Ocean Sci. J. (2016) 51(3):465–476
http://dx.doi.org/10.1007/s12601-016-0042-8

Available online at http://link.springer.com

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Article

Ocean Science Journal

pISSN 1738-5261
eISSN 2005-7172

Responses of Meiofauna and Nematode Communities to Crude Oil Contamination in a Laboratory Microcosm Experiment

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Received 27 May 2015; Revised 10 April 2016; Accepted 23 August 2016

Abstract – We examined the effects of crude oil contamination on community assemblages of meiofauna and nematodes after exposure to total petroleum hydrocarbons in the laboratory. We administered a seawater solution that had been contaminated with total petroleum hydrocarbons to seven treatment groups at different concentrations, while the control group received uncontaminated filtered seawater. The average density of total meiofauna in the experimental microcosms diluted with 0.5%, 1%, 2%, and 4% contaminated seawater was higher than the density in the control. The average density of total meiofauna in the 8%, 15%, and 20% microcosms was lower than the density in the control. The density of nematodes was similar to that of the total meiofauna. Cluster analysis divided the microcosms into group 1 (control, 0.5%, 1%, 2%, and 4% microcosms) and group 2 (8%, 15%, and 20% microcosms). However, SIMPROF analysis showed no significant difference between the two groups ($p > 0.05$). *Bolbolaimus* spp. (37.1%) were dominant among the nematodes. Cluster analysis showed similar results for nematode and meiofaunal communities. The total meiofaunal density, nematode density, and number of *Bolbolaimus* spp. individuals were significantly negatively associated with the concentration of total petroleum hydrocarbons (Spearman correlation coefficients, $p < 0.05$). Within the nematodes, epistrate feeders (group 2A: 46%) were the most abundant trophic group. Among the treatment groups, the abundance of group 2A increased in low-concentration microcosms and decreased in high-concentration microcosms. Thus, our findings provide information on the effects of oil pollution on meiofauna in the intertidal zones of sandy beaches.

Key words – meiofauna, nematode, hydrocarbon, oil spill, pollution

1. Introduction

Hydrocarbons are the most common organic contaminants in the marine environment (Louati et al. 2001). Marine ecosystems are often contaminated by petroleum hydrocarbons (Kennish 1992; Louati et al. 2001), and crude oil spills are a major source of hydrocarbon contamination (Kennish 1992). These oil spills represent a major threat that may drastically affect marine ecosystems (Dauvin 1982; Danovaro et al. 1995; Jewett et al. 1999).

It is important to understand the effects of crude oil contamination on communities of marine organisms. Although a number of studies have examined the effects on macrofauna, little is known about the effects of hydrocarbon pollution on meiofauna. Meiofauna serve as sensitive indicators of environmental biological pollution due to their small size, natural high abundance, and short generation time, as well as the fact that they complete their entire life-cycle within the sediment (Boucher 1980; Higgins and Thiel 1988; Coull and Chandler 1992). They are more rapidly affected by changes in abiotic and biotic environmental parameters than are macrofauna (Higgins and Thiel 1988).

Some studies have examined the effects of petroleum hydrocarbons on meiofauna communities due to accidental oil spills (Boucher 1980; Fleeger and Chandler 1983; Friethsen et al. 1985; Bodin 1988; Danovaro et al. 1995; Austen and McEvoy 1997; Danovaro 2000; Ansari and Ingole 2002; Commito and Tita 2002; Schratzberger et al. 2003). Many of these studies yielded different results, suggesting that different communities of meiofauna have different responses to oil pollution. Hydrocarbon contamination may influence communities of meiofauna negatively or positively. Some studies reported a reduction in the population of most meiofauna immediately following an oil spill (Boucher 1980; Elmgren et al. 1983; Coull and Chandler 1992; Danovaro et al. 1995; Peterson et

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al. 1996; Kang et al. 2014). However, other studies reported that several taxa responded positively to oil application and showed no oil-induced mortality (Fleeger and Chandler 1983; Stacey and Marcotte 1987; Feder et al. 1990).

It is easy to maintain and manipulate natural meiobenthic communities, particularly the normally dominant nematode component, in simple laboratory microcosms (Austen 1989; Sundelin and Elmgren 1991). Therefore, it is possible to explore the ecological effects of anthropogenic factors on whole communities of meiofauna in the laboratory (Warwick et al. 1988; Sundelin and Elmgren 1991; Austen et al. 1994; Carman et al. 1995; Millward and Grant 1995). Many studies have examined microcosms of meiofauna (Austen et al. 1994; Austen and McEvoy 1997; Austen and Somerfield 1997; Schratzberger and Warwick 1998; Mahmoudi et al. 2005). These studies have shown that it is possible to detect differences in the ecological effects of different types of pollutants on meiofauna.

Here, we present the results of a microcosm experiment that evaluated the effects of crude oil contamination on meiofauna and nematode communities collected on an intertidal sandy beach. The purpose of this study is to understand the effects of crude oil on densities of meiofauna and dominant taxa, species diversity, and composition of nematode communities.

2. Materials and Methods

Experimental site

Sediments containing natural meiofaunal communities were collected from Mallipo beach sand at low tide in February 2014. The study site was an intertidal sandy beach located on the Taean coast (36°47'16.69"N, 126°8'34.97"E) of the Yellow Sea in Korea (Fig. 1). The average temperature of the air was 3.5°C and that of the water was 4.3°C in February 2014. The seawater salinity was 31 psu. The sediments had a mean grain size of 1.64 phi and organic carbon content of 0.038% and were primarily composed of sand (99.5%), with some gravel (0.4%) and silt (0.1%). The total petroleum hydrocarbon (TPH) concentrations of Mallipo beach ranged between 3.5 and 35 ppb, with a mean 16.6 ppb from 2010 to 2014 according to a twice-yearly survey (KIOST 2015).

Experimental design

Contaminated water was created by mixing filtered (1.2 μm) seawater and crude oil at a ratio of 9:1 using a magnetic bar for 24 hours. Next, the seawater was separated into water and oil using a separatory funnel for 24 hours. Separated seawater was considered 100% contaminated seawater (stock solution). The TPH level of the stock solution was 2564 ppb. The oil used in the experiment was Iranian Heavy

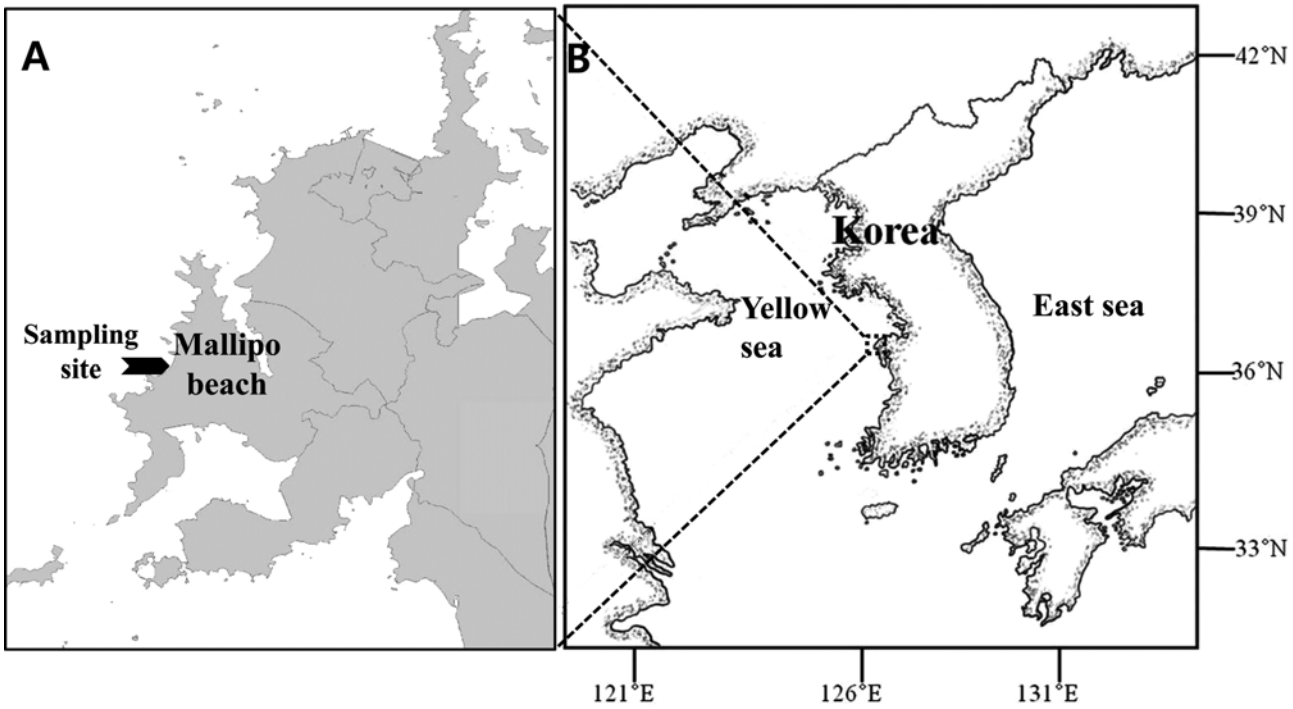

Fig. 1. Location of sampling site (A: specific location; B: general location in the Yellow Sea)

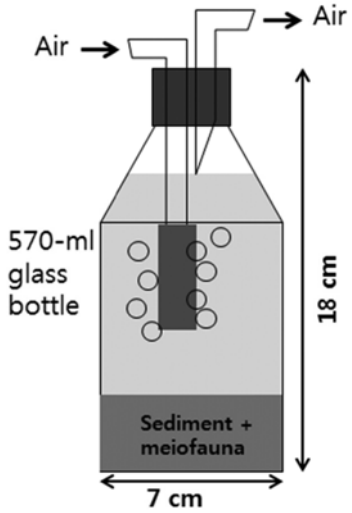

Fig. 2. Schematic diagram of microcosm

oil with a specific gravity of 0.87. The oil mostly contained hydrocarbons and some trace metals.

Microcosms were maintained in 570-mL glass bottles (Fig. 2). A microcosm set-up of the same type was successfully used to examine the effects of organic enrichment on nematode communities (Schratzberger and Warwick 1998). A control and seven experiment bottles that received water contaminated to different extents were used. There were three replications of a control microcosm that received uncontaminated filtered seawater and three replications each of the seven treatments, which received the stock solution diluted with 0.5%, 1%, 2%, 4%, 8%, 15%, and 20%, respectively, contaminated seawater. In addition, 200 g of natural sediment was added to each microcosm and the jar was filled with contaminated water of the particular TPH concentration. Each microcosm was aerated using an aquarium air-stone and was run as a closed system in a dark room. The room temperature and humidity were kept constant at 21°C and 60%. All experiments were terminated after 60 days and the sediments were fixed in 4% formalin. Immediately after the end of the experiment, the seawater of each bottle was analyzed to determine the level of TPH using a fluorometer (Turner Model 10AU).

Sample processing

In order to conduct meiofauna community analysis, the organisms were isolated from the sediment. First, samples were rinsed with tap water and filtered through a 38-μm mesh sieve. Sample material remaining on the sieve was centrifuged twice with Ludox HS40 (du Pont), with a specific gravity of 1.18 g·cm-3 (Burgess 2001). Meiofauna were retained on a 38-μm mesh sieve and preserved in 4% formaldehyde containing Rose Bengal to stain the organisms. Meiofauna were enumerated to a higher taxonomic level according to Higgins and Thiel (1988), using a dissecting microscope (Leica MZ16). All nematodes were picked out using a fine pin under a microscope, transferred from formalin to 3% glycerin, and mounted on microscope slides in anhydrous glycerine for identification. Nematodes were identified to the genus level using the pictorial keys of Platt and Warwick (1983, 1988) and Warwick et al. (1998) with the aid of an Olympus BX51 microscope. The mounted nematodes were classified according to the original groupings of Wieser (1953) into four feeding groups to investigate the trophic structure of the community.

The cluster and non-metric multidimensional scaling (MDS) analysis were carried out using the Bray–Curtis similarity measure. The average abundance data of all taxa were not transformed. The abundance of nematode species found in the samples was fourth root transformed. Cluster and MDS were applied to determine whether the meiofauna and nematode communities responded to different concentrations of oil contamination. A similarity profile (SIMPROF) test was conducted to identify community differences with cluster analysis and significance was set at $p < 0.05$. Univariate indices were computed: number of species in three replications, mean species richness, mean species evenness, and mean Shannon–Wiener diversity. Cluster analysis, MDS, SIMPROF test, and univariate indices were performed using the software package PRIMER, version 6.1.12 (Clarke and Gorley 2001).

The MI (Maturity Index) to identify colonization strategies of nematodes was calculated according to the weighted mean of the individual genus scores.

$$MI = \Sigma v(i) f(i)$$

where v is the colonizer–persister (c–p value) of genus i, as given in the appendix of Bongers et al. (1991), and $f(i)$ is the frequency of that genus (Bongers 1990). All nematode genera have a c–p scale ranging from 1 (for extreme colonizers) to 5 (for extreme persisters).

The Spearman correlation coefficient was carried out using SPSS v. 19 to test whether the oil concentration of the initial experimental water was correlated with meiofauna community parameters and dominant nematode species. For all the statistical tests, significance was set at $p < 0.05$.

3. Results

TPH concentrations in treatment groups

Initial and final TPH mean concentrations of the seawater, given as the mean of three microcosm replicates in each case, are provided in Table 1. The initial concentration of experimental samples was estimated using the stock solution (100% polluted seawater, 2564 ppb). The final concentrations of treated microcosms ranged between 9.6 and 20.7 ppb, with a mean 13.8 ppb, and did not significantly differ from that of the control microcosm, 18.1 ppb.

Meiofauna density and community structure

Total density of meiofauna is shown in Table 2 and Fig. 3. The density of total meiofauna was 115.3 ± 3.0 inds. microcosm^{-1} in the control microcosm. The total meiofauna density of experimental microcosms that received seawater contaminated below 4% ranged from 141.3 ± 19.92 to 183.0 ±

Table 1. Total petroleum hydrocarbon concentration (μg/L, ppb) of the seawater at each level of contamination

	Control	0.5%	1%	2%	4%	8%	15%	20%
Initial concentration	-	12.8	25.6	51.3	102.6	205.1	384.7	512.9
Final concentration	18.1 ± 7.4	11.3 ± 1.5	10.6 ± 3.7	9.6 ± 2.9	16.4 ± 8.8	16.5 ± 3.6	20.7 ± 4.1	11.4 ± 0.9

Table 2. Mean meiofaunal density (inds. microcosm^{-1}) in each treatment group 60 days after the start

	Control	0.5%	1%	2%	4%	8%	15%	20%
Nematodes	96.3 ± 3.1	132.7 ± 3.3	128.0 ± 1.6	102.7 ± 1.7	106.0 ± 15.5	71.7 ± 3.1	58.0 ± 5.3	46.3 ± 2.1
	83.5%	72.5%	80.7%	69.1%	75%	75.2%	61.7%	56%
Harpacticoids	16.7 ± 0.5	43.7 ± 3.8	22.7 ± 1.6	41.7 ± 2.4	29.3 ± 4.7	22.3 ± 2.8	27.7 ± 3.4	29.7 ± 1.1
	14.5%	23.9%	14.3%	28%	20.7%	23.4%	29.4%	35.9%
Nauplii								0.3 ± 0.1
								0.4%
Polychaetes	0.3 ± 0.1	1.7 ± 0.3	1.0 ± 0.2	1.3 ± 0.2	1.7 ± 0.4	0.7 ± 0.2	0.3 ± 0.1	1.0 ± 0.2
	0.3%	0.9%	0.6%	0.9%	1.2%	0.7%	0.3%	1.2%
Tardigrades	2.0 ± 0.3	5.0 ± 0.5	7.0 ± 1.5	3.0 ± 0.6	4.3 ± 0.6	0.7 ± 0.2	7.7 ± 0.9	5.3 ± 0.8
	1.7%	2.7%	4.4%	2.0%	3.0%	0.7%	8.2%	6.4%
Halacaloideans							0.3 ± 0.1	
							0.3%	
Total	115.3 ± 3.0	183.0 ± 4.9	158.7 ± 1.6	148.7 ± 3.9	141.3 ± 19.9	95.3 ± 3.2	94.0 ± 5.6	82.7 ± 3.0

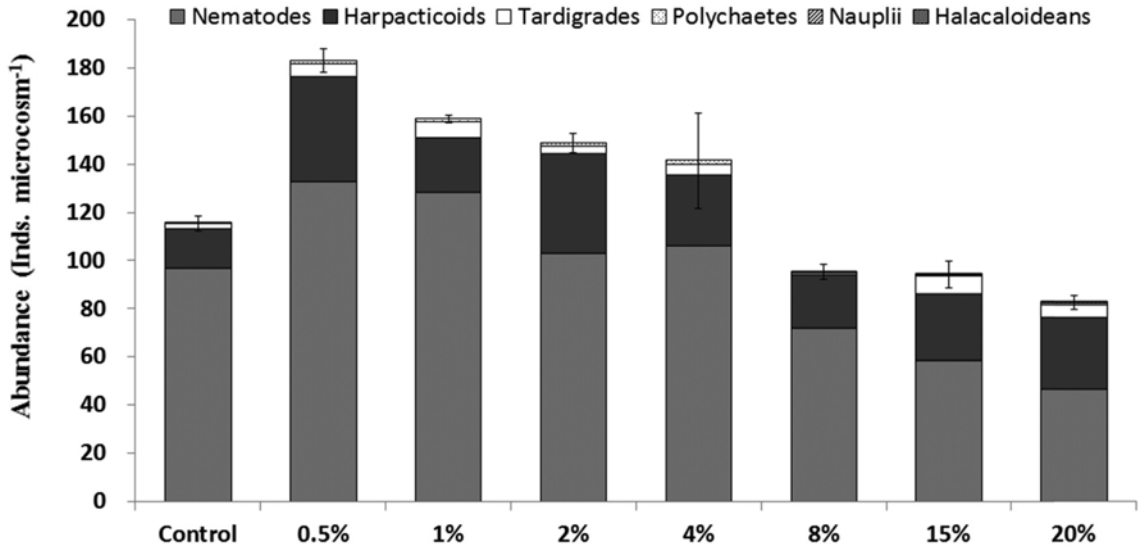

Fig. 3. Mean meiofaunal density (inds. microcosm^{-1}) in each treatment group 60 days after the start

4.9 inds. microcosm^{-1}, with a mean 157.9 inds. microcosm^{-1}. These densities were higher than the density of the control microcosm. The total meiofauna density of experimental microcosms that received seawater contaminated above 8% ranged from 82.7 ± 3.0 to 95.3 ± 3.2 inds. microcosm^{-1}, with a mean 90.7 inds. microcosm^{-1}. These densities were lower than the density of the control microcosm. The microcosm treatment contaminated with 20% TPH contained the lowest meiofauna density, 82.7 ± 3.0 inds. microcosm^{-1}, constituting only 72% of the meiofauna density in the control microcosm.

At a higher taxonomic level, nematodes clearly dominated the composition of meiofauna communities, followed by harpacticoids. Nematodes accounted for approximately 73% of total meiofauna, while harpacticoids accounted for 23%.

The density of nematodes was similar to that of total meiofauna (Fig. 3). The density of nematodes in the control group was 96.3 ± 3.1 inds. microcosm^{-1}. In the experimental microcosms that received seawater with a concentration below 4%, the nematode density exceeded that of the control microcosm, with a mean 117.3 individuals. In the experimental microcosms that received seawater with a concentration above 8%, the nematode density was lower than that of the control microcosm, with a mean 58.7 inds. microcosm^{-1}. The density of harpacticoids did not differ significantly among the treatment groups. The density of harpacticoids in the control group was 16.7 ± 0.5 inds. microcosm^{-1}, and in the experimental microcosms it ranged from 22.3 ± 2.8 to 43.7 ± 3.8 inds. microcosm^{-1}, with a mean 31.0 inds. microcosm^{-1} (Table 2).

The results of cluster analysis and MDS analysis of the Bray–Curtis similarity matrix based on the density of the meiofauna in this experiment are shown in Fig. 4. The meiofaunal communities in the experiments were divided in to two groups, those of the control and the experimental microcosms with low concentrations of the contaminated water below 4%, and those of the experimental microcosms with high concentrations of the contaminated water between 8% and 20%. However, SIMPROF analysis showed no significant difference between the two groups ($p > 0.05$).

Nematode diversity and community structure

Number of species and species richness were highest in the control microcosms, and tended to decrease as the concentration of seawater contamination increased. The number of species and species richness in the experimental microcosms that received the seawater with a concentration below 4% were higher than those for the microcosm that received seawater with a concentration above 8%.

Species evenness was low in the control group and the 0.5% and 1% microcosms but was high in the microcosms that received a concentration above 2%. There were no trends in the diversity index of various groups. Maturity Index (MI) values were similar for the control microcosm and microcosms with a concentration below 8%, and the minimum MI value was observed for the microcosm that received a concentration of 20%. The MI values ranged from 2.43 (20%) to 2.91 (4%), with a mean of 2.77 (Table 3).

A total of 2240 nematode individuals were recorded 60 days after the start of the experiments. The dominant nematode species, *Bolbolaimus* spp., accounted for 37.1% of all nematodes. The density of *Bolbolaimus* spp. was 37.3 ± 12.5 inds. microcosm^{-1} in the control microcosm. In the experimental

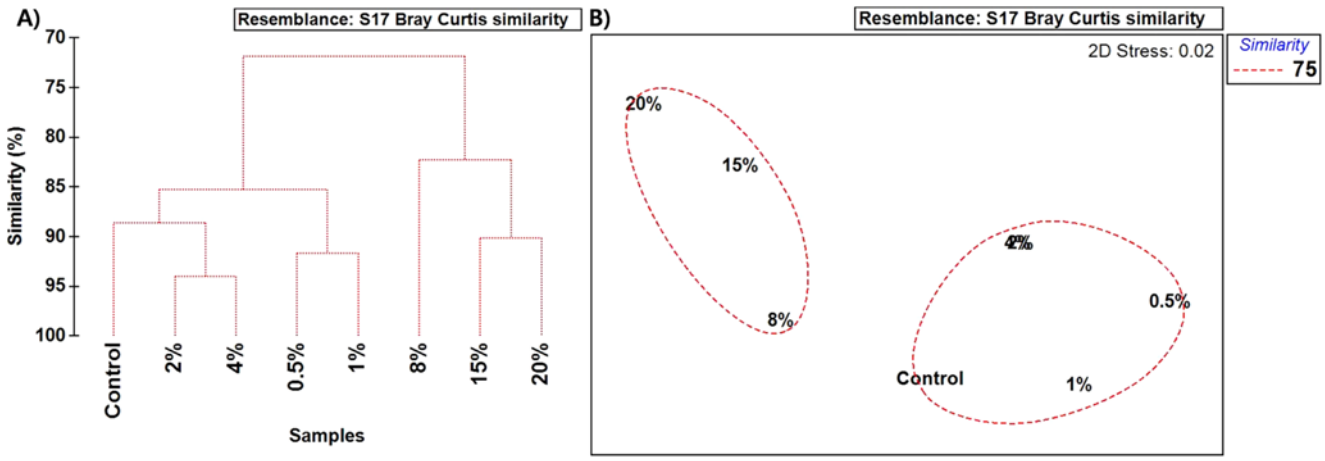

Fig. 4. CLUSTER analysis (A) and multidimensional scaling (B) in Bray–Curtis similarities among the meiofaunal communities. Broken lines indicate the same group by similarity profile (SIMPROF) analysis

Table 3. Univariate indices for nematode communities in microcosms, including number of species in three replications, mean number of individuals (inds. microcosm^{-1}), mean species richness (Margalef's index), mean species evenness (Pielou's evenness index), mean Shannon–Wiener diversity index, and Maturity Index

Microcosm	No. Species	Individuals	Richness	Evenness	Shannon Wiener index	Maturity index
Control	23	94.7 ± 27.8	2.57 ± 0.21	0.75 ± 0.08	1.89 ± 0.10	2.77 ± 0.02
0.5%	19	138.7 ± 16.2	2.44 ± 0.40	0.70 ± 0.03	1.79 ± 0.17	2.80 ± 0.16
1%	19	117.7 ± 11.9	2.52 ± 0.34	0.68 ± 0.02	1.75 ± 0.12	2.84 ± 0.03
2%	17	96.0 ± 17.7	2.33 ± 0.43	0.79 ± 0.08	1.91 ± 0.09	2.88 ± 0.20
4%	17	116.7 ± 82.1	2.56 ± 0.72	0.76 ± 0.10	1.88 ± 0.21	2.91 ± 0.17
8%	13	72.3 ± 11.2	1.94 ± 0.67	0.69 ± 0.08	1.51 ± 0.32	2.90 ± 0.30
15%	14	63.3 ± 27.7	2.12 ± 0.12	0.78 ± 0.10	1.77 ± 0.17	2.59 ± 0.04
20%	14	47.3 ± 5.5	2.43 ± 0.35	0.76 ± 0.06	1.78 ± 0.21	2.43 ± 0.15

Table 4. Feeding type[1], c–p (colonizer–persister) value, and density (inds. microcosm^{-1}) of nematode species[2] in each treatment group

	Feeding type	c–p value	Control	0.5%	1%	2%	4%	8%	15%	20%
Bolbolaimus	2A	3	37.3 ± 12.5	55.3 ± 3.2	55.0 ± 4.4	32.0 ± 16.4	42.3 ± 30.6	34.0 ± 7.5	20.3 ± 13.5	1.0 ± 0.0
Metalinhomoeus	1B	2	17.7 ± 15.1	34.3 ± 20.1	14.3 ± 3.8	15.0 ± 4.4	13.0 ± 7.9	7.7 ± 2.5	16.7 ± 7.4	16.3 ± 7.5
Enoplolaimus	2B	2	11.0 ± 4.4	9.7 ± 2.5	15.7 ± 4.0	15.0 ± 6.0	21.3 ± 17.6	13.0 ± 6.2	11.3 ± 4.0	14.3 ± 4.0
Chromadora	2A	3	5.3 ± 0.6	6.3 ± 6.0	8.3 ± 5.1	6.0 ± 2.6	4.7 ± 4.0	1.0 ± 1.0	4.3 ± 4.0	2.7 ± 1.2
Pontonema	2B	4	2.3 ± 4.0	7.0 ± 3.6	1.7 ± 2.1	2.0 ± 2.0	16.3 ± 23.3	2.0 ± 3.5	1.0± 1.0	3.3 ± 2.5
Enoplus	2B	5	1.3 ± 2.3	4.7 ± 5.5	5.3 ± 2.1	9.0 ± 8.9	5.0 ± 6.1	6.7 ± 10.7	0.3 ± 0.6	0.7 ± 1.2
Metachromadora	2B	3	4.0 ± 1.0	4.3 ± 0.6	6.3 ± 3.8	2.0 ± 2.6	5.3 ± 3.8	2.7 ± 1.2	3.7 ± 1.5	1.0 ± 1.0
Araeolaimus	1A	3	0.7 ± 0.6	2.7 ± 0.6	1.7 ± 1.5	5.3 ± 3.1	2.7 ± 2.1	1.7 ± 1.2	2.0 ± 1.0	1.7 ± 2.1
Microlaimus	2A	3	3.0 ± 3.0	7.0 ± 5.6	3.0 ± 5.2					
Pomponema	2B	3	2.7 ± 2.5	1.7 ± 2.1	1.3 ± 1.5	1.7 ± 2.1	1.0 ± 1.0	2.0 ± 0.0	0.3 ± 0.6	2.0 ± 1.0
Enoploides	2B	2	1.0 ± 1.0		0.3 ± 0.6	3.7 ± 4.0	1.0 ± 1.7		0.3 ± 0.6	1.0 ± 1.0
Steineridora	2A	3		1.3 ± 1.5	0.7 ± 0.6	1.3 ± 0.6	1.0 ± 0.0	0.3 ± 0.6	1.0 ± 1.0	1.3 ± 1.2
Euchromadora	2A	3	0.3 ± 0.6	0.7 ± 1.2	1.3 ± 0.6	1.0 ± 1.7		0.7 ± 0.6	1.0 ± 1.7	0.7 ± 0.6
Rhabdodemania	2B	4	0.3 ± 0.6	0.3 ± 0.6	0.7 ± 0.6	0.7 ± 1.2	1.3 ± 1.5	0.3 ± 0.6	0.7 ± 0.6	0.7 ± 1.2
Bathylaimus	2B	4	3.3 ± 2.9							
Rhynchonema	1B	2	0.3 ± 0.6	0.7 ± 0.6		0.3 ± 0.6	0.3 ± 0.6	0.3 ± 0.6		
MICROLAIMIDAE	2A	3	1.7 ± 2.9							
LINHOMOEIDAE	1A, 1B	2	0.3 ± 0.6		0.3 ± 0.6	0.3 ± 0.6				0.7 ± 1.2
Chromadorita	2A	3	0.3 ± 0.6	0.3 ± 0.6		0.3 ± 0.6	0.3 ± 0.6			
Eleutherolaimus	1B	2		1.3 ± 2.3						
HALIPLECTIDAE	1A	3		0.3 ± 0.6	0.3 ± 0.6		0.3 ± 0.6			
XYALIDAE	1B	2			0.7 ± 0.6		0.3 ± 0.6			
Tricoma	1A	4	0.3 ± 0.6						0.3 ± 0.6	
Leptolaimus	1A	2	0.3 ± 0.6		0.3 ± 0.6					
Daptonema	1B	2	0.3 ± 0.6							
Paramesonchium	2A	2			0.3 ± 0.6					
Coninckia	1A	3					0.3 ± 0.6			
CYATHOLAIMIDAE	2A	3	0.3 ± 0.6							
COMESOMATIDAE	1A	2	0.3 ± 0.6							
CHROMADORIDAE	2A, 2B	3		0.3 ± 0.6						
LEPTOLAIMIDAE	1A	3		0.3 ± 0.6						
IRONIDAE	2B	4				0.3 ± 0.6				

[1]Feeding types include 1A: selective deposit-feeders, 1B: non-selective deposit-feeders, 2A: epistrate feeders, 2B: predators/omnivores
[2]Uppercase taxa names are family names and lowercase ones are genus names

microcosms that received seawater at a concentration below 8%, the density of *Bolbolaimus* spp. was greater or slightly lower than that of the control microcosm. However, the density of *Bolbolaimus* spp. was decreased in the experimental microcosms that received seawater at a concentration above 15%. The density of *Bolbolaimus* spp. was 20.3 ± 13.5 inds. microcosm^{-1} in the 15% concentration and 1.0 ± 0 inds. microcosm^{-1} in the 20% concentration. The second most dominant nematode species, *Metalinhomoeus* spp., accounted for 18.1% of all total nematodes, followed by *Enoplolaimus* spp., *Chromadora* spp., *Pontonema* spp., *Enoplus* spp., *Metachromadora* spp., and *Araeolaimus* spp. A few individuals belonging to other nematode species were also recorded (Table 4).

Cluster analysis and MDS analysis results of the Bray–Curtis similarity matrix based on the density of the nematodes in this experiment are shown in Fig. 5. Cluster analysis results of nematode communities were similar to those of meiofauna communities. One group included the control group and experimental microcosms that received low concentrations (0.5%, 1%, 2%, and 4%), while the other group included microcosms that received high concentrations (8%, 15%, and 20%). However, SIMPROF test results showed no significant difference between the two groups ($p > 0.05$).

The Spearman correlation coefficients for the different TPH and meiofaunal data are presented in Table 5. The total meiofaunal density, nematode density, and number of *Bolbolaimus* spp. individuals in the microcosms were significantly negatively associated with TPH concentration ($p < 0.05$).

Within the nematode community, epistrate feeders (2A: 46%) were the most abundant trophic group, followed by predators/omnivores (2B: 32%), non-selective deposit-feeders (1B: 19%), and selective deposit-feeders (1A: 3%) (Table 5). Among the treatment groups, the abundance of group 2A increased at the low-concentration microcosms (0.5% and 1%) and decreased at the high-concentration microcosms (2%, 4%, 8%, 15%, and 20%), whereas the abundance of

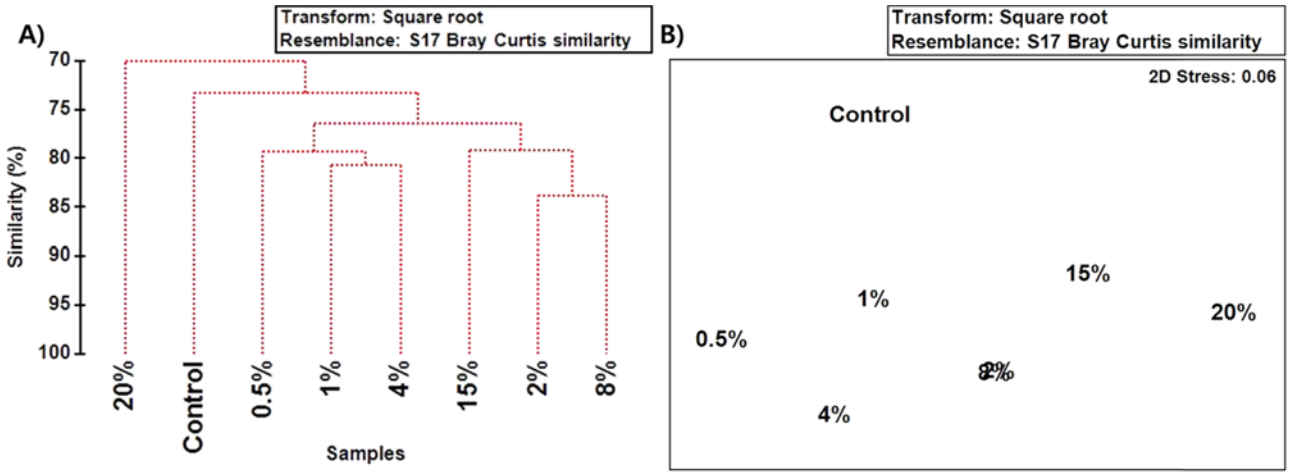

Fig. 5. CLUSTER analysis (A) and multidimensional scaling (B) in Bray–Curtis similarities among nematode species communities. Broken lines indicate the same group by similarity profile (SIMPROF) analysis

Table 5. Spearman correlation coefficients among the total petroleum hydrocarbon (TPH) level of contaminated seawater and data for major meiobenthic groups in the experimental microcosms

	TPH	Total density	Nematodes	Harpacticoids	*Bolbolaimus* spp.	*Metalinhomoeus* spp.	*Enoplolaimus* spp.
TPH	1	-.762 (*)	-.738 (*)	-.119	-.762 (*)	-.381	-.286
Total density		1	.976 (**)	.333	.857 (**)	.119	.048
Nematodes			1	.286	.929 (**)	.071	.095
Harpacticoids				1	.024	.286	-.024
Bolbolaimus spp.					1	.071	-.048
Metalinhomoeus spp.						1	-.762 (*)
Enoplolaimus spp.							1

∗ = significant relationship ($p < 0.05$)
∗∗ = significant relationship ($p < 0.01$)

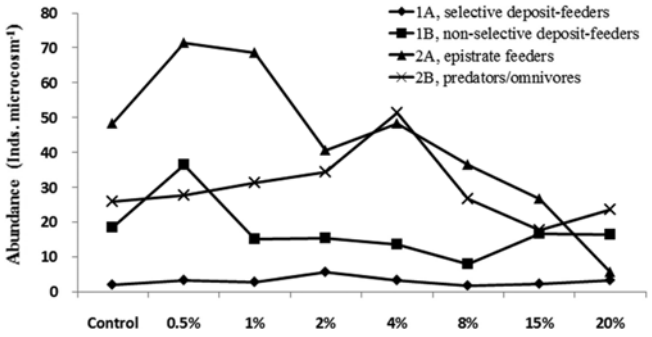

Fig. 6. Graphical summary of density of nematodes according to feeding type

nematodes with other feeding types did not significantly differ (Fig. 6).

4. Discussion

The present study was conducted to determine the effects of crude oil contamination of seawater on meiofauna and nematode communities in an intertidal sandy beach.

The initial TPH concentrations used in this study ranged up to 512.9 ppb. At the end of the experimental period, the TPH concentrations of the microcosms ranged between 9.6 and 20.7 ppb and were similar in the experimental and control groups. After the experiment concluded, the TPH concentration of the control was 18.1 ppb, which is higher than that of some of the experimental groups and slightly lower than that of others. There may be several reasons for this phenomenon. The TPH concentration in the control group after the experiment was concluded may have been caused by the background levels of TPH in sampling sediments. The mean TPH concentration of Mallipo intertidal sediments used in this study was 16.6 ppb, as reported by a twice-yearly survey from 2010 to 2014 (KIOST 2015). The TPH concentration in the control group was similar to the mean TPH concentrations in the sampling area.

After the end of the experiments, the TPH concentrations in the experimental groups were decreased to the initial levels of the control group except the experiment with 20% of the contaminated water. In general, oil components with a low boiling point evaporate within 24 hours in temperate conditions (ITOPF 2014). In addition, seawater contains a number of marine microorganisms that are capable of metabolizing oil compounds (ITOPF 2014). Hydrocarbon-degrading microorganisms are ubiquitously distributed in soil and aquatic environments. They normally constitute less than 1% of the total microbial community, but when oil pollutants are present these hydrocarbon-degrading populations increase, typically reaching 10% of the community (Atlas 1995). These organisms tend to be more abundant in chronically polluted coastal waters (ITOPF 2014). These microorganisms are present in more coastal seas than in open seas. Although the necessary microorganisms are present in relatively small numbers in the open sea, they multiply rapidly when oil is available, and degradation continues until the process is limited by nutrient or oxygen deficiency (ITOPF 2014). In this study, it is assumed that the microorganisms increased due to the hydrocarbon addition of the experiment microcosms. Microorganisms that could decompose the hydrocarbon were added to the experiments. As a result, the concentrations of TPH in the experiment microcosms showed no significant differences with those of the control microcosms at the end of the experiment.

In the present study, the total meiofauna densities of the experimental groups that received low concentrations of the contaminated seawater (0.5%, 1%, 2%, and 4%) exceeded that of the control microcosm. The total meiofauna densities of the microcosms that received higher concentrations (8%, 15%, and 20%) were lower than that of the control microcosm. Previous research on the effects of oil spills on meiofauna generated contradictory results. The effects of oil pollution seem to depend on the amount of spilled oil and the characteristics of the habitat (Renaud-Mornant et al. 1981; Fleeger and Chandler 1983). Other factors that influence the effects of oil pollution on local ecology include the type and amount of oil released, local environmental conditions, the weathering of oil, and the type of clean-up techniques employed (Jewett et al. 1999; Edgar and Barrett 2000).

In the present study, fluctuations in density were more pronounced in nematodes than in other taxa. Changes in the densities of nematode species were closely related to those in the total meiofaunal density. However, within the meiofauna and nematode communities, there were no statistically significant differences between microcosms. According to the results of cluster, SIMPROF analysis and MDS, the structure of meiofauna and nematode communities were unaffected by the oil contamination. In other words, the community structure of meiofauna and nematodes in the treated microcosms remained similar to that of the control. Because the present experiments used higher concentrations of TPH, we expected that meiofauna would decrease as the concentration increased. However, the effects of TPH

concentrations on meiofauna were less powerful than expected. Contamination with crude oil did not significantly reduce the densities of meiofauna such as nematodes and harpacticoids. The meiofauna in this study appeared to be insensitive to oil pollution, even at high TPH concentrations. Carmen et al. (1995) reported results that were similar to our findings for meiofauna in estuarine sediments exposed to polycyclic aromatic hydrocarbons. Their study showed that meiofauna communities in microcosm experimental bioassays were not very sensitive to polycyclic aromatic hydrocarbons, and the authors suggested that chronic exposure was the reason. Meiofauna in estuarine sediments are probably highly tolerant to environmental stress because they are exposed to highly variable physical and chemical conditions (Boesch and Rosenberg 1981). The organisms used in this study are also estimated to have resistance to environmental stress due to the fact that they live in an intertidal zone.

The densities of harpacticoids in the experimental groups were higher than that of the control. In general, copepods may be more sensitive than nematodes to pollution (Bouwman et al. 1984; van Damme et al. 1984; Gee et al. 1985; Warwick et al. 1988). And, copepods seem to recover slowly after a pollution event (Wormald 1976), whereas nematodes recover more rapidly (Wormald 1976; Giere 1979). However, studies of experimental oil spills also report an increase in copepod density following exposure to contamination (Naidu et al. 1978). In the present study, the density of nematodes and harpacticoids in the low-concentration treatment groups increased. Naidu et al. (1978) reported that the densities of copepods increased in experimental oil spills in an intertidal muddy site. They suggested that meiofauna increased because bacteria populations in oiled environments increased, providing greater food resources for copepods. In the present study, the oil contamination was also associated with a noticeable increase in the density of epistrate feeders among nematodes in the low-concentration treatment groups. The increase of epistrate feeders may be due to the increase of their food as a result of microorganism growth due to adding hydrocarbon. Nematode communities are generally diverse so that they can take advantage of diverse food resources (Platt and Warwick 1980). Generally, when the amount of one type of food resource increases, it is used by opportunistic nematodes. Epistrate feeders consume bacteria and unicellular eukaryotes, puncturing the cell membranes of these organisms with their teeth and ingesting only the cell contents (Jense 1987). They generally occur in greater numbers on sandy bottoms (Ansari and Parulekar 1993; Giere 1993).

A notable group of epistrate feeders is *Bolbolaimus* spp., the dominant species in this study. The density of *Bolbolaimus* spp. decreased in the experimental microcosms that received seawater with a concentration above 15%, and the density was drastically reduced in the 20% concentration. In this result, the toxicity of the oil effect on Bolbolaimusspp. is assumed in the concentration of 15% and 20% microcosoms. *Bolbolaimus* spp. are sensitive to changes and are known as good environmental indicators (Losi et al. 2012; Semprucci et al. 2015). The features of species with a c–p value of 3, such as *Bolbolaimus* spp., include a relatively longer generation time and greater sensitivity to disturbances (Ferris and Bongers 2009). The presence of sensitive or tolerant nematode genera was a particularly good indicator of the state of sediment contamination (Semprucci et al. 2015). The second and third most dominant nematodes species, *Metalinhomoeus* spp. (18%) and *Enoplolaimu* spp. (15%), were not strong indicators of the TPH concentration. These species with c–p value 2 are very tolerant of pollutants and other disturbances (Ferris and Bongers 2009). It is difficult to understand the impact of TPH on other species that are even less dominant.

Spearman correlation coefficients showed that the TPH concentration appeared to affect both meiofauna and nematodes. According to our analysis, the densities of total meiofauna, nematodes, and *Bolbolaimus* spp. were significantly negatively affected as TPH contamination increased. By increasing the mortality of the most sensitive species, hydrocarbon contamination caused a decrease in nematode densities, mean individual weight, and diversity (Beyrem and Aissa 2000; Carman et al. 2000; Kang et al. 2014).

Our experiments showed that the effects of hydrocarbon contamination were less severe than expected. The concentration of TPH that we used did not greatly exceed that of a typical oil spill, but the experimental concentrations we used were sufficiently high to represent the levels that might affect natural environments after a spill. Generally, meiofauna are expected to show a rapid response to oil pollution because of their dependence on the sediment and their short generation times. However, in the present experiments, although exposure to oil reduced the density of meiofauna, there were no statistically significant differences between the meiofauna and nematode communities exposed to different contamination levels. Our findings provide basic data on the effects of oil pollution on meiofauna in the intertidal zones of sandy beaches. Further studies should

examine the short-term impact of exposure to crude oil on various meiobenthic taxa with different ecological characteristics. In particular, experiments to evaluate the effects of oil at environmentally realistic concentrations will help to predict the impacts of oil on chronically exposed meiofauna communities.

Acknowledgments

We are very grateful to Yim UH and the staff of KIOST, who helped with the THP analysis. We wish to thank all our colleagues who were directly and indirectly involved in the sample collection and analysis. This research was partially supported by the research program of KIOST with Contract No. PM59200.

References

Ansari ZA, Ingole B (2002) Effect of an oil spill from M V Sea Transporter on intertidal meiofauna at Goa, India. Mar Pollut Bull **44**:396–402

Ansari ZA, Parulekar AH (1993) Distribution, abundance and ecology of the meiofauna in a tropical estuary along the west coast of India. Hydrobiologia **262**:115–126

Atlas RM (1995) Bioremediation of petroleum pollutants. Int. Biodeterior. Biodegradation **35**:317–327

Austen MC (1989) Factors affecting estuarine meiobenthic assemblage structure: a multifactorial microcosm experiment. J Exp Mar Biol Ecol **130**:167–187

Austen MC, McEvoy AJ (1997) The use of offshore meiobenthic communities in laboratory microcosm experiments: response to heavy metal contamination. J Exp Mar Biol Ecol **211**:247–261

Austen MC, McEvoy AJ, Warwick RM (1994) The specificity of meiobenthic community response to different pollutants: results from microcosm experiments. Mar Pollut Bull **28**:557–563

Austen MC, Somerfield PJ (1997) A community level sediment bioassay applied to an estuarine heavy metal gradient. Mar Environ Res **43**:315–328

Beyrem H, Aissa P (2000) Les nematodes libres, organisms sentinelles de l'evolution des concentrations d'hydrocarbures dans la baie de Bizerte (Tunisie). Cah Biol Mar **41**:329–342

Bodin P (1988) Results of ecological monitoring of three beaches polluted by the Amoco Cadiz oil spill: development of meiofauna from 1978 to 1984. Mar Ecol-Prog Ser **42**:105–123

Boesch DB, Rosenberg R (1981) Response to stress in marine benthic communities. In: Barrett GW, Rosenberg R (eds) Stress effects on natural ecosystems. John Wiley & Sons Ltd., Chichester, pp 179–214

Bongers T (1990) The maturity index: an ecological measure of environmental disturbance based on nematode species composition. Oecologia **83**:14–19

Bongers T, Alkemade R, Yeates GW (1991) Interpretation of disturbance-induced maturity decrease in marine nematode assemblages by means of the Maturity Index. Mar Ecol-Prog Ser **76**:135–142

Burgess R (2001) An improved protocol for separating meiofauna from sediments using colloidal silica sols. Mar Ecol-Prog Ser **214**:161–165

Boucher G (1980). Impact of Amoco Cadiz oil spill on intertidal and sublittoral meiofauna. Mar Pollut Bull **11**:95–101

Bouwman LA, Romeijn K, Admiraal W (1984) On the ecology of meiofauna in an organically polluted estuarine mudflat. Estuar Coast Shelf S **19**:633–653

Carman KR, Fleeger JW, Means JC, Pomarico SM, McMillin DJ (1995) Experimental investigation of polynuclear aromatic hydrocarbons on an estuarine sediment food web. Mar Environ Res **40**:298–318.

Carman KR, Fleeger JW, Pomarico SM (2000) Does historical exposure to hydrocarbon contamination alter the response of benthic communities to diesel contamination? Mar Environ Res **49**(3):255–278

Clarke KR, Gorley RN (2001) PRIMER v.5. User manual. PRIMER-E, Plymouth, 91 p

Commito JA, Tita G (2002) Differential dispersal rates in an intertidal meiofauna assemblage. J Exp Mar Biol Ecol **268**: 237–256

Coull BC, Chandler GT (1992) Pollution and meiofauna: field, laboratory and mesocosm studies. Oceanogr Mar Biol **30**: 191–271

Danovaro R (2000) Benthic microbial loop and meiofaunal response to oil-induced disturbance in coastal sediments: a review. Int J Environ Pollut **13**:380–391

Danovaro R, Fabiano M, Vincx M (1995) Meiofauna response to the Agip Abruzzo oil spill in subtidal sediments of the Ligurian Sea. Mar Pollut Bull **30**(2):133–145

Dauvin JC (1982) Impact of Amoco Cadiz oil spill on the muddy fine sand *Abra alba* and *Melinna palmata* community from the Bay of Morlaix. Estuar Coast Shelf S **14**:517–531

Edgar GJ, Barrett NS (2000) Impact of the Iron Baron oil spill on subtidal reef assemblages in Tasmania. Mar Pollut Bull **40**:36–49

Elmgren R, Hansson S, Larsson U, Sundelin B, Boehm PD (1983) The 'Tsesis' oil spill: acute and long-term impact on the benthos. Mar Biol **73**:51–65

Feder HM, Naidu AS, Paul AJ (1990) Trace metal and biotic changes following a simulated oil spill on a mudflat in Port Valdez, Alaska. Mar Pollut Bull **21**:131–137

Ferris H, Bongers T (2009) Indices developed specifically for analysis of nematode assemblages. In: Wilson MJ, Kakouli-Duarte T (eds) Nematodes as environmental indicators. CAB

International, Wallingford, pp 124–145

Fleeger JW, Chandler GT (1983) Meiofauna response to an experimental oil spill in a Louisiana salt marsh. Mar Ecol-Prog Ser **11**:257–264

Friethsen JB, Elmgren R, Rudnick DT (1985) Responses of benthic meiofauna to long-term, low-level additions of No. 2 fuel oil. Mar Ecol-Prog Ser **23**:1–14

Gee JM, Warwick RM, Schaaning M, Berge JA, Ambrose Jr. WG (1985) Effects of organic enrichment on meiofaunal abundance and community structure in sublittoral soft sediments. J Exp Mar Biol Ecol **91**:247–262

Giere O (1979) The impact of oil pollution on intertidal meiofauna – field studies after the La Coruna-Spill, May 1976. Cah Biol Mar **20**(2):231–251

Giere O (1993) Meiobenthology: the microscopic fauna in aquatic sediments. Springer-Verlag Berlin, Heidelberg, New York, 328 p

Higgins RP, Thiel H (1988) Introduction to the study of meiofauna. Smithsonian Institution Press, Washington DC, 488 p

ITOPF (2014) Technical Information Paper TIP 02: fate of marine oil spills. http://www.itopf.com/knowledgeresources/documents-guides/document/tip-2-fate-of-marine-oil-spills Accessed 27 July 2014

Jense P (1987) Feeding ecology of free-living aquatic nematodes. Mar Ecol-Prog Ser **35**:187–196

Jewett SC, Dean TA, Smith RO, Blanchard A (1999) 'Exxon Valdez' oil spill: impacts and recovery in the soft-bottom benthic community in and adjacent to eelgrass beds. Mar Ecol-Prog Ser **185**:59–83

Kennish MJ (1992) Polynuclear aromatic hydrocarbons. In: Kennish MJ (ed) Ecology of estuaries: anthropogenic effects. CRC Press, Boca Raton, pp 133–181

Kang TW, Min WG, Rho HS, Pack HS, Kim DS (2014) Differential responses of a benthic meiofaunal community to an artificial oil spill in the intertidal zone. J Mar Biol Assoc UK **94**(2):219–231

Kim M, Hong SH, Won J, Yim UH, Jung JH, Ha SY, An JG, Joo C, Kim E, Han GM, Baek S, Choi HW, Shim WJ (2013) Petroleum hydrocarbon contaminations in the intertidal seawater after the Hebei Spirit oil spill-effect of tidal cycle on the TPH concentrations and the chromatographic characterization of seawater extracts. Water Res **47**:758–768

KIOST (2009) Annual Report on the environmental impact assessment and environmental restoration of the *Hebei Spirit* oil spill. A Report Prepared for the Ministry of Land, Transport and Maritime Affairs, Report No. 11-1611000-000392-01, 38 p

KIOST (2015) Annual Report on the environmental assessment and restoration from oil pollution. A Report Prepared for the Ministry of Oceans and Fisheries, Report No. 11-1192000-000438-01, 65 p

Losi V, Montefalcone M, Moreno M, Giovannetti E, Gaozza L, Grondona M, Albertelli G (2012) Nematodes as indicators of environmental quality in seagrass (*Posidonia oceanica*) meadows of the NW Mediterranean Sea. Adv Oceanogr Limnol **3**:69–91

Louati A, Elleuch B, Kallel M, Saliot A, Dagaut J, Oudot J (2001) Hydrocarbon contamination of coastal sediments from the Sfax area (Tunisia), Mediterranean Sea. Mar Pollut Bull **42**:445–452

Mahmoudi E, Essid N, Beyrem H, Hedfi A, Boufahja F, Vitiello P, Aissa P (2005) Effects of hydrocarbon contamination on a free living marine nematode community: results from microcosm experiments. Mar Pollut Bull **50**:1197–1204

Millward RN, Grant A (1995) Assessing the impact of copper on nematode communities from a chronically metal-enriched estuary using pollution-induced community tolerance. Mar Pollut Bull **30**:701–706

Naidu AA, Feder HM, Norrell SA (1978) The effect of Prudhoe Bay crude oil on a tidal-flat ecosystem in Port Valdez, Alaska. In: Proceeding of 10th Offshore Technology Conference, Houston, Texas, pp 97–104

Peterson CH, Kennicutt MC, Green RH, Montagna P, Harper DE, Powell EN, Roscigno PF (1996) Ecological consequences of environmental perturbations associated with offshore hydrocarbon production: a perspective on long-term exposures in the Gulf of Mexico. Can J Fish Aquat Sci **53**:2637–2654

Platt HM, Warwick RM (1980) The significance of free-living nematodes to the littoral ecosystem. In: Price JH, Irvine DEG, Farnham WF (eds) The shore environment. Vol. 2. Ecosystems. Academic Press, New York, pp 729–759

Platt HM, Warwick RM (1983) A synopsis of the free-living marine nematodes. Part I. British Enoplids. Cambridge University Press, Cambridge, 306 p

Platt HM, Warwick RM (1988) A synopsis of the free-living marine nematodes. Part II. British Chromadorids. Cambridge University Press, Cambridge, 502 p

Renaud-Mornant J, Gourbault N, de Panafiew JB, Heleouet MN (1981). Effets de la pollution par hydrocarbures sur la meiofauna de la baie de Morlaix. In: Amoco Cadiz, Consequences d'une Pollution Accidentale par les Hydrocarbures. Actes coli. Intern. C.O.B., Brest, France, 19–22 November 1979, pp 551–561

Schratzberger M, Danie F, Wall CM, Kilbride R, Macnaughton SJ, Boyd SE, Rees HL, Swannell RPJ (2003) Response of estuarine meio and macrofauna to in situ bioremediation of oil contaminated sediment. Mar Pollut Bull **46**:430–443

Schratzberger M, Warwick RM (1998) Effects of the intensity and frequency of organic enrichment on two estuarine nematode communities. Mar Ecol-Prog Ser **164**:84–95

Semprucci F, Losi V, Moreno M (2015) A review of Italian research on free-living marine nematodes and the future perspectives in their use as ecological indicators (EcoInd). Mediterr Mar Sci **16**:352–365

Stacey B, Marcotte B (1987) Chronic effect of No. 2 fuel oil on

population dynamics of harpacticoid copepods in experimental marine mesocosms. Mar Ecol-Prog Ser **40**:61–68

Sundelin B, Elmgren R (1991) Meiofauna of an experimental soft bottom ecosystem-effects of macrofauna and cadmium exposure. Mar Ecol-Prog Ser **70**:245–255

Van Damme D, Heip C, Willems KA (1984) Influence of pollution on the harpacticoid copepods of two North Sea estuaries. Hydrobiologia **112**:143–160

Warwick RM, Carr MR, Clarke KR, Gee JM, Green RH (1988) A mesocosm experiment on the effects of hydrocarbon and copper pollution on a sublittoral soft-sediment meiobenthic community. Mar Ecol-Prog Ser **46**:181–191

Warwick RM, Platt HM, Somerfield PJ (1998) A synopsis of the free-living marine nematodes. Part III. Monhysterids, 53. Field Studies Council, Shrewsbury, 296 p

Wieser W (1953) Die beziehung zwischen Mundhohlengestalt, Ernahrungsweise und Vorkommen bei freilebenden marinen Nematoden. Eine okologisch-morphologische Studie. Ark Zool **4**(26):439–484

Wormald AP (1976) Effect of an oil spill of marine diesel oil on meiofauna of a sandy beach at Picnic Bay Hong Kong. Environ Pollut **11**:117–130