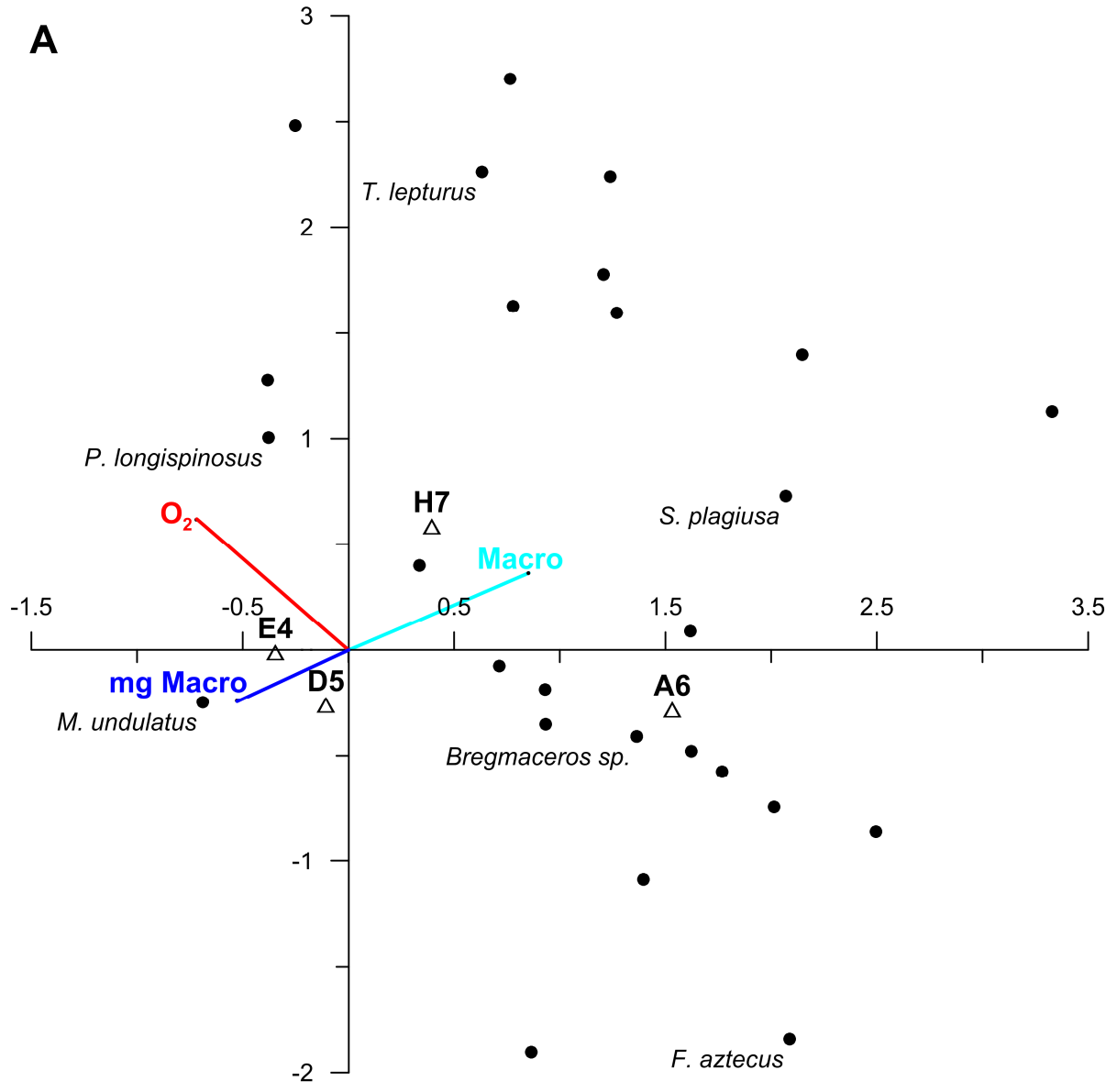




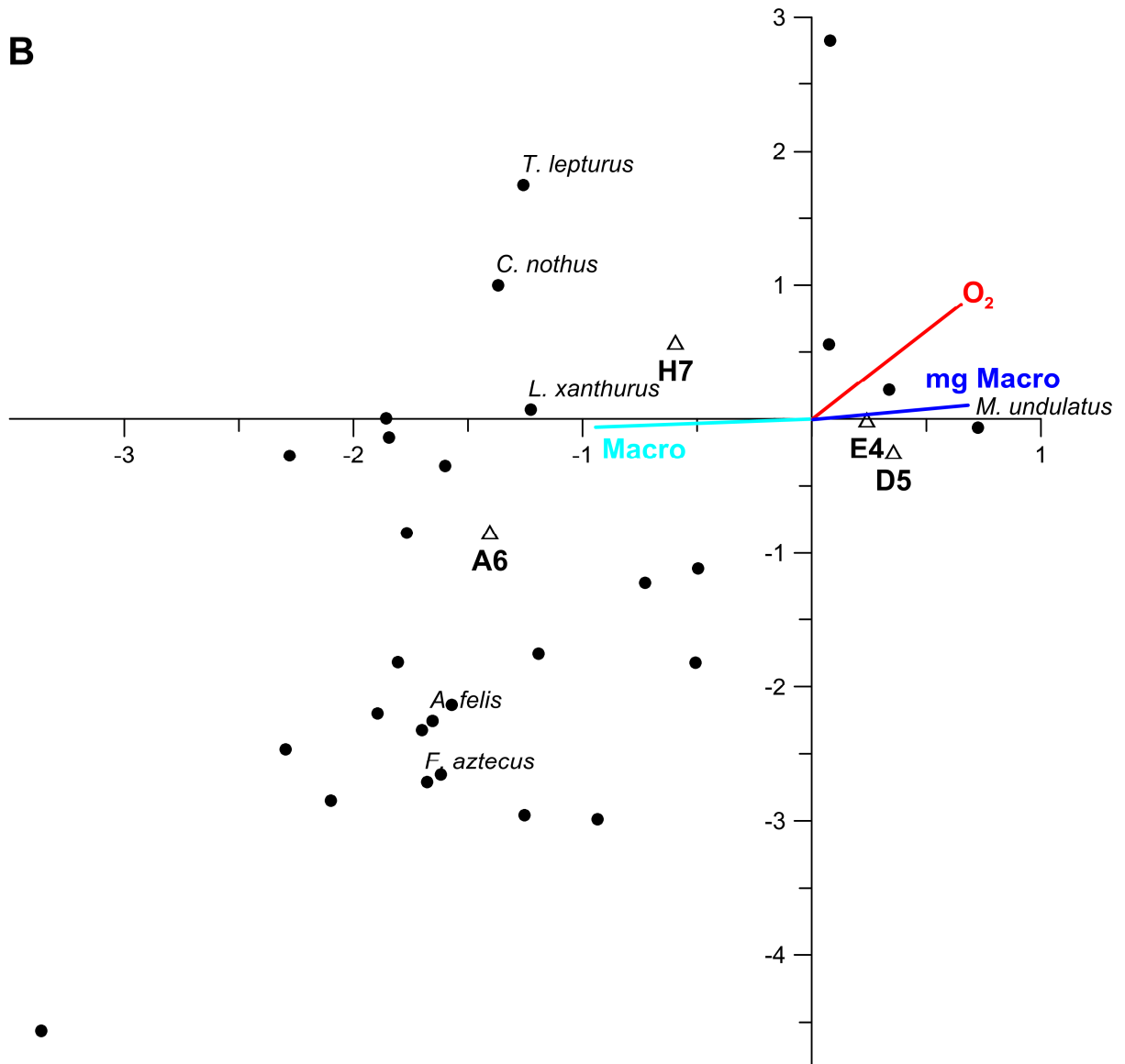
# Dissimilarity Index







**B**



- Macrobenthos assemblage composition driven by tolerance to hypoxia
- Megabenthos assemblage composition affected by migration and prey availability
- Macrobenthos are less mobile than megabenthos and show long-term hypoxia effects
- Size and depth of macrobenthos may influence abundance and biomass of megabenthos