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Effects of Crude Oil on Marine Microbial Communities in Short Term Outdoor Microcosms

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To assess the effects of crude oil spills on marine microbial communities, 10 L outdoor microcosms were manipulated over an exposure period of 8 days. The responses of microbial organisms exposed to five crude oil concentrations in 10 to 10,000 ppm (v/v) were monitored in the microcosms. The abundance of microalgae and copepods decreased rapidly upon the addition of crude oil at concentrations over 1,000 ppm, whereas the total density of heterotrophic bacteria increased dramatically at the higher concentrations. Bacterial diversity, determined by denaturing gradient gel electrophoresis, was increased at higher concentrations. In particular, the intensity of the bands representing *Jannaschia* sp. and *Sulfitobacter brevis* increased with the addition of oil. These results indicate that crude oil spills with concentrations over 1,000 ppm seriously affected the structure of the microbial communities.

***Keywords*:** DGGE, microbial communities, microcosm, oil spills, pollution effects, risk assessment

Estuarine harbours and coastal seas often suffer from significant marine oil pollution via tanker spills, tank purging, and pipelines (e.g., Stephensen *et al*., 2000). Of these, accidental oil spills from tankers are a major source of pollution. These accidents can cause extensive destruction of natural communities and adverse effects on human activities (e.g., Gordan *et al*., 1978; Marchand *et al*., 1988; Soclo *et al*., 2000). On 7th December 2007, an estimated 12,547 kL (c.a. 10,900 M/T) of crude oil were released after a collision between the oil tanker M/V *Hebei Spirit* and a barge carrying a crane, and covered an area of 6.5 nautical miles off the coast of Taean county, South Korea (Kim *et al*., 2009). The accident, which resulted in serious damage to the Korean coast, ranks alongside spills from the *Prestige* off the coast of Spain in 2002, the *Tasman Spirit* off the coast of Pakistan in 2003, and the *Solar 1* off the Philippines in 2006 as one of the largest tanker spills in recent years.

Ecological risk assessments of crude oil are intended to evaluate the detrimental effects of contaminants on the environment. Most such risk assessment studies have focused on the effects of exposure to the water-accommodated fraction (WAF) and/or chemically enhanced WAF (CEWAF) of oil on single species or populations (Berman and Heinle, 1980; Reid and Macfarlane, 2003; Ramachandran *et al*., 2004; Jung *et al*., 2009). WAF and/or CEWAF tests using standardized models of living organisms are used effectively to determine toxicity levels because they are fast, easy to use, and have high reproducibility. However, these tests have been of limited use in understanding the potential effects of oil exposure on ecosystems because interactions between biotic and abiotic factors in natural environments are very complex. In addition, investigations of oil spills in natural ecosystems face enormous logistical challenges such as high costs and the need for long-term study. To overcome these problems, mesocosm or microcosm experiments are of benefit, as they can improve our understanding of the impact of oil spills on ecosystems and possibly predict the effects of oil on entire ecosystems (Caquet *et al*., 2000; Jung *et al*., 2010).

Damage to natural resources by oil spills can be considered in terms of either chronic (long-term) or acute (immediate) injury. Organisms that are affected by chronic injury due to oil pollution are generally large in size and include macrobenthos and fishes (e.g., Widdows *et al*., 1982). On the other hand, small organisms such as bacteria, microalgae, and zooplankton are affected rapidly upon exposure to oil pollution (e.g., Bobra *et al*., 1989; González *et al*., 2009). Microbial communities form the foundation of marine open water food webs, and are the primary food source for many macroscopic open water organisms. Over the last 30 years, a number of studies have examined the effects of oil on microbial communities (Lännergren, 1978; Vargo *et al*., 1982; Gin *et al*., 2001). However, the effects of crude oil on integrated responses of microbial communities and the sub-lethal effects of oil remain largely unstudied. In particular, effects of the spilled oil in the *Hebei Spirit* incident on natural microbial communities in Korea are still unknown. Therefore, we conducted microcosm experiments to determine the sub-lethal level of concentration gradients of crude oil and monitored the responses of microbial communities, including bacteria, microalgae, and zooplankton, focusing on copepod populations.

Materials and Methods

Microcosm design

To evaluate the responses of microbial communities to crude oil

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pollution, an outdoor microcosm, comprising 12 L enclosures that contained 10 L of seawater, was set up at a monitoring site (34°59′37.48″N, 128°40′27.53″E) of the South Sea Research Institute (SSRI) of the Korea Ocean Research and Development Institute (KORDI) located on Geoje Island, Korea. Microcosms were constructed using transparent plastic materials (30 cm L×20 cm W×20 cm H). Total, 18 microcosms were kept afloat in a container (170 cm L×100 cm W×65 cm H) with natural seawater circulated continuously using an electronic pump (50 L/min). The water temperature in the microcosms was maintained through water circulation at a similar temperature to that of natural seawater. The seawater in the microcosms was filtered through a 100 μm mesh-sized net to eliminate large organisms and partitioned. Zooplankton (>200 μm size) were inoculated into each microcosm at a density of 50 ind./L using a 200 μm mesh net. Copepods predominated, making up 80% of the total zooplankton individuals identified. Crude oil (Iranian heavy crude oil, one of the spilled oils in the *Hebei Spirit* incident) was added to five treatment groups at concentrations of 10 ppm (v/v, T1 group), 100 ppm (T2), 1,000 ppm (T3), 5,000 ppm (T4), and 10,000 ppm (T5). A control microcosm (no addition of oil) was also prepared. The water in the microcosms was mixed gently for 1 min twice a day. The experiment was carried out in triplicate over a period of 8 days from 18 to 25 March, 2009.

Monitoring of biotic and abiotic factors

Sub-samples were taken daily from each microcosm at 9:00 AM. Water temperature, pH, salinity, and dissolved oxygen content were measured using a portable meter (556 MPS; YSI, USA). To analyse microalgae and heterotrophic bacteria, a 30 ml sample was collected in a 50 ml sterilised polyethylene bottle and fixed immediately with glutaraldehyde at a final concentration of 2%. The sample was stored in the dark at 4°C until analysis. At least 600 bacterial cells were counted from each sample using an epifluorescence microscope (Axioplan; Zeiss, Germany) after staining with 4', 6-diamidino-2-phenylindole (DAPI) solution at a final concentration of 1 μg/ml (Porter and Feig, 1980). Living cell counts and microalgal identification were performed for at least 500 cells per sample using a Sedgwick-Rafter counting chamber under a light microscope (Axioplan; Zeiss, Germany) at 400× magnification. To determine the viability of copepods, a 150 ml sample was collected in a 200 ml acid-cleaned PE bottle and examined immediately using a dissecting microscope (Discovery V8; Zeiss). Copepods were counted as dead when they exhibited no movement after being touched with a needle.

Significant differences among groups were assessed by one-way analysis of variance (ANOVA). Subsequently, significant differences were tested by Scheffe's post hoc test. These statistical analyses were performed using SAS v.8.12 (SAS Institute Inc., USA).

Polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis (DGGE)

Sub-samples of 1 L were taken from each microcosm on day 0 (the first day) and day 7 (the last day). Bacterial assemblages were collected on a 0.2 μm polycarbonate membrane (GTTP04700; Millipore, USA). The genomic DNA was extracted from the harvested bacteria on the membrane in accordance with the phenol-chloroform-isoamyl alcohol extraction method (Massana *et al*., 2000). The integrity of the total DNA was checked by electrophoresis on a 1% agarose gel. The DNA yield was quantified by a Hoechst dye fluorescence assay (Brunk *et al*., 1979). The DNA was stored at -70°C until analysed.

A partial 16S rDNA sequence was amplified by PCR using 10 ng of extracted DNA and the primers 341F-GC clamp (5′-CCTACGGG AGGCAGCAG-3′, which contains a 40-bp GC-rich sequence at the 5′-end) and 907RM, which is an equimolar mixture of the primers 907RC (5′-CCGTCAATTCCTTTGAGTTT-3′) and 907RA (5′-CCGTCAATTCATTTGAGTTT-3′) as described by Muyzer *et al*. (1993). The PCR mixture (total volume of 50 μl) contained 200 μmol/L of each dNTP, 1.5 mmol/L $MgCl_2$, 0.3 μmol/L each primer, 2.5 units of Taq DNA polymerase (EX Taq; TaKaRa, Japan), and the PCR buffer supplied with the enzyme. The PCR program was composed of an initial denaturation step at 94°C for 5 min followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 60°C for 1 min, and extension at 72°C for 1 min. During the final cycle, the extension step was continued for an additional 7 min at 72°C. A total of 800 to 1,000 ng of product was obtained regularly under these conditions using a Takara thermal cycler (model TP600; TaKaRa).

DGGE analysis was performed by following the protocol described by Muyzer *et al*. (1993). Samples were electrophoresed through a 0.75-mm-thick 6% polyacrylamide gel (37.5:1 acrylamide:bisacrylamide) with a linear gradient of denaturing agent from 30% to 50%, where 100% denaturing agent corresponded to 7 mol/L urea and 40% deionised formamide. Approximately 800 ng of PCR product were loaded into each lane. To determine differences in bacterial composition among treatment groups, three amplified products were mixed together, with analyses performed in triplicate for each group. Electrophoresis was carried out at 70 V for 12 h in 1× Tris-acetate-EDTA buffer (TAE; 40 mmol/L Tris, 40 mmol/L acetic acid, and 1 mmol/L EDTA, pH 7.4) at 60°C. After electrophoresis, the gel was stained with ethidium bromide (1 μg/ml) for 10 min, rinsed with distilled water, and photographed under UV transillumination with a CCD camera. The bacterial community fingerprints were compared between each pair of lanes within a gel using the photograph of the gel and similarity between the community fingerprints was calculated. A similarity and a clustering pattern were calculated by the Jaccard method and by the unweighted pair group method using average linkages (UPGMA), respectively.

To obtain the DNA sequences from the DGGE bands, polyacrylamide fragments were excised from the gel using a sterilised razor blade, resuspended in 10 μl of distilled water, and stored at 4°C for 24 h to allow the DNA to dissolve. An aliquot of the supernatant (approximately 5 μl) was used for PCR reamplification with the specific primers described above (341F without GC clamps and 907RM). Aliquots of the reamplified PCR products (30-50 ng) were sequenced with a Thermo-Sequence™ 2.0 sequencing kit (USB, USA) and a dual dye automated sequencer (Model 4200; LI-COR, USA). A BLAST search was performed to categorise the isolates phylogenetically. Reference sequences for the phylogenetic analysis were obtained directly from GenBank. The accession numbers of the partial 16S rDNA sequences determined in this study are given in Table 1.

Results

Monitoring of environmental factors

During the experiment, variations in water temperature and pH were not significantly different among all microcosms ($p>0.05$, ANOVA; Table 2). Salinity and dissolved oxygen concentration differed significantly among the treatment groups ($p<0.001$). As the concentration of oil increased, both of these variables decreased gradually.

Table 1. DGGE results of partial sequence analysis and tentative phylogenetic affiliations of bands

Band no.	Closest match (accession number)	Accession number	Sequence similarity (No. of bases)	Taxonomic group
1	*Polaribacter* sp. W-2 (DQ923436)	GU191125	100% (510)	Flavobacteria
2	Uncultured alpha proteobacterium G044 (DQ376153)	GU191126	99.8% (507)	α-Proteobacteria
3	*Erythrobacter citreus* RE35F/1 (AF118020)	GU191137	100% (474)	γ-Proteobacteria
4	*Roseobacter* sp. 01-004290 (EU819142)	GU191127	97.3% (520)	α-Proteobacteria
5	Rhodobacterales bacterium CB1051 (FJ869047)	GU191128	99.8% (505)	α-Proteobacteria
6	*Sulfitobacter* sp. DHVB8 (AJ534233)	GU191129	99.6% (507)	α-Proteobacteria
7	*Jannaschia* sp. J5–8 (FJ425223)	GU191130	99.8% (505)	α-Proteobacteria
8	*Sulfitobacter brevis* DSM11443 (DQ915633)	GU191131	97.7% (522)	α-Proteobacteria
9	*Roseobacter* sp. SOVoc28 (AM709732)	GU191132	97.8% (504)	α-Proteobacteria
10	Uncultured Rhodobacteraceae bacterium DS014 (DQ234098)	GU191133	98.0% (508)	α-Proteobacteria
11	Uncultured *Aquiluna rubra* MWH-CanK2 (AM999977)	GU191134	98.7% (477)	Actinobacteria
12	Uncultured actinobacterium CB31G02 (EF471506)	GU191135	99.4% (465)	Actinobacteria
13	*Staleya* sp. LM-7 (AJ534233)	GU191136	99.6% (507)	α-Proteobacteria

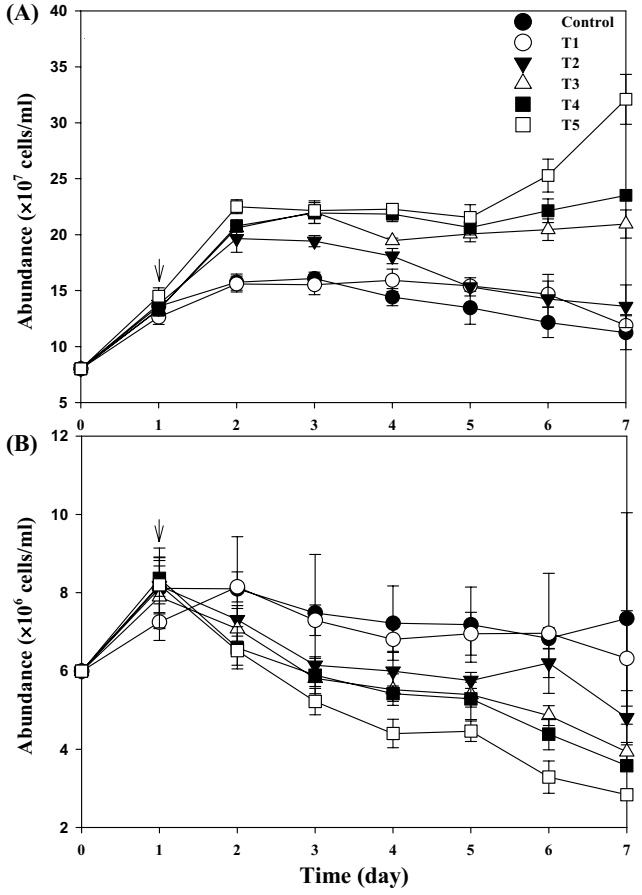

Fig. 1. Changes in bacterial density (A) and microalgal abundance (B) after the addition of oil to the microcosms. Arrows indicate the time (day) of oil addition. Data represent Means±SD from three independent assays. T1, oil concentration of 10 ppm (v/v); T2, 100 ppm; T3, 1,000 ppm; T4, 5,000 ppm; and T5, 10,000 ppm.

Effect on the heterotrophic bacterial community

Differences in the cell density of heterotrophic bacteria among treatments were statistically significant as determined by one-way ANOVA (Table 2). Before the addition of oil, the density of bacteria was similar in all microcosms, but after the oil was added, bacterial density increased with increasing oil concentration (Fig. 1A). However, the values for T1 and T2 groups were not significantly different from the control values, which did not change throughout the experiment (Table 2).

Banding patterns from the DGGE and the results of the cluster analysis are shown in Fig. 2. The sequences identified by the BLAST search were related most closely to those recovered from the prominent DGGE bands shown in Table 1. On the first day (day 0) of the experiment, samples generated simple banding patterns of six visible bands. After 7 days, the banding patterns differed among the treatment groups, indicating the existence of diverse species. The T3, T4, and T5 groups were clustered together with a value for Jaccard's coefficient of 0.8, whereas T1 and T2 were separated. This grouping was consistent with the ANOVA results for variations in the density of the bacteria (Table 2).

The DGGE fingerprints for important bands, which were present in most lanes, were scanned, and the relative intensities of the bands were recorded. Sequences obtained from the samples on day 0 were identified as groups of flavobacteria, α-proteobacteria, γ-proteobacteria, and actinobacteria (Table 1). However, among the sequences obtained from samples taken on the last day, those of α-proteobacteria and actinobacteria predominated. In particular, the band intensities of number seven [the sequence showed 99.8% similarity with *Jannaschia* sp. J5-8 (accession number AM709732)] and eight [97.7% similarity with *Sulfitobacter brevis* DSM11443 (DQ915633)] increased with increasing oil concentration. In contrast, the band intensity of lane five [99.8% similarity with Rhodobacterales bacterium CB1051 (FJ869047)] decreased with increasing oil concentration.

Effect on the microalgal community

The fluctuations in microalgal abundance upon oil exposure

Table 2. Changes in biotic and abiotic factors due to addition of oil to microcosms. Results were analysed by one-way ANOVA and Scheffe tests. Letters (A, B, and C) indicate significant differences among experimental groups

	Variables (unit)	Control	T1 (10 ppm)	T2 (100 ppm)	T3 (1,000 ppm)	T4 (10,000 ppm)	T5 (50,000 ppm)	F value
Abiotic factors	Temperature (°C)	11.43±0.10 [a]	11.57±0.13	11.63±0.12	11.90±0.13	12.25±0.23	12.35±0.26	N.S. [b]
	pH	7.81±0.02	7.85±0.04	7.87±0.02	7.86±0.03	7.77±0.03	7.78±0.04	N.S.
	Salinity (PSU)	33.32±0.04^{A}	33.26±0.04^{A}	33.17±0.05^{AB}	33.08±0.05^{B}	33.06±0.05^{B}	33.05±0.04^{B}	13.02^{*c}
	Dissolved oxygen (mg/L)	8.45±0.07^{AB}	8.48±0.09^{A}	8.55±0.09^{A}	8.34±0.16^{AB}	7.99±0.13^{BC}	7.88±0.10^{C}	8.47*
Biotic factors	Bacteria (10^7 cells/ml)	13.09±0.89^{C}	13.71±0.70^{C}	15.26±0.99^{BC}	18.11±0.61^{AB}	19.02±0.59^{A}	21.04±0.85^{A}	13.03*
	microalgae (10^6 cells/L)	7.28±1.09^{A}	6.96±0.61^{AB}	6.30±0.37^{ABC}	5.81±0.35^{BC}	5.69±0.34^{BC}	5.11±0.32^{C}	10.68*
	Survival rate of copepods (%)	73±14^{A}	67±13^{AB}	51±14^{AB}	43±12^{B}	41±8^{B}	35±13^{B}	6.94*

[a] Data: Mean±SD, [b] N.S.: No significance, [c] *: $p<0.01$

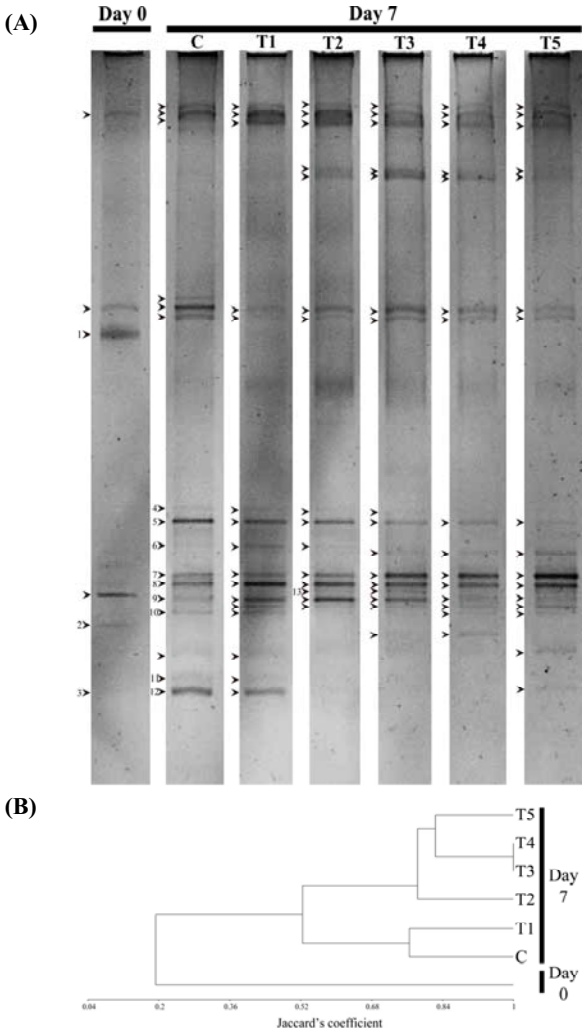

Fig. 2. Comparison of the DGGE band patterns of partial 16S rDNA fragments amplified from bulk DNA from different treatment groups (A) and cluster analysis using the UPGMA method (B) on the first day (day 0) and the last day (day 7) of the microcosm experiments. Arrowheads indicate bands. Numbered bands were analysed by DNA sequencing (see Table 1). T1, oil concentration of 10 ppm (v/v); T2, 100 ppm; T3, 1,000 ppm; T4, 5,000 ppm; and T5, 10,000 ppm.

are presented in Fig. 1B. The abundance dramatically decreased after the addition of oil at concentrations over 1,000 ppm (T3, T4, and T5 groups), whereas mean abundances of $7.0\pm0.6\times10^6$ cells/L and $7.3\pm0.6\times10^6$ cells/L were sustained in the T1 and control groups, respectively. Diatoms were the dominant microalgal group and dinoflagellates were the next most abundant group. After the addition of oil, the abundances of dinoflagellates and nanoflagellates increased slightly, whereas those of diatoms decreased rapidly (Fig. 3). The dominant diatom species were *Thalassiosira allenii* and *Skeletonema costatum* (centric diatom) before the addition of oil (Fig. 4). However, the proportions of these two diatoms changed in opposite directions after the addition of oil at concentrations over 100 ppm: the proportion of *T. allenii* decreased, whereas that of *S. costatum* increased. The proportions of *T. allenii* and *S. costatum* were similar in the control and T1 group (Fig. 4). Other microalgal components, silicoflagellates and euglenoids, did not show significant changes.

Viability of copepod populations

Copepod populations in the microcosms were dominated by four species: *Acartia omorii*, *A. steueri*, *Eurytemora pacifica*, and *Paracalanus parvus*. Copepod abundance changed rapidly depending on the concentration of oil to which they were exposed (Fig. 5). Living copepods disappeared completely in the T3, T4, and T5 groups after 6 days, while the proportions of living copepods in the T1 and T2 groups had decreased gradually to 27.3±17.3% and 6.3±8.3%, respectively, by the last day of the study. In the control group, the abundance of living copepods was sustained at the initial value.

Discussion

In this study, the oil added to the microcosms harmed microalgae and copepods, whereas rapid increases in the density of heterotrophic bacteria were observed. Pelagic organisms were affected markedly when crude oil was introduced at nominal concentrations over 1,000 ppm in the microcosm. Similarly, Dahl *et al*. (1983) stated that the sub-lethal level of crude oil for microbial communities such as bacteria, microalgae, and zooplankton was approximately 1,000 ppm. Many researchers have determined sub-lethal

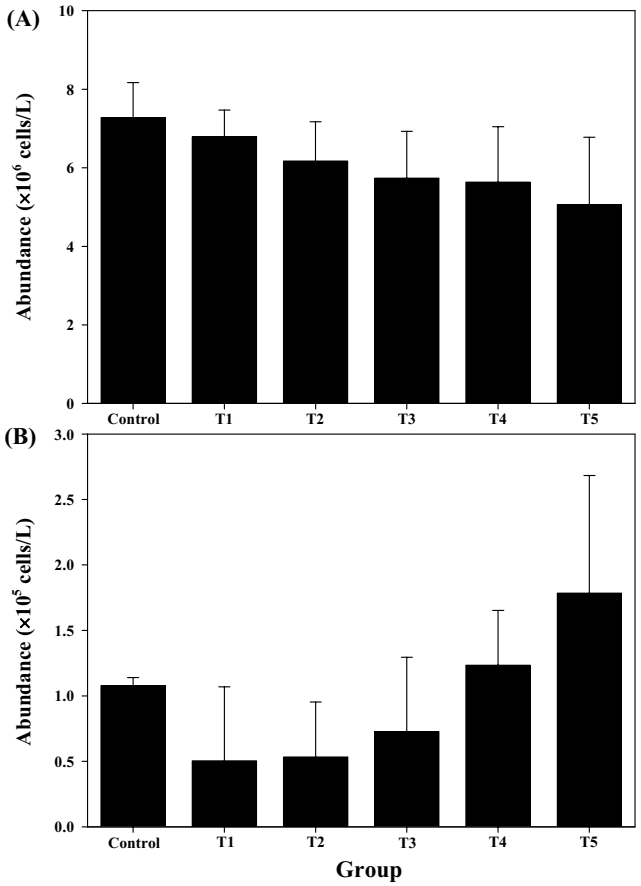

Fig. 3. Changes in the mean abundance of diatoms (A) and dinoflagellates and nanoflagellates (B) over the total time of the experiment. Data represent Means±SD from three independent assays. T1, oil concentration of 10 ppm (v/v); T2, 100 ppm; T3, 1,000 ppm; T4, 5,000 ppm; and T5, 10,000 ppm.

levels for various organisms. For example, copepods were found to be seriously affected at observed hydrocarbon concentrations of 100 ng/g (Davenport, 1982). Nayar *et al*. (2005) demonstrated that the density of bacteria increased upon the addition of petroleum hydrocarbons at a concentration of 1 ppm. Thus, the effects of petroleum hydrocarbons on specific organisms have been studied extensively in laboratory-based experiments (e.g., Wells and Percy, 1985). However, studies of the effects of crude oil on natural microbial communities are rare. Therefore, the results of the study reported herein may be important information for interpreting the effects of oil spills on the communities.

Measurements of temperature and pH confirmed that the microcosms studied were filled with nearly identical masses of water. The observed decrease in salinity can be explained by the inhibition of evaporation caused by an oil film: less water evaporated after the addition of high concentrations of oil (over 1,000 ppm) than at concentrations of 10 or 100 ppm, resulting in lower salinity in the former microcosms. This indicates that water did not easily penetrate thick oil films on the surface. Moreover, light penetration directly through an oil film on the water surface may be negligible. However, growth of microalgae may be little affected due to flank penetration of light. Dissolved oxygen decreased with increasing oil concentrations. This variation might be explained by bacterial activity (Jung *et al*., 2010). However, these minor changes are assumed to have had little effect on the microbial communities' responses in the microcosms.

The most evident effect of the addition of oil in this study was the rapid stimulation of bacterial growth similar to the findings of an oil spill microcosm study (MacNaughton *et al*., 1999). This rapid response is probably due to the immediate availability of effluent low-molecular-weight fractions as carbon and energy sources, without the requirement for any period of bacterial adaptation (Lee *et al*., 1977). Atlas and Bartha (1972) observed that the biodegradation of petroleum can increase the level of available nutrients with a resulting increase in the abundance of microorganisms. Additional nutrients can also be supplied by the degradation of dead microalgae and zooplankton. The mesocosm experiments of Jung *et al*. (2010) showed that the level of inorganic nutrients was increased by the degradation of dead organisms. Therefore, in this study, the density of bacteria might have increased due to the degradation of crude oil and dead organisms, which are stimulated by increased bacterial activity. In the DGGE analysis, the intensity of the band corresponding to *Jannaschia* sp. (band number 7) showed a prominent increase with increasing concentrations of oil (Table 1 and Fig. 2). *Jannaschia* species are known to predominate in areas exposed to oil (Röling *et al*., 2002). Harwati *et al*. (2007) reported that *Jannaschia* species are frequently present in oil-contaminated areas and can grow using some hydrocarbon compounds. The intensity of the band that corresponded to *Sulfitobacter brevis* (band number 8) also increased upon the addition of oil. This bacterium is also frequently present in areas contaminated by oil (Gerdes *et al*., 2005). Pinhassi *et al*. (1999) showed that shifts in bacterial composition can be affected by biostimulatory substances. Thus, specific bacterial populations can respond rapidly to the addition of substances, such as oil. The present results provides additional useful information about the dynamics of bacterial communities and the effects of massive inputs of organic matter, in this case crude oil, on specific bacterial populations in coastal waters.

In the microcosm, the abundance of diatoms in the microalgal community decreased upon the addition of oil. Davenport (1982) demonstrated that diatom populations are affected severely by hydrocarbon concentration. Aromatic hydrocarbons are one of the most important inhibitors of the growth of microalgae (Walker *et al*., 1975; Cerniglia, 1981; Wolfe *et al*., 1998; González *et al*., 2009). Their effects include immediate toxicity due to the soluble aromatic fraction, prolonged toxicity due to persistent products, and altered physical-chemical conditions below the floating oil (for example, temperature changes). However, at non-toxic levels of oil, as demonstrated here by the T1 group, the responses of microalgae could include enhanced metabolism due to increased nutrient availability as a result of oil degradation (Miller *et al*., 1978). With regard to dominant species, *S. costatum* increased slightly with increasing oil concentration (Fig. 4), whereas *T. allenii* showed the opposite pattern. Mahoney and Haskin (1980) reported that *S. costatum* can tolerate exposure to oil. Pulich *et al*. (1974) examined the tolerance of microalgae to oil: diatoms (*Thalassiosira* spp., in

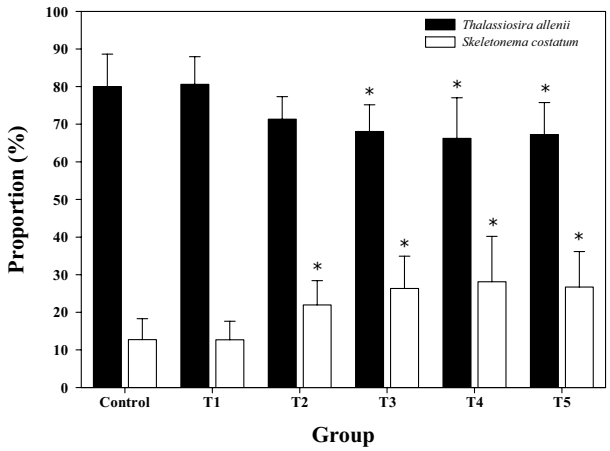

Fig. 4. Changes in the mean proportion of the dominant species, *Thalassiosira allenii* and *Skeletonema costatum* over the total time of the experiment. Data represent Means±SD from three independent assays. T1, oil concentration of 10 ppm (v/v); T2, 100 ppm; T3, 1,000 ppm; T4, 5,000 ppm; and T5, 10,000 ppm. An asterisk indicates a significant difference from the control (* $P<0.01$).

this case) were relatively sensitive to oil, whereas other blue-green algae, green algae, and a dinoflagellate were signify-cantly more tolerant to the oil tested. Thus, the response of microalgae to oil exposure might vary among species, which show different responses and tolerances to oil exposure. In the present study, the abundance of dinoflagellates and nano-flagellates was increased, while that of diatoms was decreased. Davenport (1982) found that after oil exposure the composition of microalgae in a mesocosm changed from predominantly diatoms to flagellates. Therefore, an oil spill might result in the succession of microalgae from diatoms to flagellates.

Copepods were observed to move more slowly even in the T1 and T2 groups upon the addition of low concentrations of oil, and their abundance was reduced markedly with increasing oil concentrations. The zooplankton community has been shown to be affected by the level of petroleum hydrocarbons present (Davenport, 1982). In the same manner, in the microcosm studied here, living copepods were severely affected by oil exposure in the short-term, and this effect was probably due to aromatic substances present in the oil.

The results of the present study demonstrate that the addition of crude oil at concentrations higher than 1,000 ppm might stimulate rapid bacterial growth and inhibit the growth of zooplankton and microalgae. Our results suggest that oil pollution risk assessments should be tailored to a specific level to oil exposure. The present study cannot be extrapolated fully to all marine pelagic ecosystems. Therefore, studies on scaled-up mesocosms, long-term investigations into chronic effects, and tests of various microbial communities as well as various types of oil and dispersant are needed to provide exact information.

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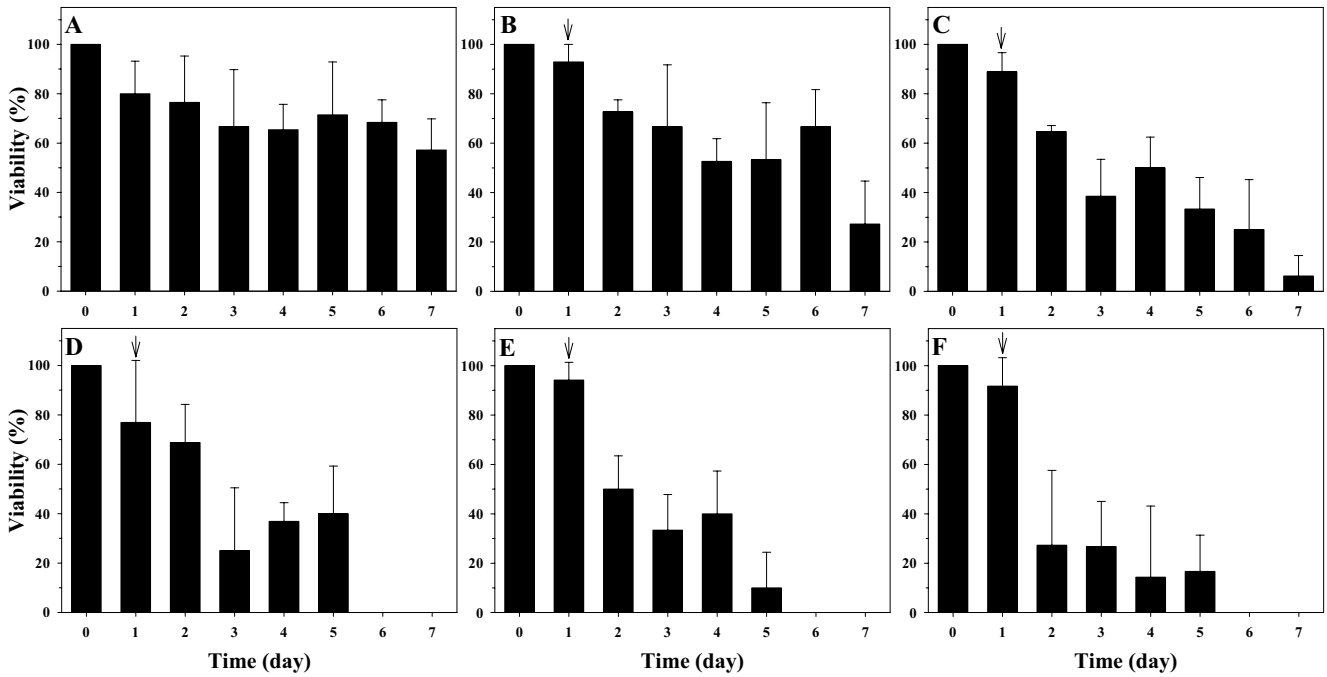

Fig. 5. Changes in the viability of copepod populations after the addition of oil. Arrows indicate the time (day) of oil addition. Data represent Means±SD from three independent assays. (A) the control, (B) T1, concentration of 10 ppm, (C) T2, 100 ppm, (D) T3, 1,000 ppm, (E) T4, 5,000 ppm, and (F) T5, 10,000 ppm.

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