

### **1.3.4 Invertebrate Population and Community Studies for Assessing Coastal Gulf of Mexico Environmental Health in the Aftermath of the *Deepwater Horizon* Oil Spill**

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#### **1.3.4.1 Abstract**

This contribution is intended to add a synthesis of information regarding programs and missions of research programs that generate baseline data that describe existing conditions of invertebrate populations and communities of the coastal GOM. First, we provide a brief history of the findings and outcomes of studies following the DWH oil spill on coastal GOM plants and animals based on selected literature from 2010–2016. Then, we describe research initiatives of the LSU entomology and the UNH genomics programs relative to the initial impact of the spill and recovery of invertebrate populations. Finally, we briefly describe objectives of current research initiatives and goals and objectives for future projects on invertebrate coastal ecology dependent upon future funding and potential international collaborations. Ultimately, we hope to help create and be active participants in comprehensive sampling programs of the invertebrates found in sediments of different coastal environments of the GOM. The goal would be to create a synthesis of reference databases and multiple-level linked metadata (geochemistry, bioinventories, and genetic information) by sharing samples and cyberinfrastructure among collaborating research groups.

#### **1.3.4.2 Introduction**

The purpose of the Gulf of Mexico Workshop on International Research (GOMWIR) was to establish international collaborations among scientists from Mexico, Cuba, and the United States and research priorities for the GOM. There were three thematic areas for open discussion, one of which was regarding recent and current studies that generate baseline data that describe existing conditions for use as future reference. The exchange of information on the research programs of the individual attendees was informal and oriented toward the goal of identifying mutual priorities in the thematic areas. These proceedings, in part, present an opportunity to share information about program missions and opportunities for collaboration.

In this article, we provide a brief history of the findings and outcomes of studies on coastal Gulf of Mexico (CGOM) plants and animals and based on selected literature from 2010 up to and including the proceedings of the GOMRI annual conference held in New Orleans, Louisiana, 6–9 February 2017. Subsequently, we describe the results of research initiatives of the LSU entomology and the UNH genomics programs relative to: 1) the initial impact of the oil spill and recovery of invertebrate populations, 2) objectives of current research initiatives, and 3) goals and objectives for future projects on invertebrate coastal ecology dependent upon future funding and potential international collaborations.

#### **1.3.4.3 Summary of Selected Literature (2010–2016) on the Effects of the DWH Oil Spill on Plants and Animals of CGOM Habitats**

The catastrophic explosion of the DWH drilling platform that occurred on 20 April 2010 caused the largest man-made marine oil spill to date with a total release of approximately 5 million barrels of oil (Whitehead et al. 2012). The physical spread of the petroleum and other contaminants was measured by large teams of scientists. The visible oil on the surface of the Gulf was tracked using aerial and satellite surveys. The locations and dates of the landfalls were recorded, catalogued, and made available to the public and scientists (ERMA 2017).

Of the 7,058 km of shoreline surveyed using the Shoreline Cleanup Assessment Technique (SCAT), 1,773 km were documented as ever having been oiled. The majority of shorelines with documented oiling occurred in Louisiana (60.6%), followed by Florida (16.1%), Mississippi (14.6%), and Alabama (8.7%). The major shoreline habitats oiled were beaches (50.8%) and marsh (44.9%). Most of the marsh oiling (94.8%) occurred in Louisiana, while the beach oiling was distributed across the four states with 32.9% in Louisiana and 21.1% in Mississippi.

Quantifying the impacts of the spill on the coastal habitats of the GOM that are of ecological and economic importance was and still is critical (Silliman et al. 2012). There have been six years of observations and detailed studies on the primary (acute), secondary (chronic), and tertiary/multitrophic effects of the oiling of pelagic and coastal ecosystems. The primary effects of the explosion of the DHW platform resulted in the loss of 11 human lives, and the subsequent acute oiling in the GOM created visible impact on prominent vertebrates, particularly birds, along the coast (Belanger et al. 2010). More than 7,000 marine mammals, turtles, and birds were found debilitated or dead during the summer months of 2010, and many of these cases were directly attributed to the animals making direct contact with the dispersing oil. For marine vertebrates initially shown to be negatively impacted, reports of recovery for whales (Tang 2017) and deepwater sharks (Gelsleichter 2017) were given at the recent GOM Oil Spill and Ecosystem Science (GOMOSSES) meeting, but secondary effects still remain for other vertebrate populations (discussed below).

During and after the spill, massive teams prowled the beaches and barrier island shores cleaning as oiling occurred. The bioavailability of polycyclic hydrocarbons (PAH) was shown to increase at stations at piers along the Louisiana coast after the explosion but returned to pre-oiling levels by March 2011 (Allan et al. 2012). Therefore, there were few studies on the effects of the oiling on organisms in the tidal zones of barrier islands other than acute impacts on vertebrates. However, examination of sediment meiofauna communities (Bik et al. 2002) indicated a dramatic and immediate change in their composition following the DWH oil spill and cleanup effort in 2010, and continued work has shown that “recovered” communities represent distinct assemblages of taxa that differ from known, pre-spill communities (Brannock et al. 2014; Rodríguez et al. 2015).

Since 95% of the oiling of tidal marshes occurred in Louisiana, most studies on the acute oiling effects on estuarine biological communities were and continue to be conducted there, particularly in Barataria Bay. The first reports on acute toxicity were made on the killifish. Subsurface water in Louisiana marshes was shown to have significant concentrations of PAH and acute toxicity to killifish with genomic and physiological changes observed (Whitehead et al. 2012). Further, Whitehead et al. (2012) reported that the PAH concentrations in subsurface water in the marsh locations remained high enough to have biological effects on killifish for up to two months, but they also provided data showing that large amounts of oil were retained in the sediment of oiled marsh at their last sample taken at 5 months after oiling.

The majority of studies on invertebrate populations started with no true pre-event population abundance data, which resulted in having to compare populations in oiled areas compared to areas that were not oiled. Of those studies that showed evidence of population abundance reductions, the majority have reported partial recovery over a 2- to 5-year period. McCall and Pennings (2012) sampled the terrestrial arthropod community and marine invertebrates found in coastal salt marshes. In 2010, intertidal crabs and terrestrial spiders and insects (classified as herbivores, sucking or stem boring parasitoids, and detritivores) were suppressed by oil exposure, but one year later, crab and arthropods had largely recovered. Fleeger et al. (2015) reported recovery of microalgae and meiofauna diversity based upon copepod richness. Our own population census and genetic studies on the horse fly *Tabanus nigrovittatus* showed population crashes in oiled locations compared to unoiled locations in 2010 and 2011 with a slow recovery up to 2016 (Husseneder et al. 2016; Husseneder et al. 2017). By using both population abundance and genetics, we were able to show not only population decline and steady recovery but also how the genetic structure

of the population changed immediately after the oil spill and then returned to patterns similar to those in non-oiled control areas.

For primary effects of oiling on plants, Hester et al. (2016) documented significant injury to the plant production and health of Louisiana salt marshes. Marsh sites with vertical oiling of plants had reduction in cover and peak standing crop particularly along the marsh edges for the majority of a 4-year study. Subsequent erosion resulted in plot loss which is consistent with other reports. Lin et al. (2017) found that initial impacts of moderate oiling were evident for *Spartina alterniflora* and *Juncus roemerianus*, but aboveground biomass and total live belowground biomass recovered within 24–30 months. Initial heavy oiling resulted in near complete plant mortality initially, and recovery of total live aboveground biomass was <50% of reference marshes six years after the spill. The *Juncus* vegetation showed no recovery six years after the spill. Furthermore, live belowground biomass of *Spartina* (0–6 cm) in heavily oiled marshes was significantly reduced compared to the reference marshes.

In addition to secondary effects of oiling on plant communities, chronic effects for vertebrates have also been reported. For example, Hicken et al. (2011) showed that sublethal exposure to crude oil causes changes in heart shape and a significant reduction in swimming performance in zebra fish, which likely would result in death in nature. Similarly, Nelson et al. (2016) showed exposure to environmentally relevant PAH concentrations impairs aspects of cardiovascular function, such as cardiac output and stroke work reductions in mahi-mahi. Smith et al. (2017) reported that studies on acute effects of the oiling based on live dolphins and necropsies confirmed lung injury and adrenal gland lesions. Subsequently, reproductive failure rates were evaluated for five years during and after the spill (2010–2015). When compared to the estimated reproductive success rates for dolphins living in areas not impacted by the DWH oil spill, the rates for unaffected areas were three-fold higher than the reproductive success rates for the animals from the affected areas.

Many of the long-term tertiary effects of the oiling will go unnoticed or undescribed due to lack of direct links. However, there have been observable and permanent tertiary effects of oiling shown for *Spartina* marsh land loss associated with the primary acute impact on the vegetation, the secondary significant impact on belowground vegetation, and ultimately the tertiary loss of the heavily oiled banks associated with the oiling and subsequent wave action of tropical storms (Rangoonwala et al. 2016). The authors measured the relative impact of wetland loss in Barataria Bay, Louisiana over a period of 1 year before the oiling to 2.5 years after oiling using synthetic aperture radar. They showed that there were significant differences in the loss of land before and after oiling (which was not observed in control areas). Specifically, land loss was greater in 2012 due to wave-induced erosion associated with Hurricane Isaac. Factors affecting this phenomenon include decreases in belowground biomass (Lin et al. 2002) and weakened soil (McClenachan et al. 2013) in salt marshes. McClenachan et al. (2013) described shoreline studies showing that soil weakened by oiling resulted in more erosion than was obvious from aboveground observations. This resulted in an overhang of even moderately oiled marsh banks with collapse of the overhang when it became too large.

#### **1.3.4.4 Research Initiatives of the LSU Entomology and UNH Genomics Programs**

##### **1.3.4.4.1 Relevant Previous Research, LSU**

From immediately after the spill in April 2010 until November 2011, we conducted studies on greenhead horse fly (*Tabanus nigrovittatus*, Figure 33) populations at four Louisiana locations west of the Mississippi River. Two locations were oiled (Grand Bayou and Grand Isle) and two were not (Cypremort Point and Cameron). Horse flies are members of the family Tabanidae which contains over 4,000 species. Insect population surveys are often used as biological barometers of the health of freshwater ecosystems. However, studies on insect biology in brackish and saline ecosystems are relatively few because there are

few insect species that are osmotolerant. The greenhead horse fly is one of the few species of tabanids that are found in these extreme environments.



**Figure 33. Larva and female adult of the greenhead horse fly, *Tabanus nigrovittatus*.**  
Photos by C. Hussender.

The rationale for selecting the greenhead horse fly as the entomological model for coastal ecology studies is that this species is native to and tightly bound to specific coastal marsh habitats that range from the Texas coast to Nova Scotia. Therefore, this insect species can be useful as a bioindicator for ecologists along both the Gulf and East Coasts. Furthermore, there are closely related species that are native to tidal marshes in many other parts of the world. The adult female flies are highly mobile, easy to catch and identify, and are highly apparent to local human populations because of their pestiferous attacks. The seasonality of the adult populations is relatively well established in most of the species' range and the life cycle is well described. Female greenhead horse flies are autogenous, which means that the first batch of approximately 150 eggs is produced before the fly pursues its first bloodmeal. This attribute results in the property of the annual populations being independent of available vertebrate hosts. There are few autogenous tabanid species and the majority of these species are found in extreme environments such as brackish marshes and subarctic zones.

We compared the population abundance and genetic structure of the adult horse flies between the unaffected and oiled locations. Adult flies were collected biweekly from June 2010 until October 2011 using at least four canopy traps per location. We also collected horse fly larvae in 2011. Horse fly abundance estimates showed severe crashes of adult tabanid populations as well as reduced numbers of larvae recovered from the soil in oiled areas (Hussender et al. 2016). Our trap data with only a range of 1.3–4.8 mean flies captured per hour in 41 trap days indicated that the adult tabanids had been affected immediately after the oil spill reached Elmer's Isle, Grand Isle (Jefferson Parish) and Grand Bayou (Plaquemines Parish); both of these areas had been notorious for greenhead attack each summer. In comparison, fly activity remained high at the unaffected locations at Cypremort Point (St. Mary Parish) and Cameron Parish (a range of 36.6 – 92.2 flies per hour in 60 trap days).

At each of the four regions the mean number of flies per hour trapped was not significantly different between the years 2010 and 2011 (Table 2). In 2010, horse fly numbers differed significantly ( $P = 0.0042$ ) between all four regions, while in 2011 catches at the two unaffected locations (Cameron, St. Mary) were equally high and catches at oiled locations (Jefferson, Plaquemines) were equally low. Overall, in both years, horse fly numbers caught at unaffected locations were significantly higher ( $P <$

0.0001) than at oiled locations (Table 2). As expected, the number of trapped flies fluctuated seasonally at each location but counts at oiled locations were in most cases orders of magnitude lower than those at unaffected locations.

**Table 2. Mean number of greenhead horse flies trapped (flies/hour) by region in 2010 and 2011 (Husseneder et al. 2016).**

Letters a, b, c, d indicate statistical differences in the mean number of horse flies trapped at each site by year; counts with the same letter are not statistically different ( $P < 0.05$ ; Tukey-Kramer).

Parish	2010			2011		
	Mean $\pm$ SE	Mean (log x+1) $\pm$ SE		Mean $\pm$ SE	Mean (log x+1) $\pm$ SE	
Cameron	82.2 $\pm$ 6.6	3.9 $\pm$ 0.2	a	53.2 $\pm$ 6.9	3.3 $\pm$ 0.2	ab
St. Mary	38.0 $\pm$ 6.6	2.6 $\pm$ 0.2	b	38.0 $\pm$ 6.5	3.3 $\pm$ 0.2	ab
Jefferson	0.8 $\pm$ 6.1	0.4 $\pm$ 0.2	d	1.3 $\pm$ 6.5	0.6 $\pm$ 0.2	d
Plaquemines	3.9 $\pm$ 5.8	1.2 $\pm$ 0.1	c	4.5 $\pm$ 5.4	1.1 $\pm$ 0.1	c

In support of the observed impact of oil intrusion on adult horse flies, we recorded lower incidence rates of larva recovery from oiled areas. At Grand Isle, no larvae were collected from any of the six collection sites; at Grand Bayou, one tabanid larva was isolated from only one of eight sediment samples. In contrast to oiled areas, there was a high probability of collecting tabanid larvae in those *Spartina* marshes in Louisiana that were not affected by the oil spill. Tabanid larvae were isolated from four of the five samples obtained in Cameron; the maximum number of larvae was 10 with an average of 3 per sample. From Cypremort Point, larvae were isolated from five of the eight samples; the greatest number of larvae was 2 with an average of 1.

Microsatellite genotyping of six non-oiled and seven oiled populations at ten polymorphic loci detected genetic bottlenecks in six of the oiled populations in association with fewer breeding parents, reduced effective population size, lower number of family clusters, and fewer migrants among populations (Table 3). This data was published by Husseneder et al. (2016) and is the first study assessing the impact of oil contamination at the level of a top arthropod predator of the invertebrate community in salt marshes.

**Table 3. Summary of the differences between populations of tabanids from oiled and non-oiled areas (Husseneder et al. 2016).**

Parameter	Non-oiled	Oiled
Adult fly counts	High	Low
Larvae recovered from marsh soil	High	Low
Effective population size	High	Low
Number of breeders	High	Low
Number of families	High	Low
Number of migrants, gene flow	High	Low
Genetic bottlenecks	No	Yes

Whether a population originated from non-oiled or oiled areas had marginally significant effects on genetic distance and significant effects on the number of migrants per generation (General Linear Model [GLM]:  $F = 4.06$ ,  $df = 1$ ,  $P = 0.069$  and  $F = 19.13$ ,  $df = 1$ ,  $P = 0.001$ , respectively). Although non-oiled populations were separated by larger geographic distance (40–105 km) than oiled populations (6–45 km), the genetic distances among them were on average marginally smaller (mean  $F_{ST} = 0.17$ ,  $SE = 0.02$ ) and thus gene flow was higher than among oiled populations (mean  $F_{ST} = 0.22$ ,  $SE = 0.01$ ,  $P = 0.090$ , 2-tailed t-test, SPSS). This indicates that the observed population crashes in oiled areas might have resulted in a

fragmentation and reduced migration/survival of immigrants. Oiling of the area also caused significantly lower numbers of parents contributing offspring to the population ( $P = 0.048$ ,  $df = 1$ ,  $F = 5.21$ ), lower effective population size ( $P = 0.03$ ,  $df = 1$ ,  $F = 6.75$ ), and lower number of offspring per parent ( $P = 0.076$ ,  $df = 1$ ,  $F = 4.02$ ). Oiling had a marginal effect on the number of family clusters being lower in oiled areas ( $P = 0.095$ ,  $df = 1$ ,  $F = 3.48$ ).

No bottleneck effects were detected in the six populations from non-oiled areas in 2010 or 2011 under any of the three mutation models tested (IAM, TPM, SMM as implemented in BOTTLENECK v.1.2.02, Piry et al. 1999). However, five out of seven populations that were hit by the oil spill showed pronounced genetic bottlenecks in 2010 and/or 2011, and an additional population showed a marginal bottleneck effect. These results reflect the observed population crashes and further emphasize the devastating impact of oiling on the top predator of the invertebrate marsh community. The populations with marginal or no bottleneck were those collected on Grand Isle that also showed genetic heterogeneity and the presence of a genetic cluster predominantly known from unaffected locations. This might be a sign of beginning recovery via immigration (Husseneder et al. 2016).

In 2016, we repeated the procedures used to compare the population abundance and genetic structure of the adult horse flies between the unaffected and oiled locations in 2010 and 2011. We found that in 2016 adult fly counts had increased in oiled areas and we were able to retrieve larvae from marsh soil in oiled locations previously devoid of larvae. Moreover, the population genetic structure showed signs of recovery. The genetic bottlenecks in populations from previously oiled areas have largely disappeared. Effective population size and the number of breeders, families and migrants have reached the levels of non-oiled populations (Husseneder et al. 2017). These studies showed the value of population genetic data for signs of impact and recovery in connection to census data or as a stand-alone method for species that are not accessible to reliable, time and cost intensive long-term population census regimes.

#### **1.3.4.4.2 Description of Current LSU Studies**

We currently are continuing to monitor the population abundance of adult tabanids in our previous study locations, and specimens are stored for future genetic analysis if required. We also have expanded our larval tabanid surveys to establish seasonality and distribution of the larval life stages and their prey. We are testing the hypothesis that the presence of the apex predator tabanid larva represents a healthy and likely diverse food chain within the marsh sediments. We are using metagenetic analyses to compare the micro- and meiofauna community in the immediate environment where tabanid larvae are found as well as the community in spartina marsh sites devoid of tabanid larvae. We also have initiated studies to analyze the gut content of tabanid larvae to identify prey species and compare their composition to that in the surrounding marsh soil. Based on this knowledge we will develop a time- and cost-efficient PCR-based diagnostic method to differentiate between healthy and biologically depleted marsh soil.

Relative to the tabanid larval food web, we have obtained 18S rRNA gene sequences (Illumina MiSeq, 2 X 300bp) of 2011 soil samples from oiled and non-oiled areas and gut contents of greenhead horse fly larva to describe the larval food web, taxa diversity across sample types, and begin the search for bioindicators of marsh health. The most abundant families are the same in the sediments and guts from oiled and non-oiled locations, although the relative abundance varies across sample type. Differences in the meiofauna composition at oiled and non-oiled locations might be due to differences in geographical location, soil chemistry, or oil tolerance. We found that hexapods and fungi are among the most abundant families across all our sample types. These taxa are also important staples in the diet of tabanid larvae.

We also have expanded our insect surveys of the coastal marshes to include other species that are native to these habitats, with “native” here defined as those species that complete their entire life cycle within these brackish marshes. These surveys include both adult and larval stages. Because there are few useful taxonomic keys for use in identification of insect larvae (including tabanids) from these marshes, we will

use DNA barcoding to aid in the identification of the life stages of the different native species. Obviously, insect larvae are staples of the tabanid larval diet resulting in overlap of these new initiatives.

#### **1.3.4.4.3 Relevant Previous Research at UNH**

The Thomas laboratory has a long history of developing molecular methods of taxonomy and biodiversity assessment including the initial development of PCR-based methods of population genetic analysis now widely adopted in metabarcoding (Kocher et al. 1989). More recently, the Hubbard Center for Genome Studies (HCGS) at UNH was among the first to apply next generation sequencing technologies to the analysis of organismal evolution and has worked with many diverse international groups toward development of novel approaches to monitor community structure with a particular emphasis on meiofauna, specifically nematodes that are among the most abundant and diverse sediment and soil animals (Bik et al. 2012; Creer et al. 2016). That emphasis included the development of novel bioinformatics tools for the analysis of next generation metabarcoding and metagenomics datasets (Gaspar et al. 2013, 2015; Westbrook et al. 2017). UNH is also home to an NSF-funded Research Coordination Network “EukHiTS” (DBI-1262470) focused on integrating research activities investigating the application of high throughput sequencing for analysis of eukaryotic biodiversity. As part of this Research Coordination Network and the NH-INBRE, Thomas and colleagues are developing a series of bioinformatics workshops with online modules suitable for research training and for implementation in course curricula. With regard to the GOM and the consequences of the DWH event, the HCGS at UNH first applied metabarcoding approaches to assay the consequences of oil contamination on benthic microbial eukaryotes (Bik et al. 2012). In addition to the observed dramatic shifts in meiofaunal community structure observed, it became obvious that the lack of robust reference datasets for the vast majority of meiofaunal species was a major impediment to developing a mechanistic understanding of biological diversity for these creatures necessary to support logical approaches to mitigation and remediation. Ultimately, those observations led to the current focus of the UNH research group.

#### **1.3.4.4.4 Description of Current UNH Studies**

To date, draft genomes have been generated for 39 species that represent 11 phyla, six with no previous genome wide sequence data. In addition, we have established new approaches for the analysis of shotgun metagenomics datasets allowing for the elucidation of population level analysis. The draft genomes revealed the standard rRNA loci and large numbers of complete single copy orthologs. These genomes will serve as references for shotgun metagenomics analysis including taxonomic characterization of communities, and description of metabolic pathways. These references support expanded metabarcoding applications by providing the sequences necessary for primer design for large numbers of universal orthologs as well as population genetic markers (e.g., microsatellite loci) for analyses of intraspecific variation and population genetic structure. Intraspecific variation also addresses some of the major shortcomings of metabarcoding namely the existence of cryptic ecologically unique species and a lack of species-level resolution afforded by standard barcoding approaches using rRNA gene sequencing.

As part of our commitment to development of resources for investigating GOM meiofaunal diversity, we established a workshop on “Benthic Invertebrate Taxonomy, Metagenomics and Bioinformatics” (BITMaB) at HRI. That workshop brought together an international group of taxonomic experts and bioinformatics specialists, as well as students, to collect and identify the specimens for the reference genomes as well as learn about the diversity of microbial eukaryotes and the training in bioinformatics skills to conduct the analysis. The 2017 workshop was cosponsored and supported by GOMRI and NSF and included 59 scientists from around the world. The participants represented all levels from undergraduate students to professors, staff scientists, and personnel from regulatory agencies.

#### 1.3.4.4.5 Description of Future LSU and UNH Studies

We have provided a synopsis of studies that describe the acute effects of the primary oiling events and both expected and unexpected secondary and tertiary effects associated with the DWH oil spill. What effects these changes have on current and future biological communities of the CGOM is an important question for the GOM scientific community. The primary issue that hindered scientists from assessing the acute effects of the 2010 oiling of different coastal habitats was the absence of pre-event, baseline data. The DWH oil spill should serve as a wakeup call for funding agencies regarding the paucity of baseline information available for the CGOM biological communities. In the future, there is always the potential for new deepwater oil exploration disasters to go along with the slow, steady decline in coastal GOM estuary health associated with climate change, sea-level rise, subsidence, and other man-made disasters; having baseline ecological data accessible to future scientists is a mandate. In this section, we present some potential goals and objectives for future projects on invertebrate coastal ecology that we hope to achieve, depending on future funding and the potential for international collaborations.

For future projects, a major goal would be to identify composition, density, and variation of meiofaunal communities across healthy intertidal zones and those impacted by the DWH oil spill as well as other geographic locations to establish baselines for changes in response to ecological conditions using quantitative PCR, DNA barcoding, metagenomics, and population genetic approaches. Projects to conduct morphological and genetic identification of macrofauna native to marshes and barrier islands also would be key elements.

We have previously used 18S metabarcoding to show effects of the DWH oil spill on a limited number of meiofaunal communities (Bik et al. 2012). A major focus of future studies would be to expand these applications for studies of the invertebrate communities of different coastal habitats of the entire GOM to provide baseline data for future monitoring activities. While the relative gain and loss of taxa from complex specious communities can provide valuable information about the consequences of environmental change, we also expect profound consequences on genetic diversity and patterns of migration, among other parameters of population structure that can be measured while not being detected at the level of species diversity (Husseneder et al. 2016).

Through the development of reference genomes, we predict that it will be possible to extend analysis of largely unstudied/understudied meiofaunal species to the population level. As shown above these reference genomes include new information to support the goal of extending metagenomics analysis to the level of population structure. Specifically, each new meiofaunal reference genome includes vast numbers of single nucleotide polymorphisms (SNPs), microsatellites and complete mitochondrial genomes. This genomic sequence data makes it possible to perform targeted assays of population structure for hundreds of invertebrate species of the GOM. For each species the reference genome allows for the selection of nuclear loci that are known to be polymorphic (such as microsatellites) and to design primers that are unique to the targeted locus. Further, the mitochondrial genomes of animals (including these meiofauna) evolve rapidly and without recombination and have served as an excellent locus for the establishment of phylogeographic structure.

In the future, we intend to use two strategies to assess intraspecific variation. One strategy would be based on traditional analysis of individual specimens using mtDNA haplotypes and microsatellite genotyping by sequencing approaches. A second strategy will be to test and validate the utility of whole metagenome shotgun data to allow parallel population genetic analysis of many species simultaneously. The individual-based approach allows the use of classical heterozygosity-based population genetic statistics (Husseneder et al. 2016), but is strongly biased towards large, abundant species that are easy to collect and identify. The novel approach of shotgun metagenomic analysis of a large number of alleles and their frequencies can provide population level assays in parallel of a community of species, including abundant, but small and less described organisms.



### 1.3.4.5 References

- Allan, SE, Smith BW, Anderson KA. 2012. Impact of the Deepwater Horizon oil spill on bioavailable polycyclic aromatic hydrocarbons in Gulf of Mexico coastal waters. *Environmental Science and Technology* 46: 20333–20339.
- Belanger M, Tan L, Askin N, Wittnich C. 2010. Chronological effects of the Deepwater Horizon Gulf of Mexico oil spill on regional seabird casualties. *Journal of Marine Animal Ecology*. 3(2): 10–14.
- Bik HM, Halanaych KM, Sharma J, Thomas WK. 2012. Dramatic shifts in benthic microbial eukaryote communities following the Deepwater Horizon oil spill. *PLoS ONE*. 7(6): e38550.
- Brannock PM, Waits DS, Sharma J, Halanaych KM. 2014. High-throughput sequencing characterizes intertidal meiofaunal communities in northern Gulf of Mexico (Dauphin Island and Mobile Bay, Alabama). *The Biological Bulletin*. 227: 161–174.
- Creer SK, Deiner S, Frey D, Porazinska P, Taberlet WK, Thomas C, Potter HM, Bik. 2016. The ecologist's field guide to sequence-based identification of biodiversity. *Methods in Ecology and Evolution*. 7(9). DOI: 10.1111/2041-210X.12574.
- [ERMA] Environmental Response Management Application. 2017. Web Application: Deepwater Gulf Response Environmental Response Management Application, National Oceanic and Atmospheric Administration [accessed: 03/03/2017] from <http://gomex.erma.noaa.gov/>.
- Fleeger JW, Carman KR, Riggio MR, Mendelssohn IA, Lin QX, Hou A, Deis DR, Zengel S. 2015. Recovery of salt marsh benthic microalgae and meiofauna following the Deepwater Horizon oil spill linked to recovery of *Spartina alterniflora*. *Marine Ecology Progress Series*. 536: 39–54.
- Gaspar JM, Thomas WK. 2013. Assessing the consequences of denoising marker-based metagenomic data. *PLoS ONE*. 8(3): e60458. DOI:10.1371/journal.pone.0060458, PMID: 23536909; PMCID: 3607570.
- Gaspar JM, Thomas WK. 2015. FlowClus: efficiently filtering and denoising pyrosequenced amplicons. *BMC Bioinformatics*. 16:105. doi: 10.1186/s12859-015-0532-1.
- Gelsleichter J. 2017. Community and population level effects of the DWH oil spill on deep demersal fishes: six years monitoring recovery in sharks, teleosts and hagfishes. Paper presented at: Gulf of Mexico Oil Spill and Ecosystem Science Conference; New Orleans, Louisiana.
- Hester MW, Willis JM, Rouhani S, Steinhoff MA, Baker MC. 2016. Impacts of the Deepwater Horizon oil spill on the salt marsh vegetation of Louisiana. *Environmental Pollution*. 216: 361e370.
- Hicken CE, Linbo TL, Baldwin DH, Willis ML, Myers MS, Holland L, Larsen M, Stekoll MS, Rice SD, Collier TK, et al. 2011. Sublethal exposure to crude oil during embryonic development alters cardiac morphology and reduces aerobic capacity in adult fish. *Proceedings of the National Academy of Sciences of the United States of American*. 108(17): 7086–7090.
- Husseneder C, Donaldson JR, Foil LD. 2016. Impact of the 2010 Deepwater Horizon oil spill on population size and genetic structure of horse flies in Louisiana marshes. *Nature Scientific Reports*. 6. doi: 10.1038/srep18968.

- Husseneder C, Park J, Foil L. 2017. Greenhead horse fly populations as bioindicators of the impact of the Deepwater Horizon oil spill on marsh health and recovery. Paper presented at: Gulf of Mexico Oil Spill and Ecosystem Science Conference; New Orleans, Louisiana.
- Kocher TD, Thomas WK, Meyer A, Edwards SV, Pääbo S, Villablanca FX, Wilson AC. 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proceedings of the National Academy of Sciences of the United States of American*. 86: 6196–6200.
- Lin QI, Mendelssohn IA, Suidan MT, Lee K, Venosa AD. 2002. The dose-response relationship between No. 2 fuel oil and the growth of the salt marsh grass, *Spartina alterniflora*. *Marine Pollution Bulletin*. 44: 897–902. doi: 10.1016/S0025-326X(02)00118-2.
- McCall BD, Pennings SC. 2012. Disturbance and recovery of salt marsh arthropod communities following BP Deepwater Horizon oil spill. *PLoS ONE*. 7: e32735.
- McClenachan G, Turner RE, Tweel AW. 2013. Effects of oil on the rate and trajectory of Louisiana marsh shoreline erosion. *Environmental Research Letters*. 8: 044030. doi:10.1088/1748-9326/8/4/044030.
- Nelson D, Heuera RM, Cox GK, Stieglitz JD, Hoenigb R, Magerb EM, Benetti DD, Grosell M, Crossley DA II. 2016. Effects of crude oil on in situ cardiac function in young adult mahi-mahi (*Coryphaena hippurus*). *Aquatic Toxicology*. 180: 274–281.
- Piry S, Luikart G, Cornuet JM. 1999. BOTTLENECK: a computer program for detecting recent reductions in the effective population size using allele frequency data. *Journal of Heredity* 90: 502–503.
- Rangoonwala A, Jones CE, Ramsey E III. 2016. Wetland shoreline recession in the Mississippi River delta from petroleum oiling and cyclonic storms. *Geophysical Research Letters*. 43(11): 652–660. doi: 10.1002/2016GL070624.
- Rodríguez LM, Overholt WA, Hagan C, Huettel M, Kostka JE, Konstantinidis KT. 2015. Microbial community successional patterns in beach sands impacted by the Deepwater Horizon oil spill. *The ISME journal*. 9(9): 1928–1940. doi:10.1038/ismej.2015.5.
- Silliman RR, van de Koppel J, McCoy MW Diller J, Kasozi GN, Earl K, Adams PN, Zimmerman AR. 2012. Degradation and resilience in Louisiana salt marshes after the BP-Deepwater Horizon oil spill. *Proceedings of the National Academy of Sciences of the United States of American*. 109(28): 11234–11239.
- Smith CR. 2017. Investigating mechanisms for reproductive failure among bottlenose dolphins in the aftermath of the Deepwater Horizon Oil Spill. Paper presented at: Gulf of Mexico Oil Spill and Ecosystem Science Conference; New Orleans, Louisiana.
- Tang T. 2017. Analysis of lethal and sublethal impacts of environmental disasters on sperm whales using stochastic modeling. Paper presented at: Gulf of Mexico Oil Spill and Ecosystem Science Conference; New Orleans, Louisiana.
- Westbrook A, Ramsdell J, Schuelke T, Normington L, Bergeron RD, Thomas WK, MacManes MD. 2017. PALADIN: protein alignment for functional profiling whole metagenome shotgun data. *Bioinformatics* 33: 1473–1478.

Whitehead A, Dubansky B, Bodinier C, Garcia TI, Miles S, Pilley C, Raghunathan V, Roach JL, Walker N, Walter RB, Rice CD, Galvez F. 2012. Genomic and physiological footprint of the Deepwater Horizon oil spill on resident marsh fishes. *Proceedings of the National Academy of Sciences* 109: 20298–20302.