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ASSESSING THE EFFECTS OF NUTRIENT RUNOFF ON BIVALVE EGG SIZE IN THE GULF OF MEXICO

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INTRODUCTION

Parts of the Northern Gulf of Mexico experience hypoxic conditions annually, due to eutrophication and seasonal stratification of the water column (Rabalais et al 2002). Eutrophic conditions are tied to an increase in agricultural runoff (Diaz and Rosenberg 2008). There are clear links between nutrient flux from the Mississippi River and primary productivity and hypoxia in the northern Gulf (Mitsch et al 2001; Diaz and Rosenberg 2008). The intensity of eutrophication and hypoxia varies among sites in the Northern Gulf (Rabalais et al 2007). Sites offshore Louisiana, for example, experience regional-scale, seasonal eutrophication (Rabalais et al 2007), whereas sites in the Mississippi Bight experience less persistent and more localized eutrophication (Brunner et al 2006 and Rabalais et al 2007). Geographic variation in the intensity of eutrophication could result in differences among marine populations in life history traits. In this study, I collected life history data to see how recent increases in nutrients affected the marine bivalve *Nuculana acuta.*

In order to determine the effects of enhanced nutrients on present day marine organisms in the Northern Gulf of Mexico, we need baseline data for what marine communities were like prior to anthropogenic nutrient enrichment. The skeletal remains of dead organisms can be used to determine biological conditions prior to human impact (Dietl and Flessa 2011). Live-dead comparisons can shed light on recent and historical changes in marine communities. Live-dead disagreement may result from natural processes such as postmortem transport (Kidwell 2007). However, recent studies have found that live-dead disagreement in species composition and relative abundance can also be caused by anthropogenic eutrophication (Kidwell 2007).

For this study, I analyzed the effects of nutrient enrichment on bivalve life history. Egg size is one of the most important life history characteristics of marine organisms and can vary in response to food availability (Moran 2004). In bivalves, egg size is positively correlated with larval shell size (PI) (Moran 2004; Malchus and Sartori 2013). A basic assumption of life history theory is that a tradeoff exists between the number of eggs and the size of eggs a mother can produce. Under lower nutrient conditions (i.e., limited food), mothers that produce larger, though fewer eggs, are expected to leave behind more offspring whereas under higher nutrient regimes (i.e., abundant food) mothers that produce more numerous, smaller eggs are expected to leave behind more offspring. Larger eggs contain greater resources for developing offspring and thus enhance survival when nutrients and food are limited. Under these conditions, mothers who produce larger eggs will have greater fitness than mothers that produce smaller eggs; if nutrients decline over time we expect to see egg size increase in a population. On the other hand, under increasing nutrient conditions, egg size will play less of a role in juvenile survival. Thus egg size will decrease in the population over time as mothers that produce more numerous, small eggs experience greater fitness. I compared live and dead larval shell sizes to see how recent increases in nutrients in the Northern Gulf of Mexico might have affected the egg sizes of *N. acuta.*

N. acuta is a bivalve that is commonly found live and dead, offshore Louisiana and Alabama (Grimmelbein this volume). Species in the family Nuculanidae are deposit feeders and are abundant in deep sea to shallow water habitats (Da Silva 2011). Intensified phytoplankton blooms (Diaz and Rosenberg 2008) resulting from anthropogenic eutrophication could provide additional food to *N. acuta* populations, and thereby affect egg size (Moran 2004). I hypothesized that increased nutrients resulted in the reduction of larval shell size over time, which would lead to a live-dead difference. I tested this hypothesis by determining the mean larval shell size for live and dead *N. acuta* populations offshore Louisiana and Alabama, to see if there was a shift in size consistent with a recent increase in nutrient levels. Geographically, I expected to see smaller PI sizes in populations in coastal Louisiana relative to Alabama, because of proximity to the Mississippi delta.

METHODS

During the summers of 2015 and 2016, students in Franklin and Marshall College's Paleobiology Lab collected live and dead samples from approximately 20 meter water depth at multiple localities offshore Louisiana and Alabama. These samples were collected from research vessels using a variety of sediment grabs, box cores, and bucket dredges. However, the majority of the samples were collected using a box core. While offshore, we sieved buckets of sediment through a 2mm mesh in order to recover all shelly material. We then picked through the >2mm size fraction to separate live and dead mollusks. Live specimens were identified by their articulated shells. However, some recently dead specimens might also have articulated shells thus there was a potential for misidentifying live specimens. Because samples were processed offshore we could not use Rose Bengal to stain live tissue. However, students did attempt to distinguish live and dead specimens by assessing whether articulated shells contained soft tissue or sediment.

We chose *N. acuta* as our study species because it was sufficiently common to allow us to make a live-dead comparison. I used a scanning electron microscope to collect larval shell (PI) measurements (Figure 1). Along with a PI measurement, every specimen was assigned a preservation grade (Table 1), so that I could factor out poor preservation as a potential bias when comparing live and dead mean larval shell size at a site, and comparing mean values among sites. Due to poor preservation of larval shells, only a small subset (AL Dead, 37%; AL Live 19%; LA Dead 7%, LA Live 27%) of specimens that I SEM analyzed were included in my final analysis.

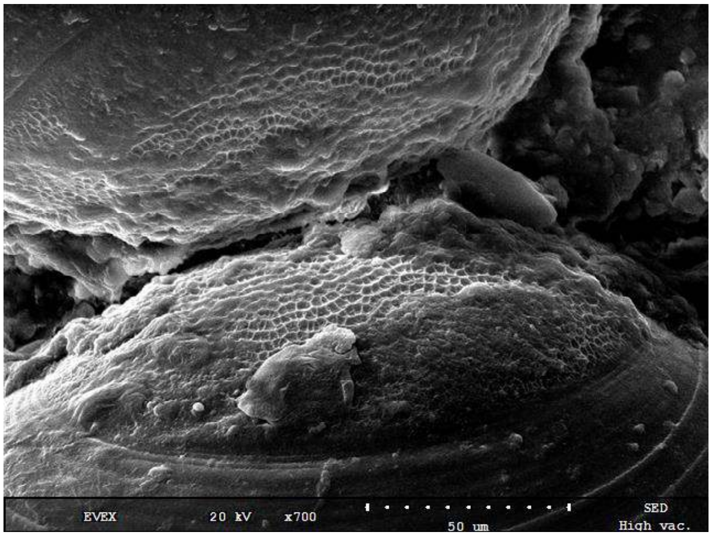

Figure 1. Scanning electron micrograph of a well-preserved, Nuculana acuta larval shell from Alabama (specimen number AL-516).

Preservation Grade	Explanation
1	**Prodissoconch Relief (Mound):** Mound is not preserved. **Prodissoconch-Nepioconch Contact**: Contact is not preserved. **External Ornamentation on Adult Shell:** Original external layer of adult shell is completely eroded.
2	**Prodissoconch Relief (Mound):** Mound is either not preserved or partially preserved. **Prodissoconch-Nepioconch Contact**: Contact is preserved on either the anterior or posterior margin of the larval shell. **External Ornamentation on Adult Shell:** Original external layer of adult shell is partially preserved, however surficial features (growth bands) are worn.
3	**Prodissoconch Relief (Mound):** Mound is partially preserved. **Prodissoconch-Nepioconch Contact**: Contact is preserved on both the anterior and posterior margin of the larval shell. **External Ornamentation on Adult Shell:** Original external layer of adult shell is completely preserved, however surficial features are worn.
4	**Prodissoconch Relief (Mound):** Mound is completely preserved. **Prodissoconch-Nepioconch Contact**: Contact is completely preserved with negligible damage. **External Ornamentation on Adult Shell:** Original external layer of adult shell is completely preserved, with detailed, undamaged surficial features. Some pitted texture of the PI is preserved.
N/A	**Prodissoconch Relief (Mound):** Orientation or some obstacle makes it difficult to see the mound. **Prodissoconch-Nepioconch Contact**: Orientation or some obstacle makes it difficult to see the contact. **External Ornamentation on Adult Shell:** Original external layer of adult shelf is irrelevant if the mound and contact are not visible due to an obstacle or orientation.

Table 1. Preservation grades used to score each specimen examined in the SEM.

I determined the mean live and dead larval shell size in Louisiana and Alabama from my SEM measurements. I ran multiple t-tests to determine if the mean larval shell sizes of the live and dead samples were significantly different. I first analyzed all specimens that had a preservation grade greater than or equal to 2. I then restricted the data to only those specimens with a preservation grade greater than or equal to 3 in order to see whether poor preservation affected mean larval shell sizes. The PI boundaries of well-preserved specimens (preservation grades 3 and 4) are well defined, whereas the poorly preserved specimens (preservation grades 1 and 2) do not have as well preserved PI boundaries. I have greater confidence in the well preserved dataset, since there was less estimation involved in making the PI measurements. I did not include any specimens with a preservation grade equal to 1, because according to the taphonomic grade system (Table 1), there should not be a PI measurement associated with these specimens.

RESULTS

Specimens with a Taphonomic Grade Greater than or Equal to 2

The mean PI size for Louisiana dead specimens (N=28) was 136.4 microns, whereas the mean PI size for Louisiana live specimens (N=10) was 133.3 microns. There was a shift towards smaller PI sizes in Louisiana over time, but this live-dead difference was not statistically significant (p=0.37).

The mean PI size for Alabama dead specimens (N=82) was 153.0 microns, whereas the mean PI size for Alabama live specimens (N=45) was 150.1 microns. There was a shift towards smaller PI sizes in Alabama over time, but this live-dead difference was not statistically significant (p=0.07).

The geographic difference in PI size between live populations in Alabama and Louisiana was statistically significant (p<0.001). The difference in PI size between dead populations in Alabama and Louisiana was also statistically significant (p<0.001).

Figure 2 presents live-dead comparisons for all Louisiana and Alabama specimens with a PI preservation grade of 2, 3 or 4.

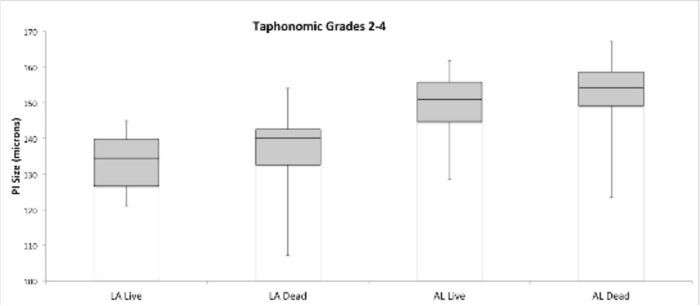

Figure 2. Larval shell size distribution of live and dead specimens (preservation grades 2-4) collected offshore Alabama and Louisiana.

Specimens with a Taphonomic Grade Greater than or Equal to 3

The mean PI size for Louisiana dead specimens (N=7) was 143.9 microns, whereas the mean PI size for Louisiana live specimens (N=9) was 134.6 microns. There was a shift towards smaller PI sizes over time in Louisiana, and this live-dead difference was statistically significant (p=0.014).

The mean PI size for Alabama dead specimens (N=37) was 154.6 microns, whereas the mean PI size for Alabama live specimens (N=17) was 151.3 microns. There was a shift towards smaller PI sizes in Alabama over time, but the live-dead difference was not statistically significant (p=0.081).

The geographic difference between PI size in live populations in Alabama and Louisiana was statistically significant (p<0.001). Similarly, the geographic difference between Alabama and Louisiana dead PI sizes was also statistically significant (p<0.001).

Figure 3 presents live-dead comparisons for all Louisiana and Alabama specimens with a PI preservation grade of 3 or 4.

DISCUSSION

Alabama Results

Regardless of preservation grade, I found that there was no statistically significant difference between live and dead larval shell sizes in Alabama, although both datasets approach statistical significance (p=0.081 for 3's and 4's; p=0.066 for 2's-4's).

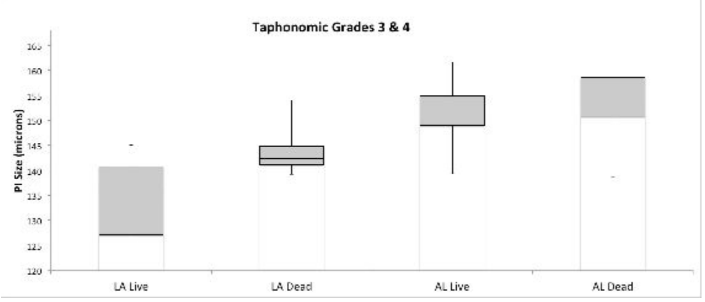

Figure 3. Larval shell size distribution of live and dead specimens (preservation grades 3&4) collected offshore Alabama and Louisiana.

According to the mean live and dead PI measurements, there is a tendency for PI size to be smaller today than in the past. Previous work suggests that this location in coastal Alabama has not experienced extensive eutrophication and hypoxia relative to other regions in the Northern Gulf (Brunner et al 2004). However, analyses of coretops indicate limited eutrophication was present offshore Alabama beginning in the 1950s (Brunner et al 2004). Because nutrient concentrations may not have changed appreciably over time, live-dead similarities in PI size are perhaps to be expected at this site.

Louisiana Results:

Regardless of preservation grade, I found there was a shift towards smaller PI sizes over time which is consistent with my hypothesis. The statistical significance of this live-dead difference in PI size depends on whether I restrict my data to only well preserved specimens. Because specimens with a preservation grade greater than 3 have well defined PI boundaries, I have greater confidence in results generated from this better-preserved subset of my data. The well preserved data suggest that *N. acuta* populations in Louisiana are responding to recent increases in nutrients in the Northern Gulf of Mexico. On average, present day *N. acuta* are producing smaller eggs, in comparison with historical dead populations. My results may also suggest that poor preservation might obscure live-dead differences in PI size.

In comparison with specimens from Alabama, microscale shell features on Louisiana specimens tended to be less well preserved (see also Regan this volume). Since preservation was so poor for some *N. acuta*, it was difficult to make reliable PI measurements. The well preserved Louisiana live (n=9) and dead (n=7) sample sizes are small, so the analysis could be strengthened in the future by increasing sample size which will necessitate additional the fieldwork.

Geographic Comparison:

Regardless of preservation grade, live and dead *N. acuta* from Louisiana have significantly smaller PI's than live and dead *N. acuta* from Alabama. This geographic difference is consistent with my hypothesis that smaller PI sizes are associated with higher nutrient conditions. Approximately 65% of Alabama's land area drains to Mobile Bay (Environmental Protection Agency 2014), whereas 31 different States drain into the Mississippi River (National Park Service). Since the Louisiana populations are closer to the mouth of the Mississippi River, they experience more nutrient-rich waters than populations offshore Mobile Bay.

The Mississippi River has always been a source of nutrients and productivity (Rabalais et al 2007), whereas, according to Brunner and coauthors (2006), the Mississippi Bight tends to be less nutrient-enriched and experiences less intense phytoplankton blooms. Because the mean Louisiana PI in the death assemblage is significantly smaller than the mean PI size in the Alabama death assemblage, we know that this difference in nutrient levels was present historically. However, the live-dead difference in larval shell size is also greater in the LA region than the AL region, which suggests a recent shift in life history in the region affected by more intense anthropogenic eutrophication.

CONCLUSION

The larval shell size of the marine bivalve *N. acuta* varies geographically and between living and death assemblages in the Northern Gulf of Mexico. Overall, live populations tend to have smaller larval shells than their dead counterparts. The well preserved Louisiana specimens, which is the more robust data subset, show a statistically significant difference in PI size between the live and dead populations. There was a significant difference in PI size between the Alabama and Louisiana specimens, which is consistent with my initial hypothesis of a smaller PI size in a region of increased primary production. Today *N. acuta* are producing, on average, smaller larval eggs in the Northern Gulf of Mexico than they did in the past, which may reflect a life history response to increased nutrient levels.

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