BIVALVE LIVE-DEAD DIFFERENCES RECORD THE IMPACT OF ANTHROPOGENIC EUTROPHICATION ON COASTAL ECOSYSTEMS IN THE NORTHERN GULF OF MEXICO

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INTRODUCTION

Human activities can reduce the health of ecosystems and threaten their biodiversity (Dietl et al., 2015). In coastal environments, anthropogenic eutrophication (AE) can be a particular stressor. Eutrophication occurs when nutrients, such as phosphorus and nitrogen, are enriched in a body of water. Eutrophication can occur naturally, but can also result from human activities such as industrial farming and urbanization (Rabalais et al., 2007). Nutrient enrichment promotes the production of phytoplankton blooms. As the phytoplankton die and decompose, oxygen is rapidly removed from the water. Hypoxic conditions can lead to the death of many organisms resulting in the formation of seasonal "dead zones" that consist of a new and less diverse biota (Rabotyagov et al., 2014).

The northern Gulf of Mexico (GOM) has suffered from AE because it serves as the drainage area for the Mississippi River. The Mississippi River basin drains much of the continental United States, including its major farming regions. Chemical fertilizer use in the Mississippi basin began in the 1950's and has continued to the present (Turner and Rabalais, 2003). Farming, urbanization, and associated run-off all contribute to the annual formation of a "dead-zone", centered on the Mississippi delta (Rabotyagov et al., 2014). Studying the effects of the "dead-zone" on coastal GOM ecosystems is complicated because studies of eutrophication and hypoxia do not predate 1985 (Rabalais et al., 2007). More broadly, biomonitoring of many marine habitats prior to human

activities is limited (Kidwell, 2015). One way to mitigate this issue is to study the skeletal remains of organisms that lived prior to specific human activities (Kosnik and Kowalewski, 2016).

Live-dead analysis involves comparing aspects of the ecology of historical death assemblages (DAs) with modern life assemblages (LAs), in order to produce a historical baseline for an ecosystem (Dietl et al., 2015). Comparisons to the baseline can help to elucidate long-term changes that have occurred in an ecosystem. Live-dead studies focus on organisms preserved in soft sediments (Kidwell, 2015). Bivalved mollusks are well-suited for live-dead studies due to their high preservation potential, high abundance, and position as key environmental indicators (Kidwell, 2008).

I studied the composition and relative abundance of bivalve species in life and death assemblages at two different locations in the northern GOM (Table 1). Each location has a unique history of nutrient enrichment. One site (LA1) was located within the "dead-zone" offshore of Cocodrie, Louisiana and the other (AL7/8) was located outside the "dead-zone" offshore of Dauphin Island, Alabama, an area less directly influenced by Mississippi River discharge and expected to have lower levels of nutrients and primary production (Rabotyagov et al., 2014). I predicted that greater eutrophication at the Louisiana locality would lead to greater live-dead disagreement in the composition and abundance of species than at the Alabama locality.

Studying live and dead assemblages

Due to their calcareous shells and benthic habitat preference, marine bivalves have a relatively high preservation potential. After death, bivalve shells can persist and become mixed into surficial sediments on the seafloor, forming a DA. This time-averaged DA may preserve communities that differ from those living today.

Several potential biases must be considered when interpreting the compositions and relative abundances of species in DAs. First, abiotic and biotic processes such as reworking by currents and burrowing by organisms can mix sedimentary layers. This mixing results in the juxtaposition of shells from organisms that lived at different times. This stratigraphic mixing, or time averaging, must be considered when discussing the age of a DA (Kosnik and Kowalewski, 2016). In addition, although bivalves have high preservation potential overall, individual species may differ in potential, due to differences in shell mineralogy, construction and size. Additionally, taphonomic processes may vary depending on the depositional environment, which can pose challenges when interpreting differences in the composition and abundance of DAs from different locations (Kosnik and Kowaleski, 2016; Lockwood and Chasnant, 2006).

Previous live-dead studies on bivalves found that eutrophication can have dramatic effects on community composition and abundance (Kidwell, 2007; Kidwell 2008). Specifically, Kidwell (2008) found that "organic-loving" bivalve species were often more abundant live than dead in areas that had undergone AE. Organic-loving bivalves were defined as all deposit feeders, chemosymbiotic species, and suspension feeders tolerant of hypoxic conditions.

METHODS

Field Methods

In the summer of 2016 I collected samples at two locations, offshore Dauphin Island, Alabama, and offshore of Cocodrie, Louisiana. Research Vessels (RVs) were chartered from the Dauphin Island Sea Lab and Louisiana Universities Marine Consortium.

Each RV was outfitted with a box core for collecting surficial seafloor sediments containing living and dead mollusks. Sampling at each location took place over the course of two separate days. The first day involved intensive replicate sampling at a single site and the second day less intensive replicate sampling at multiple sites. Sediment from each core was sieved through 2mm mesh to separate out larger live and dead organic material, and all live mollusks were picked out and stored. Once all live mollusks had been removed, the remaining dead material was stored and all live non-molluscan organic matter was returned to the ocean.

Laboratory Methods

I identified bivalve umbos in LAs and DAs to the species level where possible with the help of my research advisor, the Biodiversity of the Gulf of Mexico Database, and several GOM bivalve identification books (Table 1; Fig. 1 & 2; Moretzsohn et al., 2016; Hartmann, 2006; Mikkelsen and Bieler 2008; Tunnell et al., 2010). I used light microscopy with Leica S6D scopes outfitted with Leica DFC-425 cameras to aid in identification. After identification, I researched species' feeding habits to determine if they could be classified as organic-loving following Kidwell (2008).

To determine the degree of time-averaging, a subsample of dead Alabama and Louisiana *Nuchlana acuta* umbos were sent for radiocarbon dating to the University of California, Irvine, and Northern Arizona University.

Statistical Methods

Each pair of live-dead assemblages was statistically analyzed for four key ecological metrics: richness, rank-order abundance, evenness, and taxonomic similarity. The program Paleontological Statistics (PAST) (Hammer et al., 2001) was used for all analyses. Metrics were compared between live and dead assemblages within and among locations to quantitatively assess ecological changes potentially generated by anthropogenic impacts.

In order to measure species richness (S), I had to correct for the differing sample sizes of the live and

Table 1   30th Annual Symposium Volume, 29th April, 2017

Assemblage	Sample size (number of umbos)	Location (Site name + Latitude and Longitude)	Depth (m)	Sedimentary environment	"Organic-loving" species (%)	Median age of dead *N. acuta* radiocarbon dates
Alabama Dead	1149	AL7: 30° 05.3930'N 88° 12.7940'W	19	---	30.5	592 years
Alabama Live	447	AL7: 30° 05.3930'N 88° 12.7940'W AL8: 30° 05.396'N, 88° 12.828'W	19.8	Siliciclastic; 75/15/10% by weight in fine sand, medium to coarse sand, and silts and clays respectively	79.9	---
Louisiana Dead	152	LA1: 28° 52.3130'N, 90° 28.1235'W	19.7	---	98.7	Pending
Louisiana Live	222	LA1: 28° 52.3130'N, 90° 28.1235'W	19.7	Siliciclastic; more silt and clay relative to Alabama	99.1	---

Table 1. Characteristics of Louisiana and Alabama assemblages. All samples were collected in 2016 except for AL7 in 2015 which contained the Alabama death assemblage and the first year of the Alabama life assemblage.
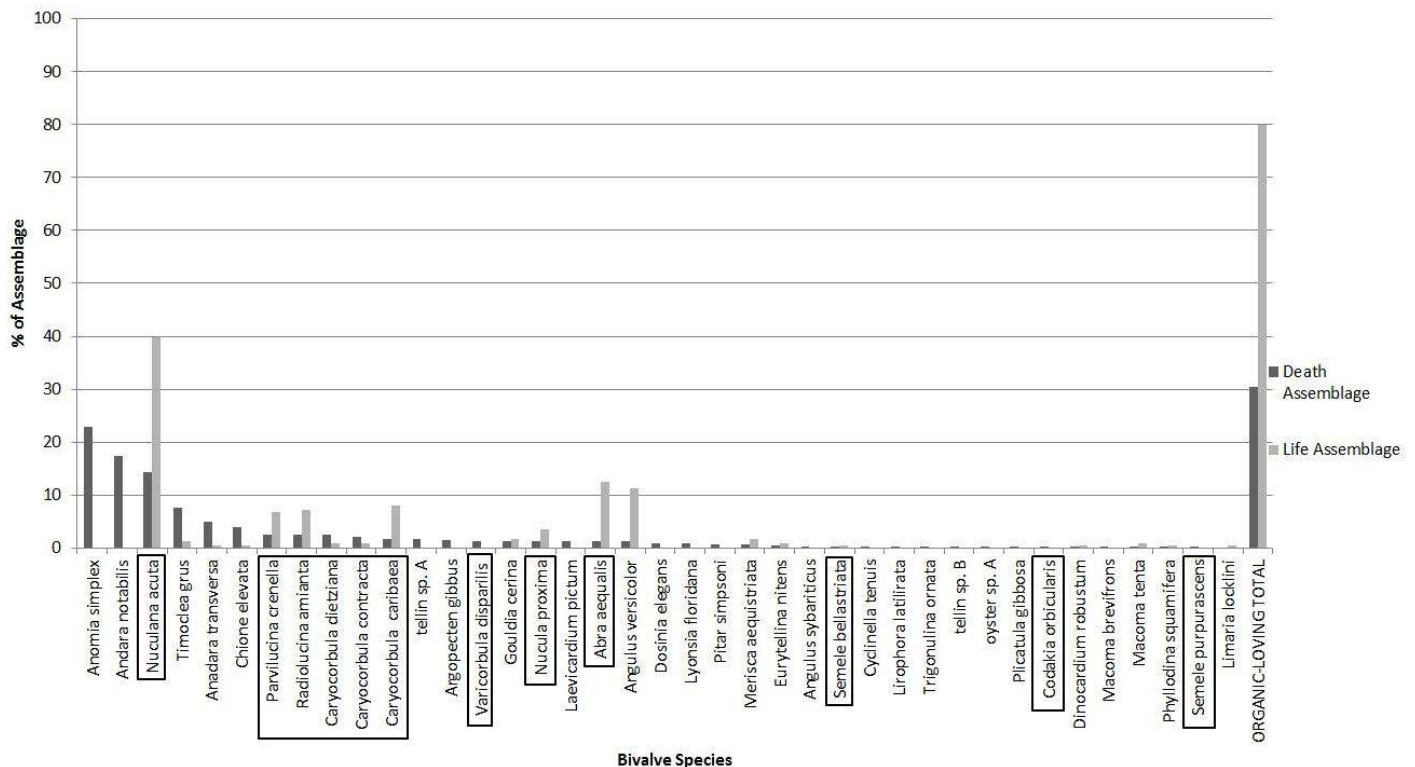

Figure 1. Bivalve live-dead comparison for Alabama site, AL 7/8. The black boxes indicate "organic-loving" species that are tolerant of low oxygen conditions, like those that might occur from AE. Notice the high proportion of Nuculana acuta and Abra aequalis in the life assemblage.
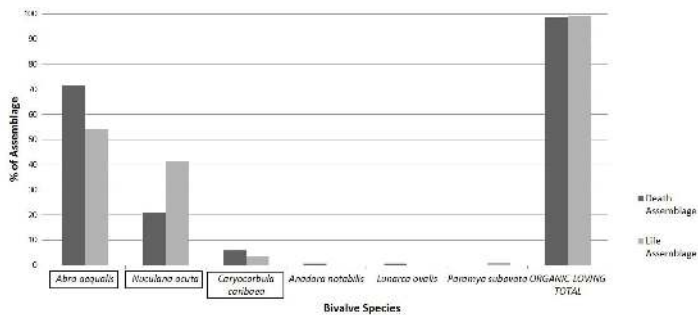
Figure 2. Bivalve live-dead comparison for Louisiana site, LA1. The black boxes indicate "organic-loving" species that are tolerant of low oxygen conditions, like those that might occur from AE. Notice the high proportion of Nuculana acuta and Abra aequalis.

death assemblages. Larger assemblages are expected to contain more taxa than smaller assemblages and that can bias comparisons of richness. I used rarefaction to rarify the larger sample down to the smaller sample size. I then compared the difference in number of taxa at the smaller sample size, by subtracting the live richness from the dead richness (Δ-S).

I calculated live-dead agreement in rank order abundance using a Spearman rank correlation test. Agreement between assemblages can range from a perfect negative correlation (-1) to perfect positive correlation (+1); e.g., in perfect positive correlation the most abundant taxon (rank 1) in assemblage A is the same as the most abundant taxon in assemblage B, the second most abundant taxon (rank 2) in assemblage A is the second most abundant taxon in assemblage B etc.

I measured evenness using Hulbert's Probability of Interspecific Encounter (PIE). Evenness measures how specimens are distributed among species in an assemblage. Perfect evenness would mean that every specimen belonged to a unique species, (i.e., PIE= 1). In a perfectly uneven assemblage, species would differ markedly in their abundance (PIE= 0). I calculated PIE for every assemblage and then the differences in PIE between each pair of live-dead assemblages (Δ-PIE or Dead PIE - Live PIE).

I measured the similarity in taxonomic composition between paired live and dead assemblages using the Jaccard Index. This is the number of shared species between the assemblages divided by the total number of species observed across both assemblages. The

Jaccard Index ranges from 0 (no taxa are shared) to 1 (all taxa are shared).

RESULTS

Each DA was significantly richer than its paired LA even after controlling for differences in sample size. Delta-S was +11.8 (significant) for Alabama and +1.07 (not significant) for Louisiana. Live and dead rank-order abundances correlated well and were significant for each live-dead pair, (Rho=0.39, p =0.01 for Alabama and Rho=0.68, p<0.01 for Louisiana). In terms of evenness, Alabama assemblages were fairly even (PIE=0.88 for the DA and 0.79 for the LA), whereas Louisiana assemblages were less even (PIE=0.48 for the DA and 0.54 for the LA). Neither the Alabama nor Louisiana assemblages had a large delta-PIE, although the Alabama live-dead difference was significant (i.e., the two evenness values fall outside each other's 95% confidence intervals) (Table 2). Taxonomic similarity was comparable for both Alabama and Louisiana assemblages (Jaccard=0.49 for Alabama, 0.5 for Louisiana).

The Louisiana assemblages and Alabama LA were composed of a high percentage of organic-loving species (Table 1). Louisiana assemblages were composed almost entirely of two organic-loving species, *Nuculana acuta* and *Abra aequalis*. *N. acuta* was also common in the Alabama LA (Fig. 1 & 2). Radiocarbon dates indicate that the median age of a *N. acuta* umbo in the Alabama DA was 590 years before present, indicating an assemblage existing prior to many human impacts including the mid-20th century development of chemical fertilizers. Louisiana radiocarbon dates are pending at the time of this publication.

DISCUSSION

The results of my study indicate a high proportion of organic-loving species in both Louisiana LAs and DAs, as well as the Alabama LA (Fig. 1 &2; Table 1). Statistical results at each location indicate mixed agreement between DAs and LAs; some, but not all, ecological metrics were significantly correlated. Species in the Alabama LA suggest adaptation to high nutrients, leading to a decrease in agreement with the Alabama DA. The most common bivalve species

found in the Louisiana LAs and DAs, and the Alabama LA indicate high nutrients. *N. acuta* and *A. aequalis* are deposit feeders meaning that they feed on organic matter within the sediment (Mikkelsen and Bieler, 2008). In a "dead-zone" environment, organic matter is input at higher rates, and this plentiful food source might contribute to the high abundance of *N. acuta* and *A. aequalis* I observed (Kidwell, 2009). Results of this study agree with previous studies that examined the impacts of AE on coastal ecosystems (Kidwell, 2008).

The high proportion of *N. acuta* and *A. aequalis* in the Louisiana life and death assemblages may represent different possible scenarios which could be distinguished with additional information about the age of the DA. First, the Louisiana assemblages could represent a recent shift in nutrient levels. If the DA is composed of relatively recent dead, then both live and dead assemblages might represent ecosystems that have experienced high nutrient levels and AE (Kidwell, 2009). Alternatively, the Louisiana dead may be much older which would suggest that the region in the vicinity of the Mississippi River delta has been subject to naturally high nutrients prior to human impacts. If so, the LA might not differ greatly from the DA because the area was already dominated by organic-loving species prior to AE. In either scenario, high sedimentation rates might mask the true nature of the DAs (Kidwell, 2009), potentially leading to faster burial of pre-AE DAs. Deeper coring may be necessary to obtain a more complete picture of DAs, prior to certain human activities.

The live-dead agreement between the Alabama assemblages was lower than expected. Evenness and rank-order abundance indicated overall positive agreement, but richness and taxonomic similarity did not (Table 2). These results suggest that AE may be affecting the benthic communities offshore of Dauphin Island.

The Alabama LA contained more *N. acuta* than the entire DA, even though the LA had a smaller sample size (Fig. 1). The higher richness of the Alabama DA may be a function of time averaging, but could also suggest a loss of diversity through time due to AE. The Alabama DA contains species representing a wider range of feeding habits relative to the Louisiana assemblages. Loss of diversity in Alabama may have affected suspension feeding bivalves, which are much more common in the Alabama DA than the Alabama LA.

The results of my study agree with the results of Kidwell (2008). Kidwell found similar results in AE-affected areas in Greece, Japan, and Sicily. Each of these locations had higher abundances of organic-loving deposit feeders in their LAs relative to their DAs. Kidwell (2008) noted that the most likely explanation for these differences was AE, and that

Table 2

Assemblage Comparison	Rank-Order Abundance (Spearman rho)	Richness (Δ S)	Evenness (Δ PIE)	Taxonomic Similarity (Jaccard Index)
Alabama Live-Dead	0.39 (p = 0.01)	+11.8 ± 1.7 (Significant)	+0.09 (Significant)	0.49
Louisiana Live-Dead	0.68 (p < 0.01)	+1.07 ± 0.29 (Not Significant)	-0.09 (Not Significant)	0.5

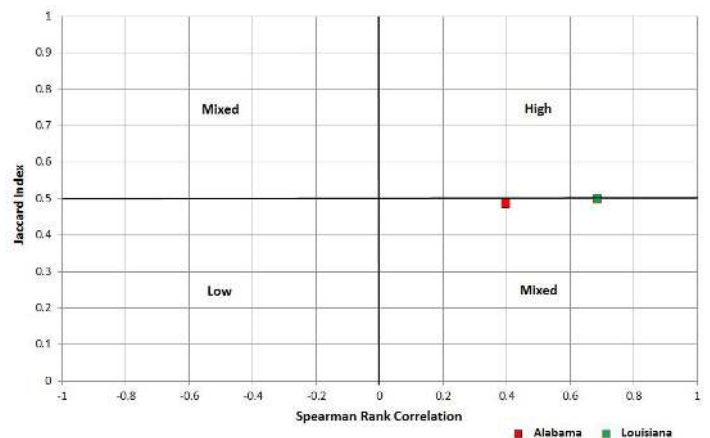*Table 2. Statistical results of Live-Dead assemblage comparisons.*

Figure 3. Rank order abundance (Spearman rank correlation) versus taxonomic similarity (Jaccard Index) for Alabama and Louisiana live-dead assemblages. This plot follows the format of the plots used by Kidwell (2007, 2009). The upper right corner represents conditions of highest live-dead agreement, and the bottom left corner the lowest live-dead agreement. The other quadrants represent mixed agreement. Note that my results for Louisiana and Alabama indicate mixed agreement.

taphonomic biases or other factors were unlikely to fully explain the discrepancies.

Kidwell (2008) characterized pristine environments as having on average 25% more species in the DA (Δ-S of 1.25 ± 0.48); having variable differences in evenness but being close to zero (Δ-PIE of +0.02 ± 0.17); having high taxonomic similarity (over 0.84 in the Jaccard-Chao index, closely related to the Jaccard Index that I used); and having positive and significant rank-order agreements (Spearman rho of +0.29 to ± 0.09). My results are mixed compared to Kidwell's baseline. The Alabama and Louisiana LAs and DAs would be pristine in rank-order agreement and evenness. The Louisiana assemblages would be classified as pristine with respect to richness, while both Louisiana and Alabama life and death assemblages would not be classified as pristine with respect to taxonomic similarity (Table 2). I created a plot of taxonomic similarity versus rank-order abundance following those of previous studies by Kidwell (2007, 2009), Alabama and Louisiana fall into an area that supports mixed agreement between the assemblages (Fig 3). Kidwell stated that several samples should be included for comparisons to this baseline, so caution is warranted in making this comparison.

Kidwell (2008) noted that a single sample of a live community usually only contains 30% of living species that are found by examining multiple years of pooled live samples. My study only considered two years of live sampling of Alabama and one year of live sampling of Louisiana. Broader geographic sampling could also improve our understanding of the diversity of bivalves across these environments. Our sampling in 2016, and previous sampling in 2015, may have only captured one habitat patch on the sea floor and other adjacent patches might represent different substrates with different associated bivalves (Kidwell, 2008). Evidence for taphonomic bias due to shell mineralogy is limited as softer aragonite shelled species are found in both DAs (Kidwell, 2008). Other factors not discussed in this paper that may also play a role in live-dead differences, such as post-mortem transportation and life-histories of different species (Kidwell, 2008). Despite these problems, the results of my study suggest that recent AE is likely having an impact on Alabama benthic communities, and possibly in Louisiana as well, though the effects of AE in Louisiana may be harder to determine due to the much longer history of natural nutrient enrichment at that locality.

ACKNOWLEDGMENTS

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