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Spatial and temporal variation of intertidal nematodes in the northern Gulf of Mexico after the *Deepwater Horizon* oil spill

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ABSTRACT

Nematodes are an abundant and diverse interstitial component of sedimentary habitats that have been reported to serve as important bioindicators. Though the 2010 *Deepwater Horizon* (*DWH*) disaster occurred 60 km offshore in the Gulf of Mexico (GOM) at a depth of 1525 m, oil rose to the surface and washed ashore, subjecting large segments of coastline in the northern GOM to contamination. Previous metabarcoding work shows intertidal nematode communities were negatively affected by the oil spill. Here we examine the subsequent recovery of nematode community structure at five sites along the Alabama coast over a two-year period. The latter part of the study (July 2011–July 2012) also included an examination of nematode vertical distribution in intertidal sediments. Results showed nematode composition within this region was more influenced by sample locality than time and depth. The five sampling sites were characterized by distinct nematode assemblages that varied by sampling dates. Nematode diversity decreased four months after the oil spill but increased after one year, returning to previous levels at all sites except Bayfront Park (BP). There was no significant difference among nematode assemblages in reference to vertical distribution. Although the composition of nematode assemblages changed, the feeding guilds they represented were not significantly different even though some variation was noted. Data from morphological observations integrated with metabarcoding data indicated similar spatial variation in nematode distribution patterns, indicating the potential of using these faster approaches to examine overall disturbance impact trends within communities. Heterogeneity of microhabitats in the intertidal zone indicates that future sampling and fine-scale studies of nematodes are needed to examine such anthropogenic effects.

1. Introduction

Nematodes are important constituents of intertidal communities (Warwick, 1976; Blome, 1983; Sharma and Webster, 1983; Nicholas, 2001; Gingold et al., 2010), and their distribution in intertidal habitats can be affected by environmental gradients of sediment granulometry, salinity, and temperature (Gingold et al., 2010). In addition, hydrodynamic and physical features that influence wave and tidal action can generate different microhabitats, such as sandbars and runnels (Maria et al., 2013), thereby affecting horizontal and vertical distributions of nematodes (Brustolin et al., 2013). Free-living nematodes are abundant and ubiquitous with short life cycles (5 days-1 year) and rapid turnover (Heip et al., 1985). As primary and secondary consumers, nematodes respond to physical, chemical, and biological properties of their food. They demonstrate a rapid response to anthropogenic disturbances such as oil spills, radiation leakage, and large amounts of total suspended solids and thus are important bioindicators (Vincx and Heip, 1991; Danovaro et al., 2009).

Ecosystem health in the northern Gulf of Mexico (nGOM) was of great concern after the explosion of the *Deepwater Horizon* (*DWH*)

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offshore oil platform on April 20, 2010. Even though this disaster occurred 60 km offshore at a depth of 1525 m, immediate attention was focused on the impact of the spill to coastal regions (Newton et al., 2013). Studies of the effect of the oil spill on intertidal to deep-sea benthos have demonstrated a decrease in abundance and diversity of meiofauna (Bik et al., 2012; Montagna et al., 2013; Landers et al., 2014; Baguley et al., 2015). As oil moved towards eastern nGOM coastline by wind and currents, it washed onto beaches and coastal regions in Alabama from early June 2010 until about mid-July 2011 (Graham et al., 2010; Hayworth et al., 2011) and continued to have an effect on the marine environment for some time (MacDonald et al., 2014). The Shoreline Cleanup Assessment Technique (SCAT) program surveys revealed that the Alabama GOM coast, including Dauphin Island, experienced heavy oiling from the *DWH* oil spill during a portion of their survey (Michel et al., 2013). Although Michel et al. (2013) reported no oil observed in Mobile Bay regions especially near Bayfront Park (BP) and Belleair Blvd (BB), satellite images of oil slicks in Mobile Bay were observed (e.g. http://response.restoration.noaa.gov/about/media/mapping-fallout-deepwater-horizon-oil-spill-developing-one-tool-bring-unity-response.htm), which are consistent with eye-witness accounts. By May 2011 oil conditions in this geographic region decreased to light or trace levels and further decreased to trace or no oil observed conditions in May 2012 (Michel et al., 2013).

Bik et al. (2012). noted a dramatic shift in intertidal meiofaunal communities along portions of the Alabama coast from a metazoan dominated community composition prior to the spill to one dominated by fungal taxa after. Subsequently, Brannock et al. (2014). reported the large portion of fungal taxa had disappeared from these locations by July 2011 and the community returned to one dominated by metazoans. Both studies (Bik et al., 2012; Brannock et al., 2014) utilized metabarcoding high-throughput sequencing approaches to examine the meiofaunal community composition. Use of metabarcoding technology to explore the composition of meiofaunal communities is less developed compared to protist and prokaryotic systems, but holds great promise and potential (Bik, 2014; Brannock and Halanych, 2015).

Previous studies examining effects of oil spills on coastal meiofaunal communities have found that oil pollution has mixed effects on nematode communities (Boucher, 1985; Danovaro, 2000; Burgess et al., 2005). Differences in the response of nematodes to hydrocarbon pollutants may be attributed to the heterogenous sedimentary environment and tolerance of some nematodes to pollutants (Giere, 1979). Studies note that nematodes are more resilient than other meiofauna taxa (Boucher, 1985), whereas other studies indicate abundance and diversity of nematodes may decrease immediately after exposure to oil contaminants, although they recover rapidly in comparison to other meiofauna (Danovaro et al., 1995; Danovaro, 2000). Only a few long-term studies have examined recovery of nematode communities following oil exposure (Giere, 1979; Gourbault, 1987; Danovaro et al., 1995), as baseline data before an oil spill disturbance is often not available. Although there are taxonomic studies of intertidal nematodes in the GOM (summarized in Hope, 2009), to date there is only one study on the ecology of intertidal nematodes from this geographic region (King, 1962).

Herein we examine spatial, temporal, and vertical distribution patterns of intertidal nematode communities along the Alabama coast and within western Mobile Bay based on morphological taxonomic methods. The main objectives of this study were to (1) examine spatial, temporal, and vertical distributions in nematode community composition within five Alabama intertidal locations, (2) explore the functional diversity as determined by nematode feeding groups within these locations, (3) compare nematode diversity within communities pre- and post *DWH* oil spill, (4) examine the pattern of recovery of nematode assemblages over time after the oil spill, and (5) briefly compare morphological and metabarcoding approaches in examining nematode communities.

2. Material and methods

2.1. Study sites

Samples were collected at five Alabama intertidal sites (Fig. 1, Table 1). Two collection sites were located within Mobile Bay (Bayfront Park: BP and BelleAir Boulevard: BB) and three sites along Dauphin Island (Ryan Court: RC, Shellfish Lab: SL, and Cadillac Avenue: CA) (Fig. 1, Table 1). BP, BB, and CA are more sheltered locations found within in low energy "Bay" type habitats, whereas RC and SL are more exposed GOM-facing beaches. These were the same sampling locations utilized by Bik et al. (2012), Williams (2013), and Brannock et al. (2014). Pre-spill sediment samples were collected in May 2010. This is after the *Deepwater Horizon* (*DWH*) oil spill commenced but prior to any oil exposure to the coast of Alabama, which did not occur until June 2010 (Graham et al., 2010; Hayworth et al., 2011). Choice of location for sample site was dictated in no small part by the ability to reach localities that were not closed off by authorities as they prepared for oil to reach the shoreline. Post-spill sediment samples were collected in September 2010 (Bik et al., 2012), March 2011 (Bik, unpublished data), and bi-monthly from July 2011–July 2012 (Brannock et al., 2014). Sediment granulometry and organic composition for a subset of the samples used in the current study have been reported previously (Williams, 2013).

2.2. Sample collection

For nematode morphological analysis, two 4-cm diameter and 10-cm depth sediment cores per collection site were taken during

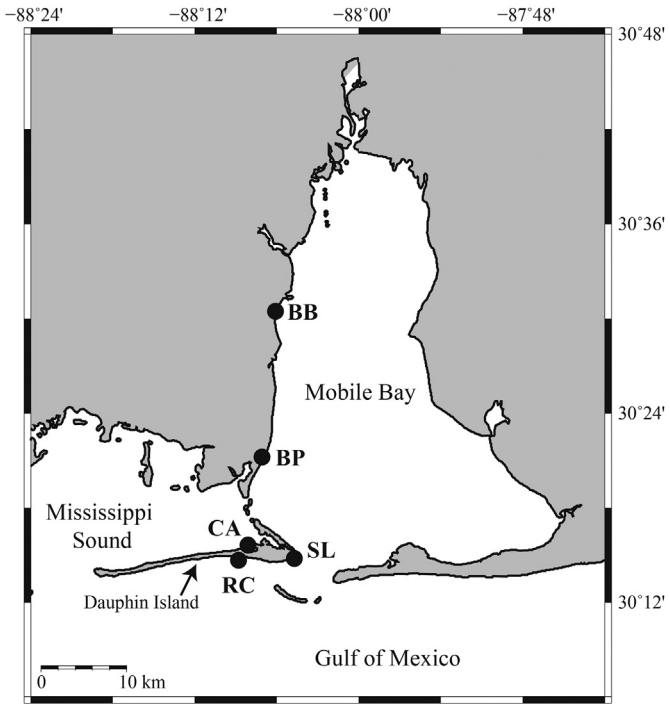

Fig. 1. Geographic representation of sampling locations within Mobile Bay and along Dauphin Island, Alabama. Site abbreviations and GPS coordinates are provided in Table 1.

Table 1
Geographic description and sediment characteristics of sampling locations.

	Ryan Court	Shellfish Lab	Cadillac Ave	Bayfront Park	BelleAir Blvd
Abbreviation	RC	SL	CA	BP	BB
Latitude	30° 15.014′ N	30° 14.793′ N	30° 15.203′ N	30° 21.235′ N	30° 30.482′ N
Longitude	88° 08.755′ W	88° 04.723′ W	88° 08.813′ W	88° 07.071′ W	88° 06.098′ W
Region	Gulf of Mexico	Gulf of Mexico	Bay	Bay	Bay
Sediment Grain size[a]	Medium-coarse sand, well sorted	Fine-medium sand, well sorted	Medium sand, well sorted	Mixed[b], heterogeneous sediment[c]	Medium-coarse sand, well sorted
Beach	Semi-dissipative	Semi-dissipative	Protected, dissipative	Protected, dissipative	Semi-dissipative

[a] Information obtained from Williams (2013).
[b] Consisted of a combination of mud, very fine to fine sand, and medium to to coarser sand.
[c] Not of one grain size.

each sampling period and preserved in DMSO EDTA Salt Solution (DESS) (Yoder et al., 2006) for nematode morphological taxonomic assignment except March 2011 when only one sample core per site was collected (Table 2). DESS was used for a preservative instead of formaldehyde to allow individuals to be used for DNA barcoding if desired. For samples collected from July 2011 to July 2012, sediment cores were divided into 0–3 cm and 3–10 cm depth fractions before preserving in approximately 125 ml DESS. Samples collected in May and September 2010 as well as March 2011 were preserved as a 0–10 cm core in approximately 250 ml of DESS.

2.3. Sample processing

Nematodes were extracted from sediment by decanting. Sediment was placed in a 1 L flask and agitated by vigorously inverting the sample five times with DESS solution up to a total of 800 ml, larger particles were allowed to settle (30-s maximum). The aqueous layer was carefully poured over a 45-μm sieve. This decantation protocol was repeated 5 times per sample. Material retained on the sieve was transferred to a petri dish to isolate nematodes. If multiple cores were initially collected, cores were processed separately and treated as biological replicates (Table 2). Depth fractions of bi-monthly (July 2011–July 2012) samples were processed separately (Table 2). The first ~100 nematodes on a gridded petri dish per core were hand-picked under a Nikon SMZ-1B stereoscope and transferred to anhydrous glycerin (Seinhorst, 1959). In samples with fewer than 100 nematodes all individuals were picked. Nematodes were mounted on slides lined with paraffin (Hooper, 1986) and identified using standard identification keys of free-living marine nematodes morphological characteristics (Platt and Warwick, 1983; Guilini et al., 2010; Schmidt-Rhaesa, 2014) with a Zeiss Axioskop. Nematodes were identified to lowest taxonomic level possible, with most specimens identified to genus level using the De Ley and Blaxter (2002) classification and where possible to putative species. Genus-level identification has been shown to be sufficient to allow significant ecological patterns to be detected as effectively as species-level identification (Vanreusel et al., 2010). Furthermore, species-level identification is often based on male characteristics and juveniles are indistinguishable beyond genus. Putative species identifications are included for monospecific genera when possible. All samples collected through the end of 2011 were processed. Due to time limitations, identification of nematodes was continued only for the RC locality collected in 2012. This location was chosen arbitrarily from the five locations. All examined nematodes have been vouchered and deposited at the Smithsonian Institution National Museum of Natural History (NMNH; accession number TM2080466).

2.4. Data analysis

For samples that had biological replicates, the mean number of nematodes for each taxonomic group of the two replicates was determined. However, all analyses were performed keeping the replicates separated. Nematode community composition was analyzed by first calculating relative abundances to take in account the differences in sample size. The relative abundance was

Table 2
All 7905 nematodes morphologically examined. Dash (–) indicates either no replicate was collected (March 2011) or samples for that time point were not processed (January–July 2012).

Date	Depth	Gulf of Mexico Locations				Bay Locations					
		Ryan Court		Shellfish Lab		Cadillac Ave		Bayfront Park		BelleAir Blvd	
		Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2
7-May-10	0-10 cm	101	55	8	90	83	93	86	131	97	92
21-Sep-10	0-10 cm	102	103	89	67	7	13	94	39	55	110
28-Mar-11	0-10 cm	111	–	103	–	102	–	71	–	105	–
20-Jul-11	0-3 cm	103	108	38	24	43	91	40	64	40	104
20-Jul-11	3-10 cm	102	106	26	71	13	30	7	6	101	108
25-Sep-11	0-3 cm	70	96	92	80	71	68	92	92	103	107
25-Sep-11	3-10 cm	93	98	101	30	79	21	7	97	105	105
16-Nov-11	0-3 cm	29	31	2	1	112	83	110	2	131	106
16-Nov-11	3-10 cm	95	100	62	72	76	78	91	15	89	97
25-Jan-12	0-3 cm	91	89	–	–	68	96	–	–	–	–
25-Jan-12	3-10 cm	78	93	–	–	43	49	–	–	–	–
21-Mar-12	0-3 cm	87	97	–	–	–	–	–	–	–	–
21-Mar-12	3-10 cm	94	90	–	–	–	–	–	–	–	–
23-May-12	0-3 cm	116	108	–	–	–	–	–	–	–	–
23-May-12	3-10 cm	103	102	–	–	–	–	–	–	–	–
25-Jul-12	0-3 cm	108	39	–	–	–	–	–	–	–	–
25-Jul-12	3-10 cm	102	31	–	–	–	–	–	–	–	–

imported into the software package PRIMER v7 (Clarke and Gorley, 2015) where data was analyzed using multivariate techniques. Bray-Curtis similarity was performed on the abundance matrix after fourth-root transformation to reduce bias when one species is very abundant (Clarke and Warwick, 1994). Similarity patterns were visualized with ordination of samples with non-metric multi-dimensional scaling (nMDS). A one-way permutational multivariate analysis of variance (PERMANOVA) was used to test the null hypothesis that there was no significant difference in nematode assemblages between samples, temporally or spatially. The contribution of individual genera to differences between the sites was determined by SIMPER (Similarity percentages) analysis (Clarke, 1993). A two fixed factor (sample location and sample date) PERMANOVA (Anderson et al., 2008) was applied to detect the statistical significance for pair-wise comparison of nematode communities at the locations at varying sampling dates.

Functional diversity of nematode communities was determined by classifying individuals to one of four feeding guilds based on the morphology of their mouthparts and pharyngeal musculature using the classification of Wieser (1953). Although more refined divisions of these feeding categories have been attempted (Moens and Vincx, 1997), here we used Wieser's (1953) scheme to allow comparison to other studies as it is a common method. The four guilds are: selective deposit feeders (1A) which have a small opening of the stoma and feed on very small food particles; non-selective deposit feeders (1B) which have a bigger buccal cavity so they can ingest varied size food particles; epigrowth feeders (2A) which have a tooth or denticles to scrape, pierce, or crack substrate for food; predators and omnivores (2B) which have a larger buccal cavity with teeth and mandibles that allow them to pierce and ingest prey. Epigrowth feeders (2A) can also be referred to as grazers. We used the original term, epigrowth feeder, to be consistent with the literature.

2.5. Morphological and metabarcoding comparison

Comparisons of nematode taxonomic assignments were made between this morphological study and the 18S metabarcoding study conducted by Brannock et al. (2014). Sediment samples for both studies were collected simultaneously. Comparison included only the subset of samples that were examined with both morphological and molecular tools. Isolation of nematode operational taxonomic units (OTUs) from Brannock et al. (2014) was conducted using the *filter_taxa_from_otu_table.py* script in QIIME v1.8 (Caporaso et al., 2010). As stated in Brannock et al. (2014), taxonomy was assigned to representative OTU sequences through obtaining the top MEGABLAST hit when compared to the SILVA v111 (Quast et al., 2013) database. Due to potential differences in taxonomic schemes used between the morphological and molecular aspects of the project, only nematode genera that were reported in the July 2011–July 2012 morphological samples and were also present within the SILVA v111 database were compared to ensure higher taxonomic levels matched between the two methods.

Samples used for both the morphological and molecular portions of the study were tested using one-fixed factor PERMANOVA to compare differences between sample location (RC, SL, CA, BP, and BB) and region (Bay and GOM) to determine if conclusions were similar between the two methods. For this test, all nematode down to morpho-species found morphologically and all nematode OTU's found molecularly within the RC July 2011 through July 2012, CA July 2011 through January 2012, and the remaining locations (SL, BP, and BB) from July 2011 to November 2011 were used. For this comparison, the average of the replicates was used to reduce heterogeneity between the various cores. Relative abundances were determined for both datasets based either on the number of individuals (morphological) or number of reads (molecular). The relative abundance datasets were imported into PRIMER. Jaccard resemblance matrix was calculated for both datasets. Bray-Curtis similarity was performed on each abundance matrix after fourth-root transformation as done previously. Similarity patterns (Jaccard and Bray-Curtis) were visualized with ordination of samples with non-metric multi-dimensional scaling (nMDS). RELATE in PRIMER was used to compare the matrices (Jaccard or Bray-Curtis) and determine whether the communities for the same locations were similar between the morphological and molecular approaches. If the resulting sample statistic (Rho) from this test is close to 1 that indicates similar patterns in both matrices.

3. Results

3.1. Taxonomic composition and diversity

This study examined 7905 nematodes (Table 2) that represented 114 genera (Table 3) distributed among 32 families over two years of sampling. Of the 105 samples examined, 32 (30.5%) had $\geq$ 100 individuals and 54 (51.4%) had $\geq$ 90 individuals. The lowest number of nematodes (2) was found in a BP replicate (Table 2). The highest generic richness (37 genera) occurred at RC in July 2011 (replicates were averaged) and lowest (5 genera) at CA in September 2010 and SL in March 2011 (Fig. 2). The number of genera decreased at all sites except SL from May 2010 to September 2010 after the oil spill. Richness of genera declined further in March 2011 at all sites except CA and BB (Fig. 2).

Community composition of nematode genera was significantly different (Pseudo-F = 5.859, p = 0.001) at the five intertidal sampling locations (Table 4). The greatest difference in genera was found between BB and RC assemblages, whereas the most similar genera composition was between BP and CA. A nMDS ordination of nematode genera illustrate RC and SL sites clustering together and indicates a separation from the other sites (BB, BP, and CA) (Fig. 3A). PERMDISP analysis indicated there was no significant difference in dispersions (t = 0.999, p = 0.362) indicating that differences seen between regions (Bay: BB, BP, and CA and GOM: RC and SL) was due to location only. Likewise, there was a significant difference in nematode genera assemblages in reference to the collection date (Pseudo-F = 2.580, p = 0.001). Pairwise comparisons indicated no significant differences after a Bonferroni multiple test correction (Table S1). A majority of differences in nematode genera were between May 2010 (pre-spill) samples and other collection dates (Table S1). Before the spill, the dominant genus across all sites was *Enoplolaimus* (18.1%; feeding guild 2B, predators and omnivores), whereas the after-spill dominant genus was *Theristus* (22.0% and 39.0%; 1B, non-selective deposit feeders) in September 2010 and March 2011, respectively. A two-factor PERMANOVA test indicates a greater difference between sampling locations (Pseudo-F = 10.824, p = 0.001) than between sampling dates (Pseudo-F = 5.776, p = 0.001). SIMPER analysis of dominant genera at the five sites that contributed to differences are noted in Table 5. The dominant genus at RC was *Mesacanthoides* (10.7%; 2B), SL was *Daptonema* (19.0%; 1B), CA was *Theristus* (20.2%; 1B), BP was *Axonolaimus* (20.9%; 1B), and BB was *Enoploides* (17.2%; 2B).

A distinct grouping of assemblages was seen among GOM stations (RC and SL) and Bay locations (BP, BB, and CA). PERMANOVA analysis indicated a significant difference between the two groups (Pseudo-F = 10.152, p = 0.001, Table 4). The five most dominant genera at Bay stations were *Theristus* (19.6%; 1B), *Axonolaimus* (15.0%; 1B), *Daptonema* (12.6%; 1B), *Viscosia* (8.9%; 2B), *Hypodontolaimus* (5.4%; 2A, epigrowth feeders), and *Metoncholaimus* (5.4%; 2B). The five most dominant genera at GOM stations were

Table 3
Nematode genera comparison between both the current morphological dataset and Brannock et al. (2014) molecular dataset. This list represents all nematode genera found within all samples examined morphologically.

Nematode genera from morphological examination	Found in July 2011–July 2012 morphological samples[a]	Found in SILVA v111 database[b]	Found in Brannock et al. (2014) molecular data	Found in Brannock et al. (2014) molecular data ONLY for sites with morphological data[a]
Actarjania				
Anoplostoma	X	X	X	X
Anticoma	X	X	X	X
Ascolaimus				
Atrochromadora	X	X		
Axonolaimus	X	X	X	X
Bathylaimus	X	X	X	X
Bolbolaimus	X			
Bradylaimus	X			
Camacolaimus	X	X	X	X
Campylaimus	X			
Ceramonema	X	X	X	X
Chaetonema	X	X		
Choanolaimus	X	X	X	X
Chromadorina	X	X	X	X
Chromadorita	X	X	X	X
Chromaspirina	X			
Cobbia	X			
Cyatholaimus	X	X	X	
Daptonema	X	X	X	X
Dasynemoides	X			
Desmodora	X	X	X	X
Desmolaimus	X	X	X	X
Desmoscolex	X	X		
Dichromadora	X	X		
Ditlevsenella	X			
Dolicholaimus				
Doliolaimus				
Dorylaimus	X	X		
Eleutherolaimus	X			
Enoploides	X	X	X	X
Enoplolaimus	X	X	X	X
Enoplus	X	X	X	X
Epsilonema	X	X		
Ethmolaimus	X	X		
Euchromadora	X			
Eumorpholaimus	X			
Eurystomina	X			
Filipjevia	X			
Gammanema	X			
Gnomoxyala	X			
Gomphionema	X			
Gonionchus	X			
Graphonema	X			
Halalaimus	X	X	X	X
Halichoanolaimus	X	X	X	X
Hypodontolaimus	X			
Innocuonema	X			
Latronema	X			
Lauratonema	X			
Leptolaimus	X	X	X	X
Linhomoeus	X			
Marylynnia	X			
Megadesmolaimus				
Mesacanthion	X			
Mesacanthoides	X			
Metachromadora	X	X	X	X
Metacyatholaimus	X			
Metadasynemoides	X			
Metalinhomoeus	X			
Metoncholaimus	X			
Meyersia				
Microlaimus	X			
Monhystera	X	X	X	
Monoposthia	X	X	X	X
Monoposthioides	X			
Nemanema	X			
Neochromadora				
Neotonchus	X			
Odontophora	X	X	X	X
Omicronema	X			

Table 3 (*continued*)

Nematode genera from morphological examination		Found in July 2011–July 2012 morphological samples[a]	Found in SILVA v111 database[b]	Found in Brannock et al. (2014) molecular data	Found in Brannock et al. (2014) molecular data ONLY for sites with morphological data[a]
Oncholaimellus		X	X		
Oncholaimus		X	X	X	X
Onyx		X			
Oxystomina		X	X	X	X
Paracanthonchus		X	X	X	X
Paracyatholaimus		X	X	X	X
Paralinhomoeus		X			
Paramonhystera		X			
Pareurystomina		X	X	X	X
Parodontophora		X			
Phanodermopsis		X	X	X	X
Polygastrophora		X			
Prochromadora		X	X		
Prochromadorella		X			
Promonhystera		X			
Prooncholaimus		X			
Pselionema		X			
Pterygonema		X			
Ptycholaimellus					
Quadricoma		X			
Retrotheristus		X			
Rhabditis		X	X	X	X
Rhabdocoma		X	X		
Rhabdodemania		X			
Rhynchonema		X			
Sabatieria		X	X	X	X
Siphonolaimus		X	X		
Sphaerolaimus			X		
Spirinia		X	X	X	X
Steineria		X			
Synonchium		X			
Syringolaimus		X	X	X	
Terschellingia		X	X		
Theristus		X	X	X	X
Thoracostomopsis		X			
Trichotheristus		X			
Tricoma		X			
Trileptium		X			
Tripyloides		X	X		
Trissonchulus		X			
Viscosia		X	X	X	X
Xyala		X			
Xyzzors		X	X		
Total	114	105	49	35	32

[a] These samples are Ryan Court (RC) July 2011–July 2012, Cadillac Ave. (CA) July 2011–January, as well as Shellfish Laboratory (SL), Bayfront Park (BP), and BelleAir Blvd (BB) July 2011–November 2011.

[b] Only genera that were found within the morphological samples from the July 2011–July 2012 time period were checked.

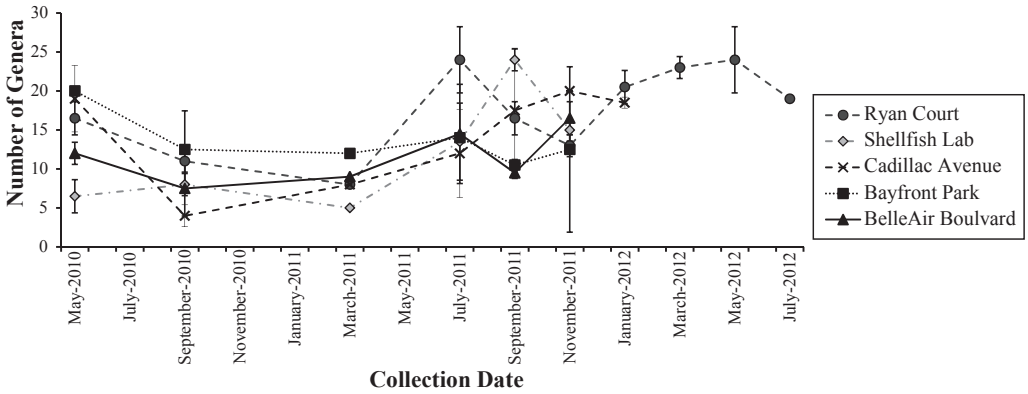

Fig. 2. Number of nematode genera present at each sample location at each time point. Bars indicate the standard deviation between the replicates.

Table 4
Nematode community structure at intertidal sites based on PERMANOVA and pairwise tests of the Bray-Curtis similarity on transformed relative abundance of genera at sampling locations and regions (Bay or GOM). Test statistic for the main test is the Psuedo-F value and the pairwise tests represents the t-value. Pairwise tests use the p-value from of the Monte Carlo test. Bold numbers show significant differences after Bonferroni corrections. Sample site abbreviations are reported in Table 1.

Comparison		Bray-Curtis Similarity	
		Test Statistic	p value
Sample Location	Main Test	**5.859**	**0.001**
	BP - RC	**3.108**	**0.001**
	BP - CA	**1.728**	**0.008**
	BP - BB	**1.996**	**0.002**
	BP - SL	**2.008**	**0.002**
	RC - CA	**2.867**	**0.001**
	RC - BB	**3.115**	**0.001**
	RC - SL	**1.662**	**0.006**
	CA - BB	**2.779**	**0.001**
	CA - SL	**1.646**	**0.007**
	BB - SL	**2.311**	**0.001**
Region	Bay - GOM	**10.152**	**0.001**

Metachromadora (12.4%; 2A), *Theristus* (11.6%; 1B), *Enoplolaimus* (8.7%; 2B), *Daptonema* (7.6%; 1B), and *Hypodontolaimus* (7.4%; 2A).

There was a significant difference in the composition of nematode families between sampling sites (Pseudo-F = 15.067, p = 0.001, Table 6) and between samples from different dates (Pseudo-F = 1.908, p = 0.001, Table S2). A nMDS ordination of families at the sampling locations shows similar separation between the sites as the genera data did (Fig. 3B). SIMPER analysis of families across sites indicated that Xyalidae was the dominant family at all locations (RC: 26.7%, SL: 44.1%, CA: 33.5%, and BP: 27.1%) except BB where the dominant family was Thoracostomopsidae (23.5%).

3.2. Vertical distribution

There was no significant difference in composition of nematode genera based on depth of sampled fraction (0–3 cm and 3–10 cm) (Pseudo-F = 0.911, p = 0.492). Dominant taxa in the upper 0–3 cm and 3–10 cm fractions were highly variable and did not display any consistent pattern (Table S3). SIMPER analysis of most abundant genera in the 0–3 cm layer (*Theristus:* 15.4%, *Axonolaimus*: 13.8% and *Daptonema*: 13.6%; all 1B) differed, with some genera more frequently present in the 3–10 cm layer (*Theristus*: 20.4% and *Hypodontolaimus*: 8.9%; 1B and 2A, respectively).

3.3. Functional composition

There was a significant difference in the composition of nematode feeding groups between sample sites (Pseudo-F = 4.769, p = 0.001, Table 7). RC had significantly different feeding group composition in comparison to BB and SL for both metrics (Table 7). There was no significant difference in feeding group in reference to region (Bay or GOM; Pseudo-F = 1.20, p ≥ 0.310, Table 7) or collection date (Pseudo-F = 1.481, p = 0.119). Predators and omnivores (2B) increased at BB immediately after the oil spill but non-selective deposit feeders (1B) dominated nematode fauna in most samples (Fig. 4). Epigrowth feeders (2A) as well as predators and omnivores (2B) were prevalent at all stations immediately after the oil spill except CA and SL. Although selective deposit feeders (1A) were almost absent at some stations (SL and BB), non-selective deposit feeders (1B) were the most common feeding group at all stations (Fig. 4). Epigrowth feeders (2A) were most prevalent at the BP and predators and omnivores (2B) were most prevalent at BB (Fig. 4).

3.4. Morphological and metabarcoding comparison

Given the 114 genera found based on morphological data here, 105 (92.1%) were found within the July 2011–July 2012 morphological samples (Table 3). The 2011–July 2012 timeframe was the period for which metabarcoding data was also available for these samples. Of the 105 genera, only 49 (46.7%) had representation within the SILVA v111 database. Therefore, a majority (56 genera; 53.5%) of genera found morphologically had no representation within the molecular database that went down to the appropriate genus level. However, representation could still be present at a higher taxonomic classification, due to the use of different taxonomic schemes at lower levels. Of those 49 genera with representation within SILVA, 35 (71.4%) were found within Brannock et al.'s (2014) entire dataset and 32 genera were found in the same samples that were examined morphologically herein. Comparing congruence of presence/absence of the 32 genera between morphological and metabarcoding approaches for the same location and time point, some genera matched between the two datasets almost all the time (≥80%; *Axonolaimus*, *Ceramonema*, *Chromadorina*, *Daptonema*, *Oxystomina*, *Rhabditis*, and *Theristus*) whereas other genera rarely matched between the two approaches (≤20%; *Anoplostoma*, *Enoplus*, and *Spirinia*).

Morphological and molecular approaches displayed similar spatial nematode community composition results (Fig. 5, Table 8). Both approaches revealed significant community differences between sample locations (RC, SL, CA, BP, and BP) and region (Bay and GOM) (Table 8). RELATE analysis showed that for both the Jaccard resemblance and Bray-Curtis similarity matrices showed similar patterns between both the morphological and molecular approaches (Rho = 0.609, p = 0.001 and Rho = 0.692, p = 0.001, respectively).

4. Discussion

Genus-level diversity of nematode assemblages decreased and community composition changed between May 2010 and September 2010 at all five intertidal sample locations within Mobile Bay and along Dauphin Island, AL (Fig. 2). Bik et al. (2012). reported a dramatic shift in meiofaunal community composition at the same sample locations for the same dates when examining higher level taxonomic (not genera) differences. They found that in May 2010 the five communities were heavily dominated by nematodes and annelids and shifted to predominately fungal communities in September 2010 (Bik et al., 2012). The current study demonstrated that nematode genera richness continued to decrease at the March 2011 time point for all locations except CA and BB. By July 2011, the number of genera increased and some locations (RC, SL, and BB) exceeded the May 2010 numbers (Fig. 2). However, no significant differences in nematode families were observed between sampling dates indicating that taxonomic differences resulted from changes in representation of genera within the same family. Decreases in nematode diversity (Gourbault, 1987; Danovaro et al., 1995, 2009) and density (Giere, 1979; Danovaro et al., 1995) have been reported in previous studies examining the effects of oil pollution on nematodes and meiofaunal community composition. Examination of an impacts on a Hong Kong beach showed that within four days of the spill most nematodes disappeared but began to recover within a month (Wormald, 1976). Danovaro et al. (1995) found negative immediate impacts (within days) of the *Agip Abruzzo* oil spill on nematode density and diversity, which recovered to similar pre-spill conditions within weeks after exposure. In contrast, nematodes had a delayed response to the *Amoco Cadiz* oil spill, showing no significant immediate (10 days) impacts, but a decrease in diversity between 7 and 19 months after the spill had occurred

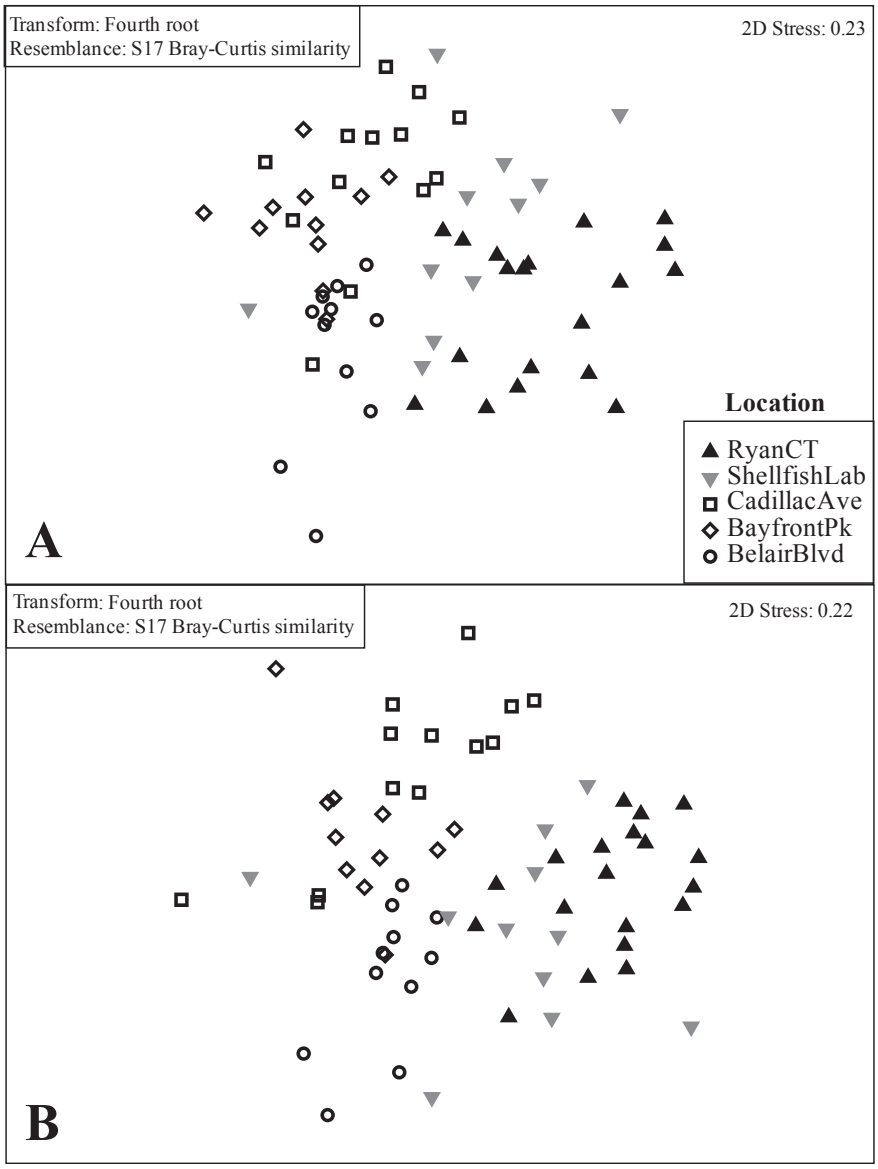

Fig. 3. Community composition of nematode genera. Non-metric multidimensional scaling (nMDS) ordination of Bray-Curtis similarity based on nematode A) genera or B) family taxonomic level.

(Renaud-Mornant et al., 1981; Gourbault, 1987; Danovaro et al., 2009). In addition, nematodes noticeably shifted in community composition after the *Amoco Cadiz* oil spill (Danovaro et al., 2009). Discrepancies in responses to oil pollution have been attributed to varying exposure levels (amount and duration) in combination with sediment properties (Danovaro, 2000; Danovaro et al., 2009). Likewise, oil composition, depth of sample collection and taxonomic level investigated most likely plays a role in the effects observed. While there is no consistent agreement among studies on the response of nematodes to oil spills, several studies have noted the recovery of nematodes after such a disturbance (Wormald, 1976; Giere, 1979; Danovaro et al., 1995). Immediate nematode response to the *DWH* oil spill along the Alabama coast is unknown. However, all five sampled locations displayed a decrease in nematode diversity 3 months (September 2010) and 9 months (March 2011) after oil reached this region (in June 2010). Nematode genera richness is partially related to the number of individuals examined as more individuals collected could potentially lead to more genera observed. In the current study, the number of nematode individuals collected was variable between sites, collection dates, and even cores. However, there was no clear pattern between the number of nematodes sampled and the number of genera found (Table S4).

The effect of oil pollution on intertidal nematodes is variable and may be affected by local hydrodynamics, microtopography, and natural conditions. Hayworth et al. (2011) noted that *DWH* tar-balls may persist in Alabama sediments and be periodically exposed due to storms. Unfortunately, no hydrocarbon specific measurements were conducted for our samples; therefore, we cannot state for certain the degree or differences in hydrocarbon exposure sampled locations experienced. However, scanning electron microscopy (SEM) and energy dispersive x-ray spectroscopy (EDS) analysis performed on a subset of these samples noted that some oil was retained in crevices of the quartz sediment particles (Williams, 2013). In addition, Williams (2013) also reported organic carbon in May 2010 (pre-spill) samples was significantly lower than in September 2010 (post-spill) samples with the exception of BB. The increase in organic carbon may be caused by hydrocarbons entering the system. Moreover, beach clean-up efforts were conducted to

Table 5
Cumulative percent contribution and feeding type of ten most discriminating species at each site from SIMPER analysis. The average similarity within a site is also presented.

Ryan Court			Shellfish Lab			Cadillac Ave			Bayfront Park			BelleAir Blvd		
Average Similarity: 35.29			Average Similarity: 29.64			Average Similarity: 37.51			Average Similarity: 39.81			Average Similarity: 45.44		
Species	Feeding Group	% Contribution	Species	Feeding Group	% Contribution	Species	Feeding Group	% Contribution	Species	Feeding Group	% Contribution	Species	Feeding Group	% Contribution
Mesacanthoides	2B	10.71	*Daptonema*	1B	19.02	*Theristus*	1B	20.18	*Axonolaimus*	1B	20.88	*Enoploides*	2B	17.17
Metachromadora	2A	10.20	*Theristus*	1B	14.80	*Daptonema*	1B	16.36	*Theristus*	1B	16.47	*Axonolaimus*	1B	14.11
Theristus	1B	8.73	*Metachromadora*	2A	14.17	*Viscosia*	2B	10.83	*Daptonema*	1B	13.65	*Theristus*	1B	11.86
Enoplolaimus	2B	6.83	*Enoplolaimus*	2B	10.43	*Camacolaimus*	2A	7.71	*Viscosia*	2B	8.03	*Metoncholaimus*	2B	10.80
Rhynchonema	1B	6.07	*Hypodontolaimus*	2A	8.71	*Axonolaimus*	1B	5.48	*Prochromadora*	2A	5.26	*Prochromadorella*	2A	9.22
Hypodontolaimus	2A	5.88	*Microlaimus*	1B	4.10	*Metachromadora*	2A	4.52	*Terschellingia*	1A	4.74	*Polygastrophtora*	2B	8.89
Axonolaimus	1B	4.98	*Metoncholaimus*	2B	3.91	*Hypodontolaimus*	2A	4.51	*Hypodontolaimus*	2A	4.53	*Oncholaimus*	2B	6.34
Enoploides	2B	4.97	*Camacolaimus*	2A	3.07	*Microlaimus*	1B	3.44	*Oncholaimus*	2B	4.29	*Viscosia*	2B	4.40
Bathylaimus	2B	4.47	*Hypodontolaimus*	2A	3.03	*Anticoma*	1A	2.91	*Chromadorita*	2A	3.85	*Daptonema*	1B	4.18
Lauratonema	1B	3.69	*Viscosia*	2B	2.82	*Paramonhystera*	1B	2.88	*Paracyatholaimus*	2A	3.80	*Hypodontolaimus*	2A	4.17

Table 6
Nematode community structure at intertidal sites based on PERMANOVA and pairwise tests of the Bray-Curtis similarity on transformed relative abundance of families at sampling locations and regions (Bay or GOM). Test statistic for the main test is the Psuedo-F value and the pairwise tests represent the t-value. Pairwise tests use the p-value from of the Monte Carlo test. Bold numbers show significant differences after Bonferroni corrections. Sample site abbreviations are reported in Table 1.

Comparison		Bray-Curtis Similarity	
		Test Statistic	p value
Sample Location	Main Test	**8.771**	**0.001**
	BP - RC	**4.104**	**0.001**
	BP - CA	**1.900**	**0.012**
	BP - BB	**2.677**	**0.001**
	BP - SL	**2.488**	**0.001**
	RC - CA	**3.585**	**0.001**
	RC - BB	**4.142**	**0.001**
	RC - SL	**1.892**	**0.007**
	CA - BB	**3.207**	**0.001**
	CA - SL	**2.102**	**0.001**
	BB - SL	**2.469**	**0.001**
Region	Bay - GOM	**15.067**	**0.001**

Table 7
Nematode community structure at intertidal sites based on PERMANOVA and pairwise tests of the Bray-Curtis similarity on transformed relative abundance of feeding groups at sampling locations and regions (Bay or GOM). Test statistic for the main test is the Psuedo-F value and the pairwise tests represent the t-value. Pairwise tests use the p-value from of the Monte Carlo test. Bold numbers show significant differences after Bonferroni corrections. Sample site abbreviations are reported in Table 1.

Comparison		Bray-Curtis Similarity	
		Test Statistic	p value
Sample Location	Main Test	**4.769**	**0.001**
	BP - RC	1.718	0.045
	BP - CA	0.427	0.835
	BP - BB	2.504	0.008
	BP - SL	1.627	0.072
	RC - CA	1.965	0.018
	RC - BB	**3.530**	**0.001**
	RC - SL	**3.153**	**0.001**
	CA - BB	2.409	0.007
	CA - SL	1.250	0.225
	BB - SL	1.500	0.137
Region	Bay - GOM	1.2078	0.310

remove oil along the Louisiana through the Florida panhandle coastal region. We do not have any information pertaining to the clean-up efforts in the region that was sampled. However, RC and SL would have been the sites most likely impacted by mechanical clean-up efforts as these sites were consistently used for summer recreation and faced the GOM (Brannock, personal observation). Beach cleaning has been reported to affect meiofaunal communities in terms of decreasing abundance and altering community structure directly through the physical removal of organisms and indirectly by changing sediment properties (Gheskiere et al., 2006; Danovaro et al., 2009). However, even if beach cleaning were to play a role in some of the shifts seen at both RC and SL, it could not explain the shifts observed at the other locations (CA, BP, and BB) where cleaning efforts were not focused.

Composition of feeding groups in the intertidal sites is likely related to the sediment grain size (Giere, 2009). Sediment analyses from all five locations showed heterogeneous grain size distribution with coarse to medium grain size and moderately to well sorted sediments at all locations except BP which had coarse, poorly sorted sediments in two samples (May 2010 and July 2011

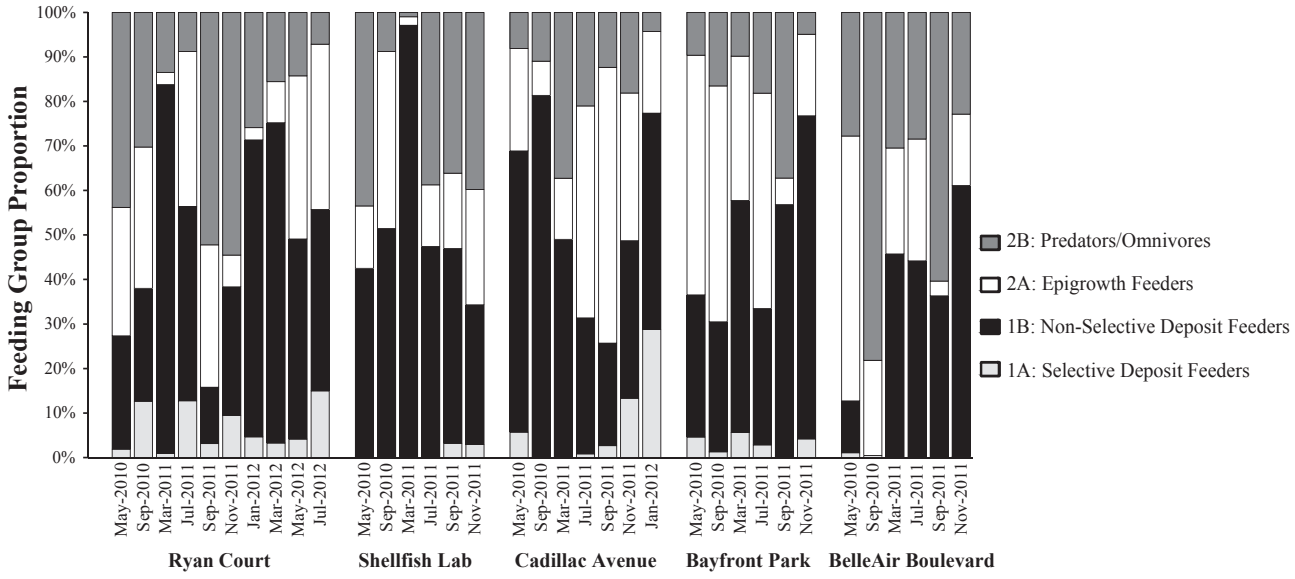

Fig. 4. Proportion of nematode feeding groups from individuals sampled at each location for the time points examined.

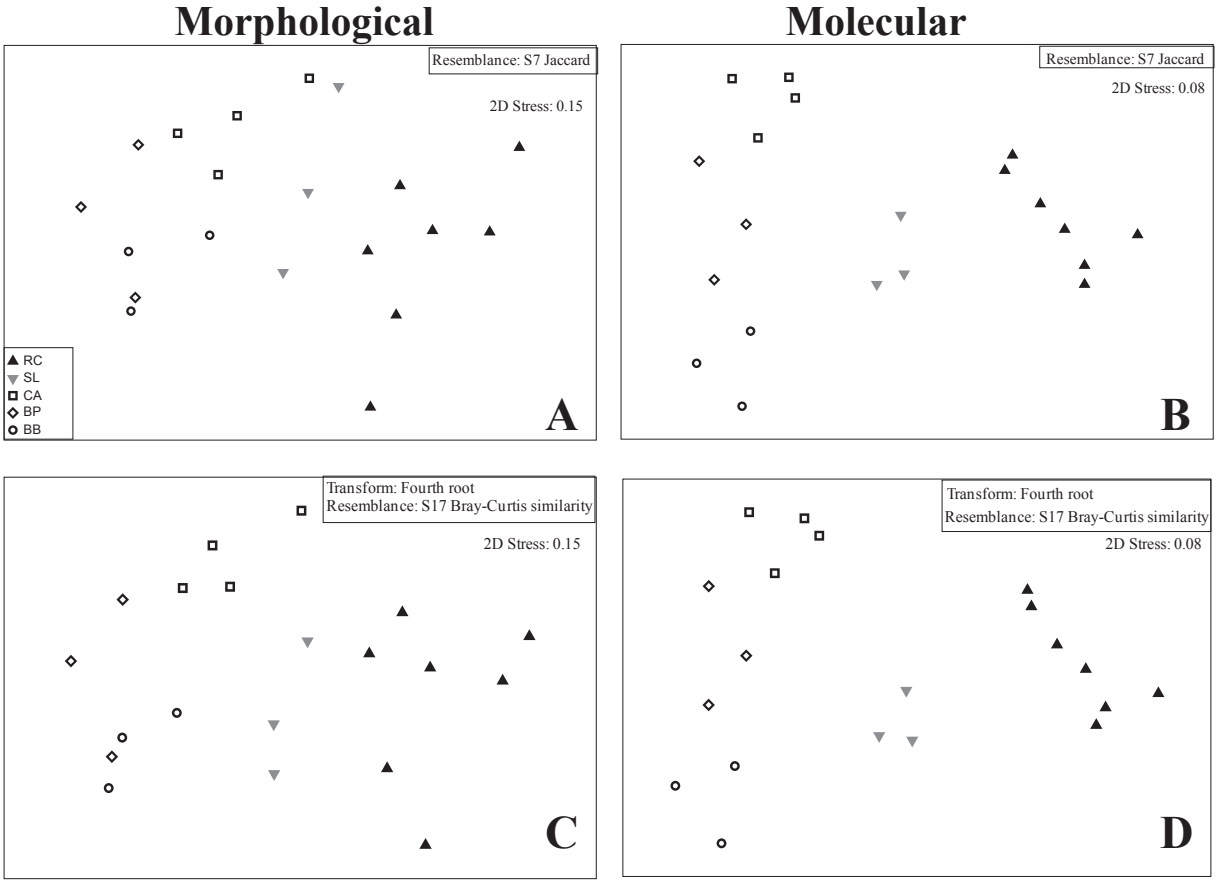

Fig. 5. Non-metric multidimensional scaling (nMDS) ordination of the Jaccard (A and B) and Bray-Curtis (C and D) beta-diversity metrics for the morphological (A and C) and molecular (B and D) approaches. Only RC July 2011 through July 2012, CA July 2011 through January 2012, and the remaining locations (SL, BP, and BB) from July 2011 to November 2011 samples are represented.

Table 8
Nematode community structure at intertidal sites based on PERMANOVA and pairwise tests of binary Jaccard and Bray-Curtis similarity metrics based on relative abundance of either operational taxonomic units (OTU) for molecular approaches or morpho-species for morphological approaches. Bold numbers show significant differences. Sample site abbreviations are reported in Table 1.

Comparison		Jaccard				Bray-Curtis			
		Morphological		Molecular		Morphological		Molecular	
		Test Stat	p	Test Stat	p	Test Stat	p	Test Stat	p
Sample Location	Main Test	**2.269**	**0.001**	**2.983**	**0.001**	**3.444**	**0.001**	**5.049**	**0.001**
	BP - RC	**1.826**	**0.007**	1.725	0.019	**2.293**	**0.004**	**2.295**	**0.007**
	BP - CA	1.406	0.025	1.310	0.149	1.672	0.454	1.535	0.082
	BP - BB	0.985	0.616	1.236	0.249	1.084	0.337	1.443	0.133
	BP - SL	1.333	0.109	1.585	0.071	1.447	0.121	1.965	0.034
	RC - CA	**1.692**	**0.016**	**1.971**	**0.003**	**2.056**	**0.004**	**2.669**	**0.001**
	RC - BB	**1.776**	**0.012**	**1.887**	**0.011**	**2.310**	**0.007**	**2.547**	**0.002**
	RC - SL	1.192	0.173	**1.830**	**0.012**	1.368	0.090	2.303	0.007
	CA - BB	1.570	0.071	1.743	0.030	**2.173**	**0.012**	2.339	0.010
	CA - SL	1.299	0.161	1.798	0.031	1.502	0.085	**2.340**	**0.009**
	BB - SL	1.337	0.168	1.773	0.057	1.623	0.075	2.262	0.023
Region	Bay - Shelf	**3.905**	**0.001**	**3.458**	**0.001**	**5.573**	**0.001**	**5.567**	**0.001**

samples; Williams, 2013). Although selective deposit feeders (1A) had a limited presence at some stations, non-selective deposit feeders (1B) were dominant at all stations (Fig. 4). This finding was surprising given that two sites were exposed and located on the nGOM coast (RC and SL) and the other three sites were more sheltered with higher sedimentation bay locations (CA, BP, and BB). However, non-selective deposit feeder guild (1B) has been reported to dominate all intertidal zones (Gheskiere et al., 2004), and selective or non-selective deposit feeders are usually associated with heterogeneous sandy sediment (Giere, 2009). Therefore, our finding of both feeding groups in high proportions throughout the study mirrors previous studies. In addition, there was no significant difference found within the abundance of nematode feeding groups between sample dates. Danovaro et al. (1995) reported a decline in non-selective deposit feeders (1B) after the *Agip Abruzzo* oil spill, but noted no difference within trophic diversity. Nematode placement in feeding groups is mainly dependent upon buccal morphology and not direct observations of prey consumption. Moreover, nematodes have been suggested to have the ability to change feeding preference dependent on food source (Moens et al., 2004, 1999). Therefore, traditional methods of placement of nematode individuals into feeding groups may not be accurately portraying trophic interactions (Guilini et al., 2010; Pape et al., 2013). However, for ease of characterization, the traditional classification of Wieser (1953) that is based on morphology of the buccal cavity is continued to be used.

Bik et al. (2012). found a substantial shift from a diverse nematode-dominated community prior to *DWH* oil impacting Alabama intertidal regions to one dominated by fungi after the exposure to oil. A majority of the fungal taxa found in post-spill samples (Bik et al., 2012) were comprised of taxa that have been previously reported to play a role in hydrocarbon degradation (Cofone et al., 1973) or contain enzymes that can potentially breakdown industrial toxins (Mtui and Nakamura, 2008; Atalla et al., 2010). Meyers and Hopper (1966) observed that *Metoncholaimus*, a predatory nematode genus (2B), is associated with fungal mats, though this varies by site. They also found that other nematode genera, *Viscosia* (2B), *Leptolaimus* (1A), and *Monhystera* (1B) could also subsist on marine fungi (Meyers and Hopper, 1966). All four genera were found within the current study (Table 3). Giere (1979) noted the predatory nematode *Enoplolaimus* (2B) ingested bacteria coated with oil droplets. These oil-coated bacteria were also observed in *Bathylaimus* (1B) and *Tripyloides* (1B) (Giere, 1979). All three of these genera were also found within the current study (Table 3). However, a majority of the seven genera mentioned above were not present in September 2010 or March 2011 samples, and if they were present they were not the dominant genera (Table S5). There was a change in nematode genera from before and after the *DWH* oiling at all five Alabama locations (Table S5). Interestingly, both locations that were more exposed and located on the GOM coast (RC and SL) were dominated by *Enoplolaimus* before and *Theristus* after the spill. *Enoplolaimus* and *Theristus* both have been shown to be intolerant to oil pollution (Kang et al., 2014). *Theristus* became abundant in these samples during the March 2011 sample (Table S5). Contrary, the three more sheltered and higher sedimentation locations (CA, BP, and BB) were either dominated by *Daptonema*, *Paracanthonchus*, or *Ethmolaimus* prior to the spill but were all dominated by *Axonolaimus*, especially in March 2011 (Table S5). *Paracanthonchus* and *Ethmolaimus* are epigrowth feeders (2A) and *Daptonema* has been observed with diatoms in gut (Sharma personal observations). Knowledge of specific life cycles and food or habitat preference of these groups is limited (Moens and Vincx, 1997; Singh and Ingole, 2011), making it difficult to interpret these findings. Gingold et al. (2010). have noted the importance of environmental gradients and habitat heterogeneity on the distribution of intertidal nematode assemblages, although such gradients have not been extensively studied across the GOM shoreline habitats.

Nematode community composition was more influenced by sample locality than time and depth from which samples were obtained (Fig. 3, Table 4, Table S1). The same finding was reported by Brannock et al.'s (2014) metabarcoding approach of the meiofaunal community composition, which employed the same sampling locations. Similarly, sample location had a larger effect on community composition than the time sampled in pelagic (Ortmann and Ortell, 2014; Brannock et al., 2016a) and subtidal (Brannock et al., 2016b; Ortmann unpublished) communities in the same geographic location. Groupings of nematodes by sample location may be attributed to variations in physical and biological factors such as salinity, granulometry, food availability, predators, and nematode life cycles. Further exploration including more physical and biological factors needs to be conducted in order to obtain a better understanding of this finding.

When comparing morphological and molecular identification of nematode communities, the lack of representation of nematode genera within the SILVA v111 database was an issue. Only 46.75% of

genera found within the overlapping samples were listed within taxonomic strings of sequences within the SILVA v111 database (Table 3). Even when searching for nematode genera within a more recent version of the SILVA database (SILVA v123-the most recent version formatted for QIIME at the time of manuscript preparation), all nematode genera that were absent in SILVA v111 were still absent in v123. This finding brings up an important point that better molecular representation of meiofauna is required to be able to determine who is present in the community (especially for genera). With taxonomic information provided down to the genus level, consistency of higher taxonomic levels could then be checked. However, of the 32 genera that matched between the two approaches, there were varying results regarding the consistency that a given genus was present within a given sample. *Axonolaimus*, *Ceramonema*, *Chromadorina*, *Daptonema*, *Oxystomina*, *Rhabditis*, and *Theristus* occurred in both datasets almost all the time. Contrary, *Anoplostoma*, *Enoplus*, and *Spirinia* rarely occurred using molecular tools where morphologically they were found. Differences in genera observed between morphological and molecular data could be caused by (A) subsampling only 100 nematodes for morphological analysis, (B) differences in clustering of sequences (97% similarity used here), (C) parameters used for taxonomic assignment, and (D) primer set used for molecular characterization. Sampling a subset of nematodes morphologically rather than the complete sample could bias against rare or less abundant taxa. Likewise, bias may factor into results as an individual is picking nematodes (e.g. selecting larger nematodes over smaller specimens, although the use of a gridded petri dish is meant to reduce the likelihood of picker bias). Clustering sequences at a higher similarity cut-off would allow for more discrimination between OTU clusters, and potentially 97% is including sequences of different genera. When assigning taxonomy, a representative (the most abundant) sequence for that OTU was used for comparison against the SILVA v111 database. The highest blast hit ($\geq$90% sequence similarity cut off) was used for the taxonomy for that OTU. If a certain genus was not present in the database (which would lead to a higher hit) that sequence could be potentially assigned as a closely related genus provided the match was sufficiently similar. Lastly, inconsistencies between the two approaches for genera that were present in both could be explained by primer bias against (or for) certain taxa. Further exploration of these issues need to be undertaken.

The intertidal habitat is a dynamic ecosystem that is subject to anthropogenic disturbances. Taxonomic composition (i.e. representation of genera) of nematode communities at five Alabama intertidal sites was different before and after *DWH* oiled beaches. Over the 2-year period of this study, some nematode genera appeared to be more resilient than others. However, the more recent collection time points showed that there is, potentially, a new steady state within the nematode genera within this geographic region. In addition, this study illustrates how metabarcoding approaches can provide an alternative faster protocol in examining shifts in nematode community composition in response to disturbances. However, due to the lack of taxonomic representation within molecular databases, metabarcoding approaches currently cannot be utilized to correctly taxonomically identify nematode genera or species. Likewise, uncertainty of how metabarcoding read number relates to abundance in eukaryotic studies limits most analysis currently to presence/absence examination. However, molecular approaches can provide insight on more diversity within the community than can be observed morphologically. Currently, research combining both molecular and morphological approaches would be highly beneficial in understanding the spatially and temporal variation.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.marenvres.2017.07.008.

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