The Deepwater Horizon oil spill: Assessing impacts on a critical habitat, oyster reefs and associated species in Florida Gulf estuaries

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SCIENCE ACTIVITIES

1) General Summary

Our primary goal was to determine if and how the Deepwater Horizon (DWH) spill could have affected survival, growth, recruitment of oyster populations and their associated faunal communities at four overall locations in peninsular Florida Gulf of Mexico estuaries ranging from Big Bend (panhandle) Florida to Tampa/St, Pete, Ft. Myers area, and Naples (Rookery Bay National Estuarine Research Reserve) To do so, at 60 reefs using the same methodologies we assessed: (a) variations in oyster populations and associated reef invertebrate assemblages along a north-south gradient (b) existing levels of oil-derived chemicals in oyster tissue; and (c) genetic diversity and connectivity among natural oyster sites. These reef sites were assessed at a time anticipated to precede any direct impact from the spill and again one year later. Although oil did not obviously contaminate any of our FL sites directly, we completed our two-time period study design by assessing oyster reef community ecology and oyster population dynamics (Coen, Kimbro, Geiger and Proffitt) including oyster growth, survival, and recruitment, and toxicology (Weinstein); and population genetics (Nance and Proffitt) the latter from not only our Florida sites, but also sites in every other U.S. state that borders the Gulf of Mexico.

These data constitute a unique and previously unavailable baseline dataset for oysters, their associated communities, and the habitat they create over a large spatial scale (hundreds of miles). The population viability and genetic diversity of Florida oysters could be affected by disturbance at other Gulf populations depending on the extent of gene flow and impacts to reproducing adults and/or the more widely dispersing larvae. Therefore, the population genetic and connectivity analyses were expanded beyond the scope of the original proposal at no additional cost to FIO by securing tissue samples from oysters from every U.S. Gulf state, and including sites in Louisiana that were oiled and/or then disturbed by diverted fresh water. Collections of adult oysters in the fall 2010 after the spill assured a pre-spill sample of oysters that settled or were living at our 60 reef sites prior to any potential DWH oiling. Collections in 2011 focused on smaller individuals that most likely recruited after the spill. Comparing the genetic makeup of the 2010 and 2011 oysters allowed us to determine if disturbance affected the genetic diversity and similarity among sites.

Project personnel participated in over 75 field days of collections of ~ 135,000 oyster measurements, 1,605 genetic samples, 600 PAH samples, and 33,000associated invertebrates in 2011. Fifty-seven of the 60 reefs were mapped using mapping grade GPS and incorporated into an ArcGIS project, with additional morphological measurements taken (e.g., reef area, maximum height, max. length and width, and rugosity).

Accomplishments:

Visible surface oil did not come ashore on any of our Florida study sites. Individual PAH analyte levels reported here were generally two orders of magnitude below any public health levels of concern for seafood. These results demonstrate that the DWH oil spill had no significant impact on oyster PAH tissue content in these four regions of the Florida Gulf Overall mean oyster densities decreased 28.1% from 2010 to 2011 (2,332.9 m⁻² Coast. +655.2 SD and 1,675.7 m^{-2} + 537.2 SD respectively), probably representing natural interannual variation in either population dynamics or the effects of local stressors. All sites had larval supply, as indicated by concurrent recruitment to deployed oyster substrate trays, that often had higher densities, but smaller mean sizes, than natural reefs. This illustrates that the oyster populations at our study sites had not developed complete size distributions within the ~8 month period studied. Oyster growth in shell height (SH) was similar between regions but highly variable within a reef site, averaging 2-4 mm / mo but ranging from essentially no growth -4 (shell lip breakage) to +12 mm / mo. Reef morphology also varied among the four Regions, at least partly due to tidal range and local features of aspect, temperatures, disease, levels of predation, etc.

The invertebrate community of the oyster reefs was abundant and speciose. Species dominance varied across the Region studied. Mud crabs, particularly, Xanthid *Eurypanopeus depressus*, and the Porcellanid anomuran *Petrolisthes armatus* were dominant many sites; and, several mussels (including *Branchidontes exustus* and *Geukensia granosissima* in southern Regions and *B. exustus* and *Ischadium recurvum* in the northern Region) shared dominance of the sessile species with oysters. Gastropods, especially *Crepidula* spp and *Cerithium* spp were important members of the assemblage at nearly all of the Sites, although both were less common in the northern Region.

Population genetic results indicate that there is a difference between Florida populations and those in the rest of the Gulf of Mexico. However, Gulf sites outside Florida were not different from one another. This suggests that current patterns and local recruitment may combine to keep Florida somewhat isolated from larvae originating from other sites. However, further studies are needed on this point. There were no differences in genetic diversity from 2010 pre-spill, and 2011 post-spill, even in sites in Louisiana where oil and/or freshwater discharges caused high mortalities. This suggests that the larval pool available for the initial recolonization process in disturbed areas was sufficiently diverse in genetic makeup to represent the local / regional population in that area.

2. Results and Scientific Highlights

Defining the Problem: Eastern oyster, Crassostrea virginica, forms intertidal and subtidal reefs along the Atlantic and Gulf of Mexico coasts from Canada to Mexico and have undergone significant declines since the early 20th century (e.g., Kirby et al. 2004, Beck et al. 2009, 2011, Seavey et al. 2012, zu Ermagassen et al. 2012). Oyster reefs are essential habitats in estuaries as they provide numerous valuable ecosystem services and functions. (Coen et al. 1999, 2007; Coen ASMFC 2007). As biofilters, ovsters reduce contamination in the water column by removing phytoplankton, sediments, and pollutants through filter-feeding (reviewed in Dame 1996, Coen and Luckenbach 2000). Through the filtration process, ovsters can sequester carbon via shell production and produce concentrated biodeposits (feces or pseudofeces) which serve as a food source for other benthic organisms (reviewed in Dame 1996, Coen et al. 1999, Coen and Luckenbach 2000, Dame et al. 2001, Newell et al. 2005, Grizzle et al. 2006, 2008, zu Ermagassen et al. 2013). They also are involved in nutrient sequestration (e.g., Piehler and Smyth 2011). Further, the complex biogenic reefs formed by oysters provide hard substratum for other species to settle on or take refuge within and have been demonstrated to increase species abundance and diversity (Coen et al. 1999, Coen and Luchenbach 2000) and to stabilize shorelines (e.g., Meyer et al. 1997, Grizzle et al. 2002, Piazza et al. 2005, Coen et al. 2007, Grabowski and Peterson 2007, Grabowski et al. 2013). On Florida's Gulf coast, there is also a significant oyster fishery in the Big Bend region. In response to the DWH spill, we studied the ecology and genetics of oysters and the habitat they form that were presumed to be at risk from oiling.

We still have a poor understanding of the status and changes associated with either critically valuable intertidal or subtidal oyster habitats throughout the Gulf of Mexico (see Beck et al. 2009, 2011, zu Ermagassen et al. 2012, Seavey et al. 2012) or for that matter many marine habitats (Beck et al. 2001, 2003). For oyster reef habitats we are just starting to quantify rigorously the services rendered by these important ecosystem engineers (e.g., Peterson et al. 2003a,b, Piehler and Smyth 2011, Grabowski et al. 2013, zu Ermagassen et al. 2013). Although research has shown that oysters provide critical biogenic habitat, most sites in the Gulf of Mexico lack historical or current spatial reef data, including quantitative information on population demographics and the biologically-diverse reef communities. Additionally, limited historical data exists from past studies, and even if available, studies used many different methodologies, often with little or no replication. Baseline data are critical to adequately assess potential impacts from discharges related to the Deepwater Horizon (DWH) spill, related restoration and post-recovery status, as well as anticipated future stressors such as climate change, HABs, pollutants, eutrophication, low DO and demographic shifts. In terms of restoration science for oyster habitats, clearly we still have a long way to go (e.g., NRC 2010, Kennedy et al. 2011, Baggett et al. 2013)

A. FIELD ECOLOGY

Study system

Site selection: Our assessment of the DWH spill on oyster reefs and their associated species within Florida Gulf estuaries began in November 2010. For oyster populations, reef

communities, and PAH analyses, we divided the coast into four latitudinal "Regions". These, from north to south were Big Bend (FSU), Tampa Bay/St. Petersburg (FWRI), Ft. Myers (FAU, FGCU), and Rookery Bay (FAU). At each Region, we established three "Sites", and at each Site choose five distinct oyster "Reefs" (see subsection below on Reef Selection). The nine study sites targeted by this study ranged from Apalachee Bay to the Ten Thousand Islands of FL (~800 km). Within each of the four Regions, the three clustered Sites typically represented different embayments over a wide spatial scale in that Region. Maps of all sites are shown in a series of pages constituting Fig.1.

<u>Reef Selection:</u> Within each Site, we then selected five replicate oyster Reefs that were at least 400 m² in area (where possible) and were spaced at least 100 m apart. Overall, these reefs comprised a diversity of conditions in terms of shoreline slope, tidal inundation, and sediment characteristics that we assessed during the study, the first of its kind using identical methods for sampling. Furthermore, within each site, we strove to select a mixture of reef types so that our sampling reflected all possible reef conditions. For instance, within each of the northern Florida sites, we partitioned the five replicate reefs between those that fringed salt marsh (*Spartina alterniflora*) or mangroves (Rookery Bay and Ft. Myers sites, and those that were isolated or were non-fringing oyster patch reefs. Most reefs tended to be the latter isolated patch reefs.

Figure 1. Maps of each study site are shown by Region (arranged from north to south) in the ensuing pages.

























Climate of the Four Regions:

We accessed data from <u>www.sercc.com/climateinfo/historical/historical_fl.html</u> to develop summaries of climate (Table 1). The climate data indicate similar rainfall in the four Regions, and similar temperature patterns in the three southern Regions, while the northernmost Big Bend region had a greater number of months with cool temperatures.

regions from www.serce.com/ennacemro/instorical/instorical_fi.itim.											
	Long Terr	ong Term Averages									
	Ann. Max Temp (F)	Ann. Min. Temp (F)	Aver Tot Precp (in)	Number of months/yr GE 90 F	Number of months/yr LE 60 F	Number of months/yr LE 50 F					
Big Bend (St Marks)	78.6	57.7	53.76	3	8	5					
Tampa Bay (St. Pete)	81.4	66.2	51.7	2	4	0					
Ft Myers	83.6	64.5	53.67	3	4	0					
Rookery Bay (Naples)	84.7	64.7	53.45	4	4	0					

Table 1. Long term averages of patterns of temperature and rainfall in the four Gulf Coast Regions from <u>www.sercc.com/climateinfo/historical/historical_fl.html</u>.

Salinities:

Sites were typically chosen to include reefs relatively near passes or the mouth of estuaries with the assumption that these would be most likely to be exposed to oil, had any arrived.

- At Rookery Bay, mid-estuary salinities near our Sites had a mean of 30.7 +/- 3 psu (Surge and Lohmann 2002).
- Based on a limited number of discrete sample events, the salinities at Ft. Myers site were 30.2 +/- 6.12 (SCB), 32.2 +/- 3.63 (TB) and 32.8 +/- 3.80 (PIS). A nearby datalogger (SCCF RECON), reports salinity up-estuary from our study sites had a maximum range of ~ 24 33 psu during 2011. The sites are exposed to managed outflows from the Caloosahatchee River which can be extreme during wet years but were not in 2011.
- In the Tampa Bay region annual averages of water salinity were 33.2 +/- 0.84 ppt (FD), 31.9 +/- 2.47 ppt (GP), and 31.1 +/- 2.90 ppt (PP), very little variability for an oyster reef. The source of FW is almost entirely local precipitation. The Gulfport reefs receive the most urban runoff of any of the 12 sites included in this study.
- In the northern Big Bend region annual averages of water salinity at these sites were 32.3 psu, 25.2 psu, and 18.2 psu, respectively. This broad range of salinity most likely reflects spatial variation in the amount of fresh water discharge. While Alligator Harbor's source of FW reflects the influence of local precipitation, the oyster reefs of Shell Point and St. Marks annually receive 604.7 ft³/sec and 722.1 ft³/sec of fresh water from the Ochlockonee and St. Marks Rivers, respectively (based on 2010 and 2011 annual averages, USGS).

Annual Assessment of Oyster Reefs:

<u>Community Structure of Natural Oyster Reefs:</u> In December 2010, we sampled the community structure of oyster reefs in a manner that accounted for spatial variation in oyster reef size both within and among sites. The standardization of where to sample on reach involved three steps. First, we calculated a reef's specific dimensions and, perpendicular to the shoreline, bisected the reef into left and right-hand halves. Second, and based on the width of each half of the reef (i.e., distribution of oysters from low to higher intertidal), we bisected both halves so that they contained and upper and lower intertidal section. Third, we measured the distance of the intersection of the upper and lower sections and placed a 0.14 m^2 quadrat at the midpoint of this line.

Before excavating all of the reef material within each quadrat, we recorded the GPS coordinates and typically marked them with PVC poles in order to prevent sampling the same location the following year. All contents were then harvested and were typically transported back to the lab or field station for sorting/processing.

Mensurative Experiments

<u>Colonization of oyster reef community (Tray data)</u>: Trays (0.14 m²) were filled with recently dead oyster shell obtained in Tallahassee (M. Berrigan, FL DACS). In Jan.-Feb. 2011,

replicated plastic trays (n=2 per reef) for recruitment of organisms and oysters were placed in the field into depressions made in the reefs so that elevations were similar to the natural reef. Large mesh (1 ¼", XB-1133 polyethylene covered the trays (top and bottom) to keep shell in, but still allowing many predator species access. Tray locations were adjacent to locations sampled by quadrat and to the cages (see below). Colonization by oysters and associated invertebrates was recorded as replicate trays were collected at different times.

<u>Growth and survivorship of individual oysters (cage data)</u>: In January 2011, we manufactured cages (1/8" polyethelyene mesh, black color, held together with cable ties) to keep oyster predators out. At each site, we collected oysters from each quadrat (see above) and brought them back to the lab. At the lab, we cleaned, tagged, and measured the size of 5 oysters (2-4cm or smaller) were tagged and measured and placed in cage. Cages were placed directly next to quadrat extraction using gps coordinates, and Cages were staked or weighed down with half of brick at each end (cage n=2 per reef; reef n=5/site; 3 sites). Every six weeks, each team checked on the size and survivorship of each surviving individual oyster (January 2011 – September 2011).

<u>Contamination of oyster reefs by PAH:</u> Six individual oysters were collected from each reef and sent to Co-PI Weinstein for toxicity processing. Sampling occurred in Fall 2010 and Fall 2011.

<u>Genetic structure of oyster population</u>: November 2010 and Oct. 2011: A minimum of 45 oysters were collected from reefs in each Region. These were shipped to FAU colleagues (Nance and Proffitt) for population genetic analyses. Oysters from 11 additional locations around the Gulf of Mexico were obtained and also used in genetic analyses.

<u>Counting and Taxonomic Identification Methods</u>: Oyster Population and Associated Species Analyses: Quadrat and tray samples were sieved through a 1 mm mesh. Taxa were placed in jars of formalin and later transferred to ethanol. All individuals were identified to lowest practicable taxon, usually species, and selected identifications were checked by professional taxonomists at Applied Ecology, Inc. A voucher collection is maintained at FAU (Proffitt lab). Herein, only selected numerically abundant species are discussed.

Field Ecology Results:

Oyster Reef Analyses

The average reef size in our study was larger in the Big Bend region (1144 m^2) and Pine Island Sound (673 m^2) than Tampa Bay (333 m^2) and Fort Myers (265 m^2) . Despite relatively small variation in tidal range, from 1 - 2 m over our entire study area, we can detect a correlation between tide (x) and relief (y) (r=0.72), see Fig. 2. The Big Bend region also had the highest elevation, highest tidal ranges and rugosity. However, Rookery Bay, which had the smallest reefs, did have the steepest slopes, perhaps due to the surrounding sediment type, soft mud, which would restrict lateral reef growth. While not exhaustive, the results presented here serve as a template for describing aspects of the morphology of Florida's reefs, and suggest physical

characters may define limits and characteristics which could be useful in assessing change and restoration success. All oyster and community data are subject to some degree of change as we recheck some samples and IDs to ensure accuracy.



Figure 2. The relationship between tidal range and reef topographic relief.

The mean abundance of oysters at all Regions and Sites was 1544 per m², ranging ~ 142-6722 for individual reefs sampled by quadrats. The abundance of oysters on natural reefs was highest in the Ft. Myers region in both sample periods but among-site and among-reef variation was also high in all regions. Density on reefs at three of the four Gulf coast regions declined slightly between years, but these changes did not appear biologically important, probably reflecting variability in the timing of larval settlement in each estuary. Trays (containing recently dead shell) were rapidly colonized and much more densely populated (mean was 3650 / m²; ~ range 39 – 12,328), and had mean oyster shell height sizes considerably smaller than the natural reefs. Recruits were filling vacant ecological space (unoccupied shell) and had not reached full population development during the ~ 8-months deployment.

In cages where live oysters were deployed, oyster growth averaged 1.74 mm per month with individual variability exceeding regional or localized variability. Mean growth for individual sites and sample periods ranged from -1.93 to +6.75 mm / mo, with no easily discernible spatial pattern. Individual variability was even higher, again with no apparent pattern. Growth was higher in March through August, as well as October, than in September or January-February.

Invertebrate Assemblage Dominance

Numerical dominance of mussel and decapod species changed substantially among Regions. The mussels *Brachidontes exustus* and *Ischadium recurvum* that were in high abundances in northern Regions, were largely replaced by *Geukensia demissa* in the south.

Moreover, the mud crab *Eurypanopeus depressus* very abundant in the Big Bend, become much less abundant further south (Table 1). The invasive and apparently non-native green

porcelain crab, *Petrolisthes armatus*, occurred at each Region but was in somewhat lower abundances in more southerly Regions.

Gastropods (e.g., *Melongena corona* and *Boonea impressa*) known to prey on oysters occurred in samples only in some regions, but probably exist at all of them. Crepidulids also occurred attached to oysters in each region, but were not quantified in all Regions, so abundances are not presented here. Several species of the small gastropod *Cerithium* occurred in all regions, and in some cases these mud-and-micro algae eaters were in extremely high abundances ($\approx 17,000 / m^2$).

Table 1. Mean (1 S.D.) numbers $/ m^2$ of selected mussel and decapods species showing substantial shifts in abundance among Regions. Bottom, the 2010 and 2011 mean oyster abundances in 2010 and 2011 are provided as background information on abundance of the systems' foundation species.

	Big Bend		Tampa E	Bay	Ft. Myeı	S	Rookery	Bay
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Brachidontes								
exustus	372.1	14.3	137.1	92.1	50.0	59.3	0.0	0.0
Geukensia								
demissa	7.1	20.0	377.9	476.4	22.9	26.4	0.0	0.0
Geukensia								
granosissima	0.0	0.0	0.0	0.0	0.0	0.0	50.0	60.6
Ischadium								
recurvum	162.9	97.1	27.9	57.1	60.7	70.7	0.0	0.0
Eurypanopeus								
depressus	3007.1	102.9	304.3	315.7	282.1	123.6	198.8	89.0
Petrolisthes								
armatus	131.4	105.7	34.3	25.0	32.1	30.0	347.9	180.2
	Region Me	ans (2010	& 2011)	-				
2010-2011	2010	2011	2010	2011	2010	2011	2010	2011
Crassostrea								
virginica	2329.3	1104.3	1847.1	1933.6	3259.3	2339.3	1810.7	1416.7

B. POPULATION GENETIC ANALYSES:

The genetic component of this project covered a wider geographic area, from Rookery Bay in south Florida to Aransas Bay, Texas (Fig. 3), but also included the fine geographic scale of the reefs defined in the Field Ecology portion of the study (Fig. 1). The goal of this study was to assess the impacts of the BP oil spill on reefs across this range by characterizing genetic diversity and population structure among C. virginica before and after the spill. As we did not collect oysters for these genetic analyses before the 2010 spill, our 'pre-spill' sample consisted of oysters collected in fall 2010 that were sufficiently large (>60mm total length) to ensure they had been spawned, completed their entire larval stage, and settled prior to the spill. In this way, these 'pre-spill' oysters represent the genetic composition of reefs prior to the spill. Our postspill samples came from the same reefs sampled in fall 2010 (with the exception of Sabine Lake and Calcasieu Lake, LA), but were collected in fall 2011 and were sufficiently small (<60mm total length) to ensure they were spawned after the spill. With these two samples, we characterized patterns of population genetic structure (differentiation) both within and between estuaries, and between sampling years. We also compared genetic diversity (a proxy for effective population size) spatially and temporally to determine whether regions that had been heavily oiled had experienced a loss of diversity post-spill.

Genetic variation was characterized by genotyping 763 individual oysters across 17 reefs in 2010 and 842 oysters in 2011 at six nuclear microsatellite loci (Brown et al 2000, Reece et al 2004). We also sequenced 542 basepairs of the mitochondrial (mtDNA) gene cytochrome oxidase I (COI) from 418 individuals collected in 2011. With these genetic data, we first characterized patterns of genetic structure using F-statistics calculated with Arlequin v. 3.5 (Excoffier and Lischer 2010). Genetic variation at the microsatellite loci, as measured by F_{ST} , was weak but statistically significant across the entire Gulf region ($F_{ST} = 0.01$, p = 0.00 for 2010, $F_{ST} = 0.03$, p = 0.00 for 2011). There was significant genetic variation both within and between estuaries, as measured by F_{CT} [genetic variation between circled estuaries (Figure 2)] and F_{SC} [genetic variation between reefs within an estuary (Figure 2)]; $F_{CT} = 0.01$, p = 0.00 in 2010, F_{CT} = 0.01, p = 0.00 in 2011; F_{SC} = 0.01, p = 0.02, F_{SC} = 0.01, p = 0.00 in 2011. Pairwise F_{ST} characterizing genetic variation between all possible pairs of reefs in 2010 and 2011 showed a genetic 'break' between the northern Gulf region (TX, LA, AL) and the Florida Gulf coast, with the majority of significant [after sequential Bonferroni correction of alpha (Rice, 1989)] differences found between Florida and the northern Gulf coast region (see Figures 3, 4, and 5 for pairwise F_{ST} values based on microsatellite analyses on 2010 and 2011 oysters, and mtDNA analyses from 2011 oysters).

The large-scale pattern of genetic differentiation between the northern Gulf region and the Florida Gulf coast was further shown using the population assignment method implemented by STRUCTURE (Hubisz et al 2009). This method relies on microsatellite genotype data to group individuals such that Hardy-Weinberg and Linkage equilibrium are maximized (Hubisz et al 2009), and therefore shows population subdivision across sampled individuals. In both 2010 and 2011, STRUCTURE found two groups across the Gulf region – one from Florida and the other from the remaining northern Gulf region, with northern Florida (reefs at St. Marks and Alligator Harbor) representing a transition zone with individuals of 'mixed' Florida and northern Gulf ancestry (Fig. 6).

Temporal patterns of structure were found between most reefs sampled using Arlequin v. 3.5 (Excoffier and Lischer 2010), as F_{ST} values for oysters collected from the same reefs across years (2010 and 2011) were low but statistically significant (Table 2). Despite these temporal differences in the allelic composition of reefs before and after the spill, levels of gene diversity (Θ) as calculated in Arlequin v. 3.5 (Excoffier and Lischer 2010), were neither significantly different among reefs across the entire Gulf region, nor among reefs across sample years (Θ ranges from 0.78 (TAB2010) to 0.89 (SAB2011)).

These genetic analyses suggest that large-scale patterns of genetic variation exist among Gulf of Mexico populations of C. virginica, primarily between the Gulf coast of Florida and the remaining northern Gulf region comprising AL, LA, and TX. This large-scale pattern of genetic structure was found with both nuclear microsatellite markers and mtDNA, and was temporally stable across our sampling period (2010 and 2011, representing 'pre' and 'post' spill, respectively). Temporal stability in this broad pattern of genetic structure, and the lack of significant differences in gene diversity among reefs across the entire Gulf region and between individual reefs across years, suggest that either the impact of the BP oil spill was minimal among reefs that we sampled, or (more likely) genetic connectivity is sufficiently high and populations are sufficiently large such that high mortality from the spill in one area is buffered by the large number of recruits coming from other nearby areas. Although there were weak patterns of population structure between some reefs and estuaries, as shown with F-statistics, the major patterns evident in the pairwise matrices (Figs. 4-7) and STRUCTURE results (Fig. 8) suggest that gene flow is high both within the Florida region and the northern Gulf region, but low between these two regions. This genetic 'break' was temporally stable and evident at both nuclear and mitochondrial markers. Given the high connectivity within, but not between, these two regions in the Gulf, mitigation of future oil spills and fishery management should consider the oysters in these two regions as distinct stocks.



Figure 3. Reefs sampled for genetic analyses in 2010 and 2011. Reef names and abbreviations are as follows, from west to east starting in Texas: Texas: Lap Reef (LAP), Causeway South (CWS), Halfmoon Reef (HMR); Louisiana: Sabine Lake (SAB), Calcasieu Lake (CSL), Caillou Lake (CAL), Grand Bayou DuLarge (GBD), Breton Sound (BS); Alabama: Sandy Bay (SBW), Perdido Pass (PP); Florida: Alligator Harbor (ALH), St. Marks (SMA), Tampa Bay reef 1 (TB1), Tampa Bay reef 2 (TB2), San Carlos Bay (SCB), Tarpon Bay (TAB), Cat Claw Trail (CCT),

Rookery Bay dock (RBD). Note: Sabine Lake (SAB) and Calcasieu Lake (CSL) were only sampled in 2011.



Figure 4. F_{CT} characterized genetic variation between estuaries (circled regions); F_{SC} characterized genetic variation between reefs within an estuary or smaller region (red circles represent reefs within a circled estuary).

	RBD CCT TAB SCB TB1 TB3 SMA RD * -						A	L		LA			ТΧ			
	RBD	ССТ	TAB	SCB	TB1	TB3	SMA	ALH	РР	SBW	BS	GBD	CAL	HMR	CWS	LAP
RBD	*															
ССТ	0.01	*														
TAB	0.00	0.01	*													
SCB	0.00	0.01	0.01	*												
TB1	0.00	0.00	0.01	0.00	*											
твз	0.00	0.01	0.01	0.01	0.00	*										
SMA	0.00	0.01	0.01	0.01	0.00	0.00	*									
ALH	0.00	0.01	0.01	0.01	0.00	0.01	0.00	*								
PP	0.02	0.03	0.03	0.01	0.01	0.01	0.01	0.02	*							
SBW	0.02	0.03	0.03	0.02	0.01	0.02	0.01	0.01	0.01	*						
BS	0.01	0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.00	*					
GBD	0.02	0.04	0.04	0.03	0.02	0.02	0.01	0.03	0.00	0.01	0.00	*				
CAL	0.02	0.04	0.03	0.02	0.02	0.01	0.02	0.03	0.01	0.01	0.01	0.00	*			
HMR	0.02	0.03	0.02	0.02	0.02	0.01	0.01	0.02	0.01	0.00	0.01	0.00	0.01	*		
CWS	0.01	0.01	0.02	0.01	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.01	0.00	*	
LAP	0.01	0.02	0.02	0.01	0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.01	*

Figure 5. Pairwise F_{ST} values for all possible pairs of reefs sampled in 2010, based on microsatellite data. Reef abbreviations are listed in Figure 3. Values in bold are significant at the 0.05 level; values highlighted in green are significant after sequential Bonferroni correction of alpha (Rice 1989).

				F	Ľ				Α	L			LA				ГХ	
	RBD	ССТ	ТАВ	SCB	TB1	TB3	SMA	ALH	РР	SBW	BS	GBD	CAL	CSL	SAB	HMR	CWS	LAP
RBD	0.00																	
CCT	0.01	0.00																
TAB	0.00	0.01	0.00															
SCB	0.00	0.00	0.00	0.00														
TB1	0.01	0.00	0.01	0.00	0.00													
TB3	0.01	0.01	0.01	0.01	0.01	0.00												
SMA	0.01	0.00	0.02	0.01	0.01	0.02	0.00											
ALH	0.01	0.01	0.01	0.01	0.02	0.03	0.00	0.00										
PP	0.02	0.01	0.02	0.01	0.02	0.03	0.01	0.01	0.00									
SBW	0.02	0.01	0.01	0.01	0.01	0.02	0.01	0.02	0.00	0.00								
BS	0.03	0.01	0.02	0.01	0.01	0.03	0.01	0.02	0.01	0.01	0.00							
GBD	0.03	0.02	0.03	0.02	0.02	0.03	0.01	0.02	0.00	0.01	0.01	0.00						
CAL	0.03	0.01	0.02	0.02	0.02	0.03	0.01	0.01	0.00	0.01	0.00	0.00	0.00					
CSL	0.02	0.02	0.02	0.02	0.02	0.02	0.03	0.04	0.02	0.01	0.03	0.02	0.02	0.00				
SAB	0.02	0.02	0.02	0.01	0.02	0.02	0.02	0.03	0.01	0.01	0.03	0.02	0.02	0.01	0.00			
HMR	0.02	0.02	0.02	0.01	0.02	0.03	0.01	0.02	0.00	0.01	0.01	0.00	0.01	0.02	0.02	0.00		
CWS	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.02	0.02	0.01	0.01	0.00	0.01	0.00	
LAP	0.03	0.02	0.02	0.01	0.02	0.03	0.01	0.02	0.00	0.01	0.01	0.00	0.01	0.02	0.02	0.00	0.02	0.00

Figure 6. Pairwise F_{ST} values for all possible pairs of reefs sampled in 2011, based on microsatellite data. Reef abbreviations are listed in Figure 3. Values in bold are significant at the 0.05 level; values highlighted in green are significant after sequential Bonferroni correction of alpha (Rice 1989).

				I	FL					4L			LA				ТΧ	
	RBD	ССТ	TAB	SCB	TB1	TB3	SMA	ALH	PP	SBW	BS	GBD	CAL	CSL	SAB	HMR	CWS	LAP
RBD	*																	
CCT	0.00	*																
TAB	0.02	0.00	*															
SCB	0.00	0.00	0.01	*														
TB1	0.02	0.01	0.00	0.03	*													
TB3	0.03	0.00	0.00	0.01	0.01	*												
SMA	0.10	0.09	0.14	0.14	0.06	0.15	*											
ALH	0.06	0.07	0.12	0.11	0.05	0.13	0.00	*										
PP	0.19	0.18	0.23	0.24	0.12	0.24	0.01	0.04	*									
SBW	0.26	0.23	0.28	0.32	0.17	0.29	0.04	0.09	0.00	*								
BS	0.30	0.26	0.32	0.38	0.19	0.33	0.05	0.12	0.00	0.00	*							
GBD	0.39	0.33	0.39	0.47	0.26	0.40	0.11	0.20	0.03	0.02	0.00	*						
CAL	0.31	0.28	0.33	0.38	0.20	0.34	0.07	0.13	0.01	0.00	0.00	0.00	*					
CSL	0.23	0.21	0.26	0.29	0.15	0.27	0.03	0.07	0.00	0.00	0.00	0.01	0.00	*				
SAB	0.22	0.22	0.26	0.29	0.16	0.27	0.04	0.08	0.00	0.00	0.00	0.00	0.00	0.00	*			
HMR	0.25	0.23	0.28	0.32	0.16	0.29	0.04	0.09	0.00	0.00	0.00	0.01	0.00	0.00	0.00	*		
CWS	0.19	0.19	0.23	0.25	0.13	0.24	0.02	0.06	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	*	
ΙΔΡ	0 31	0.28	0 33	0 38	0.21	0 34	0.08	0 14	0.02	0.01	0.01	0.01	0.00	0.01	0.00	0.01	0.01	*

Figure 7. Pairwise ϕ_{ST} values for all possible pairs of reefs sampled in 2011, based on mtDNA data (ϕ_{ST} is mtDNA analog to F_{ST} , and a similar measure of genetic variation among groups). Reef abbreviations are listed in Figure 3. Values in bold are significant at the 0.05 level; values highlighted in green are significant after sequential Bonferroni correction of alpha (Rice 1989).



Figure 8. Graphical representation of STRUCTURE (Hubisz et al 2009) results, based on microsatellite genotypes from oysters in 2010 (top) and 2011 (bottom). Numbered bars represent individual reefs, starting with 1 at Rookery Bay, FL and going northwest to reefs in Texas. Individual, thin red and/or green lines within bars represent the proportion of ancestry for each individual oyster. Both figures show that there are two distinct groups, red and green, corresponding to oysters collected along Florida's Gulf coast and oysters collected along the Gulf coasts of Alabama, Louisiana, and Texas, respectively. Note red bars 7 and 8, which correspond to reefs at St. Marks and Alligator Harbor, FL. These reefs in Florida's northern Gulf coast region, represent a transition zone as individual oysters have a mixed ancestry consisting of 'red' Florida and 'green' northern Gulf coast.

Table 2. F_{ST} values for oysters collected from the same reefs in 2010 and 2011, and corresponding p-values. F_{ST} values in green are significant at the 0.05 level. Reef abbreviations are listed in Figure 3.

	F _{ST}	P-value
RBD	0.005	0.008
ССТ	0.022	0.000
ТАВ	0.014	0.000
SCB	0.004	0.021
TB1	0.003	0.058
TB3	0.001	0.337
SMA	0.016	0.000
ALH	0.025	0.000
PP	0.010	0.000
SBW	0.000	0.754
BS	0.020	0.000
GBD	0.000	0.469
CAL	0.002	0.129
HMR	0.005	0.022
CWS	0.000	0.953
LAP	0.029	0.000

C. **PAH ANALYSES:**

Results

- Review of the PAH data suggests that there were little to no oil residues present in any of the oysters sampled during this study. In general, levels of Total PAHs from the various regions were consistent with background levels established in previous studies (Fig. 9). None of the oysters sampled in this study had levels of PAHs above those established by the FDA as levels of concern.
- Among regions (see Field Ecology) and sampling dates, Tampa Bay oysters sampled in April 2011 (379.0 ± 156.7 ng/g) had significantly higher levels of Total PAHs compared to all other regions and sampling dates (165.7 ± 35.3 ng/g). These elevated levels are probably related to local suburban and urban sources, rather than the oil residues resulting from the Deepwater Horizon oil spill. Future isomer ratio analysis will be conducted to determine the potential sources of these elevated PAHs.

- Within regions, several site differences were noted with regard to Total PAHs (Fig. 9). At Big Bend sites sampled in October 2011, St. Mark Reef oysters (157.3 ± 62.6 ng/g) had significantly higher levels compared to Alligator Harbor Reef oysters (106.0 ± 31.1 ng/g). At Pine Island sites sampled in May 2011, San Carlos Bay oysters (217.6 ± 104.2 ng/g) had significantly higher levels compared to Tarpon Bay oysters (88.2 ± 24.4 ng/g). At Tampa Bay sites sampled in both December 2010 and December 2011, Gulfport oysters (361.4 ± 711.5 ng/g and 329.2 193.7 ng/g) had significantly higher levels compared to Fort Desoto (165.1 ± 126.2 ng/g and 67.3 ± 34.1 ng/g) and Pinellas Point oysters (96.8 ± 41.8 ng/g and 95.5 ± 34.2 ng/g).
- These data establishes a baseline of PAH levels within these four regions such that the impact of any future oil spills affecting Florida Gulf Coast oysters may be assessed



Figure 9. Mean Σ PAH₁₆ levels in oysters collected from sites in the Big Bend, Tampa Bay, Pine Island, and Rookery and Johnson Bay regions. Those sites within a sampling period sharing the same letter (A or B) within a region are similar. Differences between regional means in this study and that of the10-year regional means for NOAA Mussel Watch locations are indicated by an asterisk. Error bars = +1 SD. n.s.= not significant.

Report Acknowledgments

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We thank Dr. Donna Devlin who led the team doing the first field sampling in Rookery Bay. Moreover, her keen and insightful editorial comments greatly improved the final version of this report.

We appreciate all the hard work by Elizabeth Salewski (former FAU graduate student) in identifying most of the invertebrates collected at nearly all sites in the project, overseeing further confirmation of ID's by Ecological Associates, Inc., and for assisting in the field and lab with oyster counting and measuring.

Two FAU graduate students, Pedro Lara and Dana Smith, assisted in much of the field and lab work. Tampa Bay sampling was largely conducted by Richard Radigan, Ashley Hurley, Michael Drexler, Anthony Vasilas, Bethany Pierce and Janessa Cobb.

Dr. Susan Laramore assisted in supervising the genetics post doc.

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Bryan Piazza - Nature Conservancy Bill Walton and Glen Chaplin - Auburn Univ. Megan and Jerome Lapeyre - LSU Earl Melancon - Nicholls State Univ. Jennifer Pollack and Brittany Bloomberg - TAMU Corpus Christie.

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- Beck, M.W., R.D. Brumbaugh, L. Airoldi, A. Carranza, L.D. Coen, C. Crawford, O. Defeo, G.J. Edgar, B. Hancock, M. Kay, H. Lenihan, M.W. Luckenbach, C.L. Toropova, and G.

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3. **Peer-Reviewed Publications**, if planned (Note: a special section will focus on student and post-doctoral publications)

Manuscripts on both field ecology and population genetics will be prepared once all data are rigorously analyzed by the respective investigator teams.

4. **Presentations and Posters**, if planned (Please provide copies of each) (Note: a special section will focus on student presentations). PDFs attached.

Title	Presenter	Authors	Meeting or Audience	Abstract	Date
				published	
				(\mathbf{Y}/\mathbf{N})	
Population genetics	H. Nance	Nance, H & C.E.	Coastal & Estuarine	Y	11/11
response to the		Proffitt	Research Federation		
DWH oil spill in					
Eastern oysters,					
Crassostrea					
virginica					
Baseline assessment	S. Geiger	S. Geiger, C.E.	National Shellfish	Y	3/12
of oyster reefs and		Proffitt, L.D.	Association		
their associated		Coen, H. Nance,			

fauna in multiple		D.L. Kimbro,			
estuaries		J.E. Weinstein			
Spatial and temporal J.		J. Weinstein, J.	Gulf of Mexico Oil	Y	1/13
trends in PAH levels W	Veinstein	Miller, C.E.	Spill & Ecosystem		
of oysters from the		Proffitt, D.L.	Science Conference		
Florida Gulf Coast		Kimbro, S.			
following the		Geiger, L.D.			
Deepwater Horizon		Coen			
oil spill					
Florida Gulf Oysters C	Coen	LD Coen, CE	Coastal & Estuarine	?	11/11
and Associated		Proffitt, SP	Research Federation		
Fauna: Effects of the		Geiger, DL			
Deepwater Horizon		Kimbro, & JE			
Oil Spill		Weinstein			
and Setting Baseline					
Conditions For					
Future Disturbances					
An Overview of L	L. Coen	L. Coen	FMFEI meeting	Ν	7/11
Current Oyster					
Restoration-					
Assessment					
Efforts Across the					
U.S. with FL					
Emphasis: Related					
Goals, Metrics and					
Methodologies					

5) Other Products or Deliverables

Our information was used in the development of:

Baggett, L.P., S.P. Powers, R. Brumbaugh, L.D. Coen, B. DeAngelis, J. Green, B. Hancock, and S. Morlock, 2013 A handbook of monitoring guidelines and criteria for judging the performance of oyster restoration projects. TNC Report

Boswell, J.G. J.A. Ott, and A. Birch, 2012, Charlotte Harbor National Estuary Program Oyster Habitat Restoration Plan, Charlotte Harbor National Estuary Program, Technical Report, December 2012, 169pp plus appendices.

6) Data

Data, once collections are completed and data compiled and analyzed, will be submitted to GRI data management).

PARTICIPANTS AND COLLABORATORS

7) Project Participants

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							р
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				m			
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		superv.	FAU	u			
Holly	Nance	Co-	FAU	hnance@hboi.fau.edu	F	С	USA
		PI/Postdoc					

MENTORING AND TRAINING

8) Student and Post-Doctoral Participants

First	Last Name	Post-doc /	Thesis or	Institution	Supervisor	Expected	Gender	Race	Citizens
Name		PhD / MS /	research topic			Completion			hip
		BS				year			
Holly	Nance	Post-doc	Oyster	FAU	Proffitt/Lar	2012	F	С	USA
			population		amore				
			genetics						
Pedro	Lara	MS	Oysters, dermo,	FAU	Proffitt	2013	М	С	USA
			and reef vs						
			mangrove prop						
			roots						
Elizabeth	Salewski	MS	Oysters in	FAU	Proffitt	Graduated	F	С	USA
			St.Lucie/invert.			summer			
			community BP			2012			

9) Student and Post-Doctoral Publications, if planned

Nance, Holly. and C.E. Proffitt. Journal article on spatial and temporal differences in population genetics of oysters. Manuscript in prep.

Lara, Pedro. Journal article on red mangrove and other intertidal refuges from Dermo. Pending completion of thesis.

10) **Student and Post-Doctoral Presentations and Posters**, if planned (Please provide copies of each)

Presentation by Nance listed above

11) Images

Please attach high-resolution image and provide details including a description of the image, location, credit, date, etc. Of note: Image may be used in GoMRI promotions, make sure you have rights to use the image. Note: GoMRI will establish a Flicker site to share these images through the GoMRI website and with media and the public.

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