La mer 45 : 105-116, 2007
Société franco-japonaise d'océanographie, Tokyo

Mesocosm studies on phytoplankton community succession after inputs of the water-soluble fraction of Bunker A oil

Hideaki NOMURA[1]*, Keita TOYODA[2], Mihoko YAMADA[3], Ken OKAMOTO[4], Minoru WADA[1], Masahiko NISHIMURA[1], Akihiro YOSHIDA[1], Akira SHIBATA[1], Hideshige TAKADA[3] and Kouichi OHWADA[5]

Abstract: We monitored the succession of a phytoplankton community for 10 days in an enclosed meso-scale seawater tank (mesocosm) , into which the water-soluble fraction of Bunker A oil was spiked, with or without a chemical dispersant. Diatoms such as *Chaetoceros* spp. and *Skeletonema costatum* contributed more than 50% of the total phytoplankton abundance for the first 3 days in all tanks. In the seawater tank that was devoid of the oil contamination, phytoplankton abundance fluctuated greatly, but diatoms predominated until day 8. However, in the oil-spiked tanks, autotrophic flagellates predominated over diatoms by day 5 after the oil addition. Daily monitoring of sediment trap contents revealed that the oil-spiked seawater resulted in a significantly reduced flux of diatom cells compared with the seawater tank. The decline in the diatom contribution to total phytoplankton abundance in both seawater and trap contents was more pronounced in the presence of the dispersant than in its absence. From these results, the mesocosm experiments clearly demonstrated adverse effects of Bunker A oil components on planktonic diatom assemblages under experimental conditions similar to those found in natural coastal environments. A combination of careful observation of the succession within the phytoplankton population in the water column and analysis of sediment trap samples have provided insights into the possible impacts of low levels of oil contamination on grazing food webs in natural marine environments.

Keywords: *diatoms, flagellates, phytoplankton, succession, oil, dispersants, mesocosms*

Introduction

Accidental oil spillage is one of the severe problems facing marine ecosystems, because the chemical components that diffuse from spilled oil often show acute or chronic eco–toxicity to diverse types of plants and animals (ALBERS, 1995). Evaluation of the environmental damage caused by such spilled oil has been a substantial challenge because of the lack of baseline information about the species present at a site, and their interactions and physiological states prior to the oil contamination. Although laboratory experiments on the effects of oil contamination on particular species of marine organisms are necessary, it is often difficult to apply the results of these studies directly to predict population– or community–level impacts that would affect the structure and function of the natural ecosystem.

Field experiments in a meso–scale, controlled ecosystem (mesocosm) have been conducted to overcome such difficulties in evaluating the impacts of pollutants on biological processes (LEE and TAKAHASHI, 1977; OVIATT *et al.*, 1982; PARSONS *et al.*, 1984; LINDÉN *et al.*, 1987). In

1. Ocean Research Institute, The University of Tokyo
2. Laboratory of Aquatic Science Consultant Co., Ltd.
3. Institute of Symbiotic Science and Technology, Tokyo University of Agriculture and Technology
4. Graduate School of Agricultural and Life Science, The University of Tokyo
5. Faculty of Environmental and Symbiotic Sciences, Prefectural University of Kumamoto

*Corresponding author
Ocean Research Institute, The University of Tokyo, Minami-dai, Nakano, Tokyo 164-8639, Japan
Tel: +81-3-5351-6483, Fax: +81-3-5351-6482
E-mail address: nmr@ori.u-tokyo.ac.jp

contrast to a glass flask in a laboratory, a mesocosm tank is designed to hold a water mass large enough to allow a diverse array of planktonic organisms at different trophic levels to exist under conditions similar to the natural environment in terms of temperature, sunlight, wind, and rainfall (MENZEL, 1977; PARSONS *et al.*, 1984). An enclosed marine mesocosm near the mouth of Lake Hamana (Hamana–ko), a seawater lake in Shizuoka prefecture, Japan, has been used to study the impacts of contamination from the water–soluble fraction (WSF) of Bunker A oil on the microbial food web in seawater (OHWADA *et al.*, 2003; TOYODA *et al.*, 2005; NISHIMURA *et al.*, 2006; YOSHIDA *et al.*, 2006). One of the conclusions from these studies was that even low levels of oil contamination can disturb species composition and trophic interactions in the microbial food web.

In the present paper, we report on the succession of the phytoplankton community in the same mesocosm system, into which the WSF of Bunker A oil was added, with or without a chemical dispersant. We also describe the phytoplankton composition in sediment trap samples retrieved daily from the mesocosm. From these results, we discuss the possible impacts of low concentrations of the oil components on the phytoplankton community and grazing food web in natural marine environments.

Materials and methods

The mesocosm experiments were carried out from 23 May to 2 June 2001 using three experimental tanks. Each cylindrical tank has a 1.5–m diameter, a 3.0–m depth, and a 5000–L capacity (OHWADA *et al.*, 2003). Surface water from Hamana–ko was introduced into the two reservoirs for three tanks by using an electric submersible pump (OMORI and JO, 1989), and then distributed equally into the experimental tanks. On 22 May, nutrients (KNO_3, 100 μg N L^{-1}; KH_2PO_4, 10 μg P L^{-1}; Na_2SiO_3–$9H_2O$, 10 μg Si L^{-1}) were added to each experimental tank to maintain biological activity during the experiment, and the tanks were stirred for 0.5 h using stainless–steel blades (YOSHIDA *et al.*, 2006).

We prepared a mixture of the WSF of Bunker A oil and autoclaved seawater from Hamana–ko (YAMADA *et al.*, 2003). We also prepared a mixture of the WSF and a chemical dispersant (nonionic surfactant; Taiho Self Mixing S–7, Taiho Industries, Tokyo, Japan). Details of the procedure for the preparation and handling of the WSF and the dispersant were described in YAMADA *et al.* (2003) and YOSHIDA *et al.* (2006). After introducing water into the experimental tanks, either the WSF or the mixture of WSF and chemical dispersant was added to the mesocosm tanks, which were designated as the "OIL" tank or the "OD" tank, respectively. The last tank was kept free of contamination from both oil and dispersant as a control and was designated as the "SEA" tank.

Oil concentration just after the addition of the WSF in the OIL tank was estimated to be 224 μg L^{-1}, as measured by fluorometric analysis according to the method of Integrated Global Ocean Services System (IGOSS, 1974; referenced in: the Oceanographic Society of Japan, 1979). This oil concentration is comparable to that found in the inner part of the port of Tokyo Bay and is similar to that of MARL experiments in the early 1980s (OVIATT *et al.*, 1982). Determination of the oil concentration in the OD tank failed due to a poor extraction efficiency caused by the dispersant used (M. YAMADA, personal communication). Concentrations of four representative polyaromatic hydrocarbon (PAH) compounds– naphthalene ($C_{10}H_8$), phenanthrene ($C_{14}H_{10}$), fluoranthene ($C_{16}H_{10}$), and chrysen ($C_{18}H_{12}$) –were determined by gas chromatography–mass spectrometry (YAMADA *et al.*, 2003).

Before pouring the oil–water mixture into the tanks, a sample of tank water was collected and subjected to microscopic observation of phytoplankton. For chemical analysis, another water sample was collected just after the introduction of the oil–water mixture. These are referred to as "day 0" samples. Water samples were siphoned to collect periodically from day 0 through day 10 from a depth of 0.5 m, using teflon tubing attached to the stainless–steel pipe, carefully introduced into glass bottles, and stored at appropriate temperature until analysis. At the same time as water sampling, a portable STD system (model 610–DM; YSI,

Table 1. Changes of water temperature, salinity, and PAHs in the subsurface water during the mesocosm experiments.

Time (days)		0	1	2	3	4	5	10
Temperature (℃)	SEA	19.6	19.7	20.4	22.6	23.6	22.7	22.0
	OIL	20.1	20.0	20.2	21.4	22.4	22.1	23.1
	OD	20.1	20.0	20.2	21.4	22.3	22.0	22.8
Salinity	SEA	31.63	31.54	31.44	31.40	30.92	31.42	31.43
	OIL	31.54	31.21	30.92	31.18	31.13	31.20	31.22
	OD	31.53	31.28	31.23	31.19	31.20	31.09	30.99
PAH s (ngL^{-1})								
Naphthalene	SEA	149.6	168.9			190.2		152.8
	OIL	3777.1	3606.8	276.9	113.5		190.8	205.9
	OD	5059.7	5613.0	349.1	159.6		146.1	158.9
Phenanthrene	SEA	7.3	6.6			7.4		9.7
	OIL	600.8	489.4	6.7	11.1		11.2	13.7
	OD	999.6	1159.0	82.2		11.1	12.1	19.6
Fluoranthene	SEA	3.6	3.4			4.6		3.6
	OIL	14.5	13.2	6.9	6.4		8.0	3.0
	OD	19.6	25.3	24.0	15.1		10.4	6.5
Chrysene	SEA	0.3	0.2			0.3		0.3
	OIL	2.9	2.6	2.7	2.5		2.3	1.5
	OD	4.0	5.0	4.8	4.7		6.3	4.9

SEA:natural seawater without oil and/or chemical dispersant
OIL:seawater with oil alone
OD:seawater with oil plus chemical dispersant

Yellow Springs, OH, USA) was used to make a depth profile of temperature, salinity, and dissolved oxygen. Water temperature and salinity in all the experimental tanks ranged from 19.6 to 23.1 ℃, and from 30.9 to 31.6, respectively, during the 10 days of incubation (Table 1).

Water samples were fixed with formalin (final concentration: 1%, v/v) and observed with an inverted Nomarsky–type microscope (model TE300; Nikon, Tokyo, Japan) to taxonomically identify and enumerate phytoplankton cells. A 25% glutaraldehyde solution (final concentration: 2%, v/v) was used to gently fix autotrophic nanoflagellates (ANFs) in water samples. The ANF cells were stained with FITC and DAPI (SHERR and SHERR, 1983), filtered onto a polycarbonate Nuclepore (Whatman, Springfield Mill, UK) membrane (pore size, 0.8 μm), and counted under an epifluorescence microscope (type E800; Nikon) in a dark room. ANFs were distinguished from heterotrophs by the autofluorescence of photosynthetic pigments observed under blue light excitation. To minimize the masking effect of the protein–binding dye FITC to the autofluorescence of photosynthetic pigments (SHERR *et al.*, 1993), we empirically set the staining time (5 minutes) and the final concentration (3 μg ml^{-1}).

To measure the concentration of chlorophyll *a* (Chl), a 100–ml water sample was filtered through a Whatman GF/F glass–fiber filter (Whatman, Springfield Mill, UK), and the filters were soaked in N,N–dimethylformamide (SUZUKI and ISHIMARU, 1990). Filters were stored in the dark at approximately −20 ℃for fewer than 10 days before Chl content was measured with a fluorometer (type 10R; Turner Designs, Sunnyvale, CA, USA). A calibration curve was obtained using a Chl standard (Sigma, St. Louis, MO, USA). The filtrate collected during Chl sample preparation was used to analyze for nitrogen (NO_2^- + NO_3^-) and phosphate (PO_4^{3-}) concentrations using an auto–analyzer (model AAAC3; BRAN+LUEBBE, Norderstedt, Germany).

Every day, four glass vials (120–mm height, 30–mm inner diameter, and 100–ml volume) were suspended in the tanks using a thread at a depth of 2.5 m to collect sinking particles. Vials were gently retrieved after 1 day and used

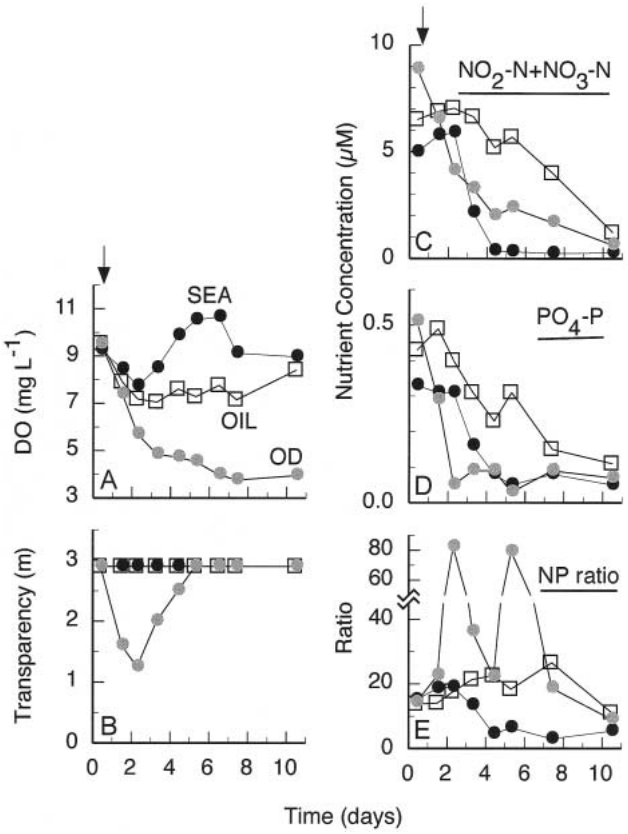

Fig. 1. Temporal variation of concentration of dissolved oxygen (DO) (A), transparency (B), nitrogen (N: NO_2-N + NO_3-N) (C), phosphate (P: PO_4-P) (D), and NP ratio (E) during the mesocosm run from 23rd of May to 2nd of June in 2001. The arrow denotes the time of oil contamination. SEA: natural seawater in the mesocosm tank, OIL: natural seawater with WSF mixture, OD: natural seawater with WSF-chemical dispersant mixture.

for analysis of phytoplankton abundance and Chl content. The phytoplankton trapped in the vials were fixed immediately by formalin (final concentration: 1%, v/v) and stored in the dark at 4 ℃ until microscopic analysis. Identification and enumeration were conducted in the same way as described for the water samples.

We measured water transparency by using a white ceramic disc 80 mm in diameter (smaller than a standard Secchi disc, which is 200 or 300 mm in diameter). According to PREISENDORFER (1986), transparencies measured with the smaller disc and the standard ones are practically the same. The compensation depth (*CD*, m) was calculated using transparency depth (*Ds*, m) with the equation described in ARUGA (1986) : $CD = 2.67Ds$.

Results and discussion

Changes in physico–chemical parameters

The highest concentrations of PAHs were found in the tank to which seawater and the WSF of Bunker A oil and a dispersant (OD) were added (Table 1). Relatively lower molecular weight (LMW) PAHs, naphthalene and phenanthrene, dramatically decreased to less than 10% of the initial concentrations in the first 2 days, whereas higher molecular weight (HMW) PAHs, fluoranthene and chrysene, decreased more slowly. The rapid decrease in the LMW–PAHs can be ascribed to microbial degradation, and the slower removal of the HMW–PAHs (and HMW–alkanes and hopanes; data not shown), from the water column presumably results from settling or sedimentation

(YAMADA *et al.*, 2003).

Dissolved oxygen (DO) concentration in all the tanks was initially 9.5 mg L^{-1}, decreasing by day 7 to 7.2 mg L^{-1} in the OIL tank and to 3.8 mg L^{-1} in the OD tank (Fig. 1A). DO concentrations in the OIL tank became to initial level by day 10, while those in the OD tank continued to decrease. In the control seawater (SEA) tank, DO fluctuated greatly, but it remained above 7.5 mg L^{-1} during the entire incubation period. It is likely that bacterial respiration during the degradation of hydrocarbons in the WSF, the dispersant, or both, substantially contributed to the decline in DO (YOSHIDA *et al.*, 2006).

Initial concentrations of inorganic nitrogen ($NO_2^- + NO_3^-$) and phosphate (PO_4^{3-}) in the tanks ranged from 5 to 9 μM and from 0.3 to 0.5 μM, respectively (Fig. 1C, D). Although both nitrogen and phosphate disappeared almost entirely by day 10, the patterns of disappearance varied between the nutrients and between the tanks. Nitrogen decreased more slowly in both the OIL and the OD tanks compared with that in the SEA tank. Phosphate tended to disappear more rapidly than nitrogen, particularly in the OD tank during the first 2 days. These differences in nutrient removal from the tanks were reflected in the molar ratio of nitrogen to phosphorus (N:P ratio) (Fig. 1E). The N:P ratio was about 14:1 in all of the tanks at day 0 and decreased to less than 10 by day 5 in the SEA tank. In contrast, the N:P ratios in the OIL and OD tanks remained above the initial value through through day 7, with larger fluctuations in the OD tank. Because the N:P ratio of the natural phytoplankton community is 16:1 (REDFIELD *et al.*, 1963), the SEA tank was under N–limited conditions from day 5, whereas the OIL and OD tanks were under P–limited conditions for almost the entire period of the experiment.

Differences in phytoplankton community structure

A total of 62 species of phytoplankton, comprising 47 diatoms, 10 dinoflagellates, and 5 other species, was found in the mesocosm tanks during the experiment (Table 2). While most of them (59 species) were found in the SEA tank, about half were found in the OIL and the OD tanks, suggesting that phytoplankton species richness was higher in the SEA tank.

During the initial phase of the incubation (from day 0 to day 3), planktonic diatoms, including some chain–forming species such as *Chaetoceros seiracanthus, C. debilis, C. dydimus*, and *S. costatum*, contributed more than 50% of the total phytoplankton abundance in all of the tanks (Fig. 2). The rest of the phytoplankton population during this period was mainly composed of autotrophic flagellates, such as *Prorocentrum minimum*, and coccolithophorids.

After day 3, the relative contribution of diatoms to the total phytoplankton population became less pronounced. Although diatoms continued dominating total phytoplankton abundance in the SEA tank until day 8, autotrophic flagellates, namely *P. minimum*, gradually increased after day 5 and finally became dominant (75% of the total phytoplankton population) by day 10. In addition to flagellates, coccolithophorids contributed about 20% of the total phytoplankton population at day 10 in the SEA tank. A succession from diatoms to flagellates in a phytoplankton population is a typical phenomenon in an enclosure system devoid of water turbulence (LEE and TAKAHASHI, 1977; EGGE and AKSNES, 1992). In contrast, autotrophic flagellates, particularly ANFs, rapidly dominated the phytoplankton populations in the OIL and OD tanks after day 5. In the oil–contaminated tanks (with and without dispersant), coccolithophorids disappeared from the water column by day 6.

Beneficial or adverse effects of petroleum substances on phytoplankton should vary depending on the phytoplankton species. Based on previous studies certain autotrophic flagellates appeared to be petroleum–insensitive (PULICH *et al.*, 1974; PARSONS *et al.*, 1976; LEE and TAKAHASHI, 1977; KARYDIS, 1981; MORALES–LOO and GOUTX, 1990; SIRON *et al.*, 1991; OKUMURA *et al.*, 2003), although coccolithophorids suffered severely under oil–contaminated conditions. In another set of mesocosm studies using the same tanks, we confirmed by a vital FDA (fluorescein

Table 2. List of phytoplankton species occurred from the water column of mesocosm tanks during experiments. Water from the surface layer of the mouth part of Hamana-ko was directly brought into tanks via an under-ground tubing water supply pump on May 22, 2001.

Species	Tanks SEA	OIL	OD
Division Dinophyta			
Class Dinophyceae			
Order Prorocentrales			
Prorocentrum compressum	+	−	−
P. dentatum	+	+	−
P. micans	+	−	−
P. minimum	+	+	+
P. triestinum	+	+	+
Order Dinophysiales			
Oxyphysis oxitoxoides	−	−	+
Order Gymnodiniales			
Gymnodinium sp.	+	−	−
Order Gonyaulacales			
Ceratium kofoidii	−	+	−
Order Peridiniales			
Peridinium quinquecorne	+	+	+
Protoperidinium spp.	+	−	+
Division Heterokontophyta			
Class Chrysophyceae			
Order Dictyochales			
Ebria tripartita	+	−	−
Dictyocha fibula	+	−	−
Distephanus speculum	+	+	+
Class Bacillariophyceae			
Order Centrales			
Actinoptychus senarius	+	−	−
Bacteriastrum delicatulum	−	−	+
Chaetoceros atlanticus	+	−	−
C. borealis	+	+	−
C. compressus	+	+	+
C. coronatus	+	−	−
C. curvisetus	+	+	−
C. debilis	+	+	+
C. decipens	+	−	−
C. densus	+	+	+
C. dydimus	+	+	+
C. lorenzianus	+	+	+
C. seiracanthus	+	+	+
C. simplex	+	−	−
Chaetoceros spp.	+	+	+
Coscinodiscus gigas	+	+	−
C. perforatus	+	−	−
Dactyliosolen bravyanus	+	+	+
D. fragilissimus	+	+	−
Detonula pumila	+	−	−
Dytilum brightwellii	+	−	−
Eucampia zodiacus	+	−	+
Guinardia delicatula	+	+	−
G. striata	+	+	+
Hemialus hauckii	+	+	−
Lauderia annulata	+	−	−

+: occurred, −: not occurred

Table 2. continued.

Species	Tanks SEA	OIL	OD
Leptocylindrus minimus	+	+	+
Neostreptotheca subindica ?	+	−	−
Odontella longicruris	+	−	−
Pseudosolenia calcar-avis	+	+	−
Rhizosolenia hebetata f. *hebetata*	+	+	+
R. setigera	+	+	+
R. styliformis	+	−	−
Skeletonema costatum	+	+	+
Thalassiosira anguste-lineata	+	−	−
T. hyalina	+	−	−
T. rotula	+	+	+
T. weissflogii	+	−	−
Thalassiosira spp.	+	+	+
Order Pennales			
Asterionellopsis gracialis	+	+	−
Cylindrotheca closterium	+	+	+
Nitzschia longissima	+	+	+
Nitzschia spp.	+	+	−
Pleurosigma spp. ?	+	−	−
Pseudo-nitzschia spp	+	+	+
Thalassionema frauenfeldii	+	−	+
T. nitzschioides	+	+	+
Division Haptophyta			
Class Coccosphaerales			
unidentified species	+	+	+
Division Euglenophyta			
Class Euglenophyceae			
unidentified species	+	+	+

+: occurred, −: not occurred

diacetate) staining (BENTLEY–MOWAT, 1982) that most autotrophic flagellates were active even at the same levels of WSF of Bunker A oil used in the present study (data not shown).

Phytoplankton in sediment trap samples

Daily monitoring of sediment trap samples from the mesocosm tanks revealed that downward fluxes of Chl in the SEA, OIL, and OD tanks averaged 8.7 (1.0–19.3), 2.8 (0.4–5.6), and 1.7 (0.8–3.7) mg m^{-2} d^{-1}, respectively (Fig. 3). The total phytoplankton and diatom fluxes in the SEA tank averaged 2.3×10^{9} and 2.2×10^{9} cells m^{-2} d^{-1}, respectively, which were one order of magnitude higher than those in the OIL and OD tanks. Although Chaetoceros vegetative cells mainly contributed to the fluxes in the SEA tank during the entire incubation period, their resting spores clearly increased in the latter half of the incubation period, exhibiting a peak of more than 5×10^{7} cells m^{-2} d^{-1} on day 7. In contrast, the number of the resting spore in the trap samples from the OIL and OD tanks was extremely low, fewer than 1×10^{6} cells m^{-2} d^{-1} on average, whereas autotrophic flagellates contributed more to the total phytoplankton fluxes in the oil–contaminated tanks than in the SEA tank.

Species of *Chaetoceros* produce resting spores immediately following blooms (e.g., ODATE and MAITA, 1990), and nitrogen depletion is one of the essential factors inducing spore formation in centric diatoms (FRENCH and HARGRAVES, 1980; KUWATA and TAKAHASHI, 1990; OKU and KAMATANI, 1995, 1997; MCQUOID and HOBSON, 1996). Our results are consistent with these observations; resting spore formation by *Chaetoceros* in the SEA tank became intensive after nitrogen was depleted from the seawater. Besides nitrogen, phosphate depletion can be a factor leading to resting spore formation in diatoms (OKU and KAMATANI, 1995). However,

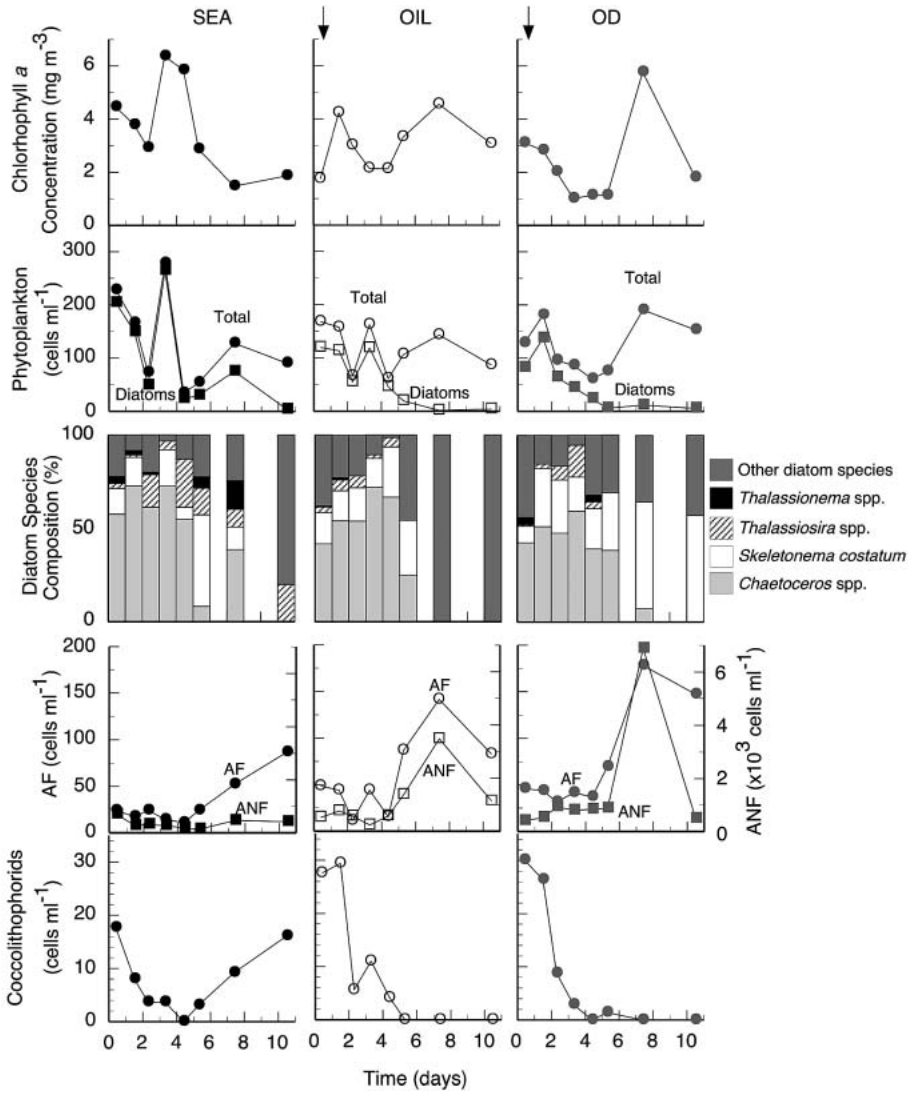

Fig. 2. Temporal variation of concentrations of chlorophyll *a* concentration, phytoplankton cell density, diatom species composition, cell density of autotrophic flagellate (AF) and autotrophic nanoflagellate (ANF) and coccolithophorid during the mesocosm run from 23rd of May to 2nd of June in 2001. The arrows denote the time of oil contamination. SEA: natural seawater in the mesocosm tank, OIL: natural seawater with WSF mixture, OD: natural seawater with WSF–chemical dispersant mixture.

this probably did not occur in our mesocosm tanks, because phosphate was depleted faster in the OD tank than in the SEA tank (see Fig. 1D) and yet the OD tank had fewer spores than the SEA tank (Fig. 3). Although the lower numbers of resting spores in the OIL and OD tanks may be partly explained by the relatively slow depletion of nitrogen compared with the SEA tank, it is more likely that chemical components in the WSF of Bunker A oil were responsible for limiting spore formation.

Because the spore formation by planktonic diatoms in the SEA tank is adequately explained by nitrogen limitation alone, it is unlikely that other elements such as silicon (Si) were limiting for diatom growth and spore formation in the present study. In support of this, we did not observe any Si depletion in the

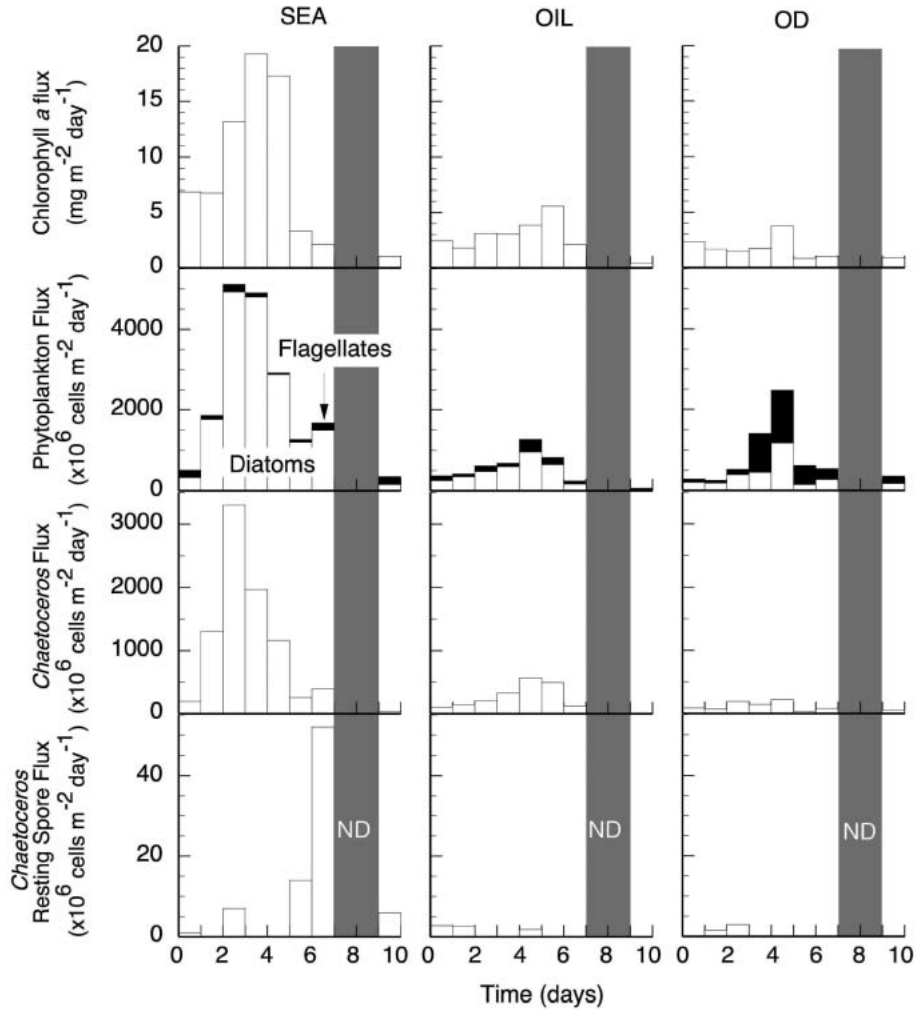

Fig. 3. Temporal variation of downward flux, chlorophyll *a*, total phytoplankton cell, *Chaetoceros* cell, and *Chaetoceros* resting spore, during the mesocosm run from 23rd of May to 2nd of June in 2001. SEA: natural seawater in the mesocosm tank, OIL: natural seawater with WSF mixture, OD: natural seawater with WSF –chemical dispersant mixture. ND: no data.

previous mesocosm study with the same experimental conditions. For instance, the Si:N ratio in the seawater tank in the previous experiment (autumn 2000) ranged from 0.9:1 to 1.2:1 (average = 1.1:1) during the initial 9 days of incubation (data not shown). Actively growing diatom cells have a Si:N composition ratio of 1.2:1 (BRZEZINSKI, 1985), so it is likely that diatoms in the SEA tank did not face serious Si depletion either in the present or in the previous experiments.

MORINAGA and ARAKAWA (2000) reported that an oil slick on the sea surface attenuated the photosynthetically available radiation (PAR) below the sea surface. Such changes in the optical environment can be detrimental to autotrophic plankton, although the optimum PAR may vary depending on the species. PARSONS *et al.* (1984) reported depressed primary production in the mesocosm tank resulting from light attenuation after the input of dispersed oil. In the present study, the transparency of the OD tank immediately decreased from 3 to 1.3 m after the addition of the

mixture of the WSF of Bunker A oil and a dispersant. It took 5 days to return to the original level of transparency, whereas transparency remained constant in the SEA tank and the OIL tank (see Fig. 1B). Based on ARUGA's equation, the compensation depth of the OD tank on day 2 was estimated to be 3.3 m. However, it was more than 7.7 m in the SEA and OIL tanks. Because sunlight attenuates drastically with depth, diatoms that cannot maintain their vertical position at a depth of optimum light conditions are likely to be outcompeted by motile flagellates.

Spilled oil has both inhibitory and stimulatory impacts on phytoplankton, which vary with the type of oil and concentrations of the petroleum components (ALBERS, 1995). Results of the present study suggest that the WSF of Bunker A oil is detrimental to diatoms, whereas it is less inhibitory to the flagellate population. Consequently, flagellates gained better access to the light and nutrients in the water column. Dispersants increase the concentrations of oil components in the water, thereby creating more harsh conditions for the diatom population (PARSONS *et al.*, 1984; YAMANE *et al.*, 1984; SIRON *et al.*, 1996). In the context of grazing food webs, the WSF of Bunker A oil altered not only the community structure but also the functions of the primary producers. The decreased flux of diatom cells from the upper layer implies a reduced transport of organic matter in the water column, which could interrupt the plankton–benthos coupling in natural environments (AMBROSE and RENAUD, 1995).

The physical microturbulence in a water mass is removed or weakened in an enclosed mesocosm (LALLI and PARSONS, 1993), therefore, it might be difficult for planktonic diatoms to remain suspended in the water column in mesocosm tanks such as those used in the present study, which could complicate interpretation of our results. Due to this intrinsic characteristic of these tanks, they may only be suitable for short–term observations of diatom populations in the water column. Nonetheless, the combination of careful observations of the phytoplankton population in the water column and in the sediment trap samples makes it possible to determine with great sensitivity the impacts of low levels of spilled oil on both the phytoplankton community structure and the trophic interactions in the grazing food webs in aquatic environments.

Acknowledgements

We are grateful to professors Dr. Akinori HINO and Dr. Yuzuru SUZUKI of the Fisheries Laboratory, Graduate School of Agricultural and Life Sciences, The University of Tokyo, and to several staff members of the Fisheries Laboratory, for providing experimental space and accommodations. We thank Dr. Osamu OKU of Meikai University for his important information and comments, and Mr. Jay Melton of Prefectural University of Kumamoto for proofreading our English in an earlier draft of this manuscript. We also thank Showa Shell Sekiyu Co. for kindly supplying us with the Bunker A heavy oil. This work was supported by the Research for the Future Program sponsored by the Japan Society for the Promotion of Science (JSPS).

References

ALBERS, P.H. (1995) : Petroleum and individual polycyclic aromatic hydrocarbons. *In* Handbook of Ecotoxicology. Hoffman, D.J., B.A. Rather, G.A. Burton, Jr. and J. Cairns, Jr. (eds.), Lewis Pub., Florida, p. 330–347.

AMBROSE, W.G., Jr. and P.E. RENAUD (1995) : Benthic response to water column productivity patterns: evidence for benthic–pelagic coupling in the Northeast Water Polynya. J. Geophys. Res., Oceans, **100**, 4411–4421.

ARUGA, Y. (1986) : Production ecology of marine phytoplankton. *In* Sohrui–no–seitai (Algal Ecology). Akiyama, M., Y. ARUGA, M. Sakamoto and Y. Yokohama (eds.), Uchida Rokakuho, Tokyo, p. 81–121. (in Japanese)

BENTLEY–MOWAT, J.A. (1982) : Application of fluorescence microscopy to pollution studies on marine phytoplankton. Bot. Mar., **25**, 203–204.

BRZEZINSKI, M.A. (1985) : The Si:C:N ratio of marine diatoms: interspecific variability and the effect of some environmental variables. J. Phycol., **21**, 347–357.

EGGE, J.K. and D.L. AKSNES (1992) : Silicate as regulating nutrient in phytoplankton competition. Mar. Ecol. Prog. Ser., **83**, 281–289.

FRENCH, F.W. and P.E. HARGRAVES (1980) : Physiological characteristics of plankton diatom

resting spores. Mar. Biol. Lett., **1**, 185–195.
KARYDIS, M. (1981) : The toxicity of crude oil for the marine alga *Skeletonema costatum* (Greille) Cleve in relation to nutrient limitation. Hydrobiologia, **85**, 137–143.
KUWATA, A. and M. TAKAHASHI (1990) Life–form population responses of a marine planktonic diatom, *Chaetoceros pseudocurvisetus*, to oligotrophication in regionally upwelled water. Mar. Biol., **107**: 503–512.
LALLI, C.M. and T.R. PARSONS (1993) : Biological oceanography: an introduction. Butterworth–Heinemann, Oxford, 301pp.
LEE, R.F. and M. TAKAHASHI (1977) : The fate and effect of petroleum in controlled ecosystem enclosures. Rapp. P. –v. Rèun. Cons. int. Explor. Mer, **171**, 150–156.
LINDÉN, O., A. ROSEMARIN, A. LINDSKOG, C. HÖGLUND and S. JOHANSSON (1987) : Effects of oil and oil dispersant on an enclosed marine ecosystem. Environ. Sci. Technol., **21**, 374–382.
MCQUOID, M.R. and L.A. HOBSON (1996) : Diatom resting stages. J. Phycol., **32**, 889–902.
MENZEL, D.W. (1977) : Summary of experimental results: controlled ecosystem pollution experiment. Bull. Mar. Sci., **27**, 142–145.
MORALES–LOO, M.R. and M. GOUTX (1990) : Effects of water–soluble fraction of the Mexican crude oil "Isthmus Cactus" on growth, cellular content of chlorophyll a, and lipid composition of planktonic microalgae. Mar. Biol., **104**, 503–509.
MORINAGA, T. and H. ARAKAWA (2000) : Distribution of underwater irradiances and estimated light attenuation by oil slick in the ROPME Sea area. La mer, **37**, 173–181.
NISHIMURA, M., A. YOSHIDA, K. TOYODA, M. YAMADA, H. NOMURA, M. WADA, K. OKAMOTO, A. SHIBATA, H. TAKADA and K. OHWADA (2006) : Mesocosm experiment on the succession of microbial community in response to oil contamination to coastal seawater. La mer, **44**, 59–65.
Oceanographic Society of Japan, The (1979) : Kaiyo–Kankyou–Chosa–Ho (Methods of Marine Environmental Research). Kouseisha–Kouseikaku, Tokyo, 666pp.
ODATE, T. and Y. MAITA (1990) : Seasonal distribution and vertical flux of resting spores of *Chaetoceros* (Bacillariophyceae) species in the nertic water of Funka Bay, Japan. Bull. Fac. Fish. Hokkaido Univ., **41**, 1–7.
OHWADA, K., M. NISHIMURA, M. WADA, H. NOMURA, A. SHIBATA, K. OKAMOTO, K. TOYODA, A. YOSHIDA, H. TAKADA and M. YAMADA, (2003) : Study of the effect of water–soluble fractions of heavy–oil on coastal marine organisms using enclosed ecosystems, mesocosms. Mar. Poll. Bull., **47**, 78–84.
OKU, O. and A. KAMATANI (1995) : Resting spore formation and phosphorus composition of the marine diatom *Chaetoceros pseudocurvisetus* under various nutrient conditions. Mar. Biol., **123**, 393–399.
OKU, O. and A. KAMATANI (1997) : Resting spore formation of the marine planktonic diatom *Chaetoceros anastomosans* induced by high salinity and nitrogen depletion. Mar. Biol., **127**, 515–520.
OKUMURA, Y., J. KOAYAMA and S. UNO (2003) : The relationship between logPow and molecular weight of polycyclic aromatic hydrocarbons and EC50 values of marine microalgae. La mer, **41**, 182–191.
OMORI, M. and S.–G. JO (1989) : Plankton sampling system with a new submersible vortex pump and its use to estimate small–scale vertical distributuion of eggs and larvae of *Sergia lucens*. Bull. Plankton Soc. Japan, **36**, 19–26.
OVIATT, C., J. FRITHSEN, J. GEARING and P. GEARING (1982) : Low chronic additions of No. 2 fuel oil: chemical behavior, biological impact and recovery in a simulated estuarine environment. Mar. Ecol. Prog. Ser., **9**, 121–136.
PARSONS, T. R., W. K. W. LI and R. WATERS (1976) : Some preliminary observations on the enhancement of phytoplankton growth by low levels of mineral hydrocarbons. Hydrobiol., **51**, 85–89.
PARSONS, T. R., P. J. HARRISON, J. C. ACREMAN, H. M. DOVEY, P. A. THOMPSON, C. M. LALLI, K. LEE, G. LI and X. CHEN (1984) : An experimental marine ecosystem response to crude oil and Corexit 9527: part 2 – biological effects. Mar. Environ. Res., **13,** 265–275.
PREISENDORFER, R.W. (1986) : Secchi disk science: visual optics of natural waters. Limnol. Oceanogr., **31**, 909–926.
PULICH, W. M. Jr., K. WINTERS and C. VAN BAALEN (1974) : The effects of a No. 2 fuel oil and two crude oils on the growth and photosynthesis of microalgae. Mar. Biol., **28**, 87–94.
REDFIELD, A.C., B.H. KETCHUM and F.A. RICHARDS (1963) : The influence of organisms on the composition of seawater. *In* The sea, vol. 2. M.N. Hill (ed.), Wiley Interscience, New York, p.26–77.
SHERR, E.B. and B.F. SHERR (1983) : Double–staining epifluorescence technique to assess frequency of dividing cells and bacteriovory in natural populations of heterotrophic microprotozoa. Appl. Environ. Microbiol., **46**, 1388–1393.
SHERR, E.B., D.A. CARON and B.F. SHERR (1993) : Staining of heterotrophic protists for visualization via epifluorescence microscopy. *In* Handbook of methods in aquatic microbial ecology, Kemp, P.F., B.F. SHERR, E.B. SHERR and J.J. Cole, (eds.), CRC Press LLC, Florida, USA, 213–

227.

Siron, R., G. Giusti, B. Berland, R. Morales–Loo and É. Pelletier (1991) : Water–soluble petroleum compounds: chemical aspects and effects on the growth of microalgae. Sci. Total Environ., **104**, 211–227.

Siron, R., É. Pelletier and S. Roy (1996) : Effects of dispersed and adsorbed crude oil on microalgal and bacterial communities of cold water. Ecotoxicol., **5**, 229–251.

Suzuki, R. and T. Ishimaru (1990) : An improved method for determination of phytoplankton chlorophyll using N,N–dimethylformamide. J. Oceanogr. Soc. Japan, **46**, 190–194.

Toyoda, K., A. Shibata, M. Wada, M. Nishimura, H. Nomura, A. Yoshida, K. Okamoto, M. Yamada, H. Takada, K. Kogure and K. Ohwada (2005) : Trophic interactions among marine microbes in oil–contaminated seawater at mesocosmic scale. Microb. Environ., **20**, 104–109.

Yamada, M., H. Takada, K. Toyoda, A. Yoshida, A. Sibata, H. Nomura, M. Wada, M. Nishimura, K. Okamoto and K. Ohwada (2003) : Study on the fate of petroleum–derived polycyclic aromatic hydrocarbons (PAHs) and the effect of chemical dispersant using an enclosed ecosystem, mesocosm. Mar. Poll. Bull. **47**, 105–113.

Yamane, A. N., M. Okada and R. Sudo (1984) : The growth inhibition of planktonic algae due to surfactants used in washing agents. Water Res., **18**, 1101–1105.

Yoshida, A., H. Nomura, K. Toyoda, T. Nishino, Y. Seo,M. Yamada, M. Nishimura, M. Wada, K. Okamoto, A. Shibata, H. Takada, K. Kogure and K. Ohwada (2006) : Microbial responses using the denaturing gradient gel electrophoresis to oil and chemical dispersant inputs in enclosed ecosystems. Mar. Poll. Bull., **52**, 89–95.

Received October 27, 2005
Accepted November 26, 2007