D-1.2.1 Methods for Evaluating the Influence of Pollutant Loading on Marine Ecosystems using a Floating Mesocosm

Contact Person Shigeki Harada
Water Environment Engineering Section
National Institute for Environmental Studies
Environment Agency
Onogawa 16-2, Tsukuba, Ibaraki, 305-0053 Japan
Tel: +81-298-50-2388 Fax: +81-298-50-2576
E-mail: sharada@nies.go.jp

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Abstract To understand effects of pollutant loading from the Changjiang River on the estuarine plankton ecosystem, phosphorous- and oil-enrichment mesocosm experiments were carried out in the Changjiang estuary. A floating mesocosm system was developed that could endure the strong tidal currents and waves of over 1 m in the Changjiang estuary. The mixing conditions in the mesocosm water mass were determined using a dye (Rhodamine WT). Wave heights from 30 to 60 cm resulted in a well-mixed water mass (vertical diffusion coefficient ca. 11 cm^2 sec^{-1}) in the mesocosm. The phosphorous enrichment experiments in the Changjiang estuary in autumn and spring revealed that phytoplankton blooms could be easily raised by the addition of only phosphorous to the estuary. The phytoplankton that formed the blooms were diatoms (autumn) and dinoflagellates (spring). Since silica was rich in both seasons, there seems to be a factor other than silica concentration in the selection of the dominant phytoplankton species. It was also shown that control of phytoplankton blooms by zooplankton grazing was more effective for diatoms than for dinoflagellates. The oil enrichment experiment using water-soluble parts of fuel oil showed that photosynthetic activity was clearly affected by the oil. Ciliates and noctiluca drastically decreased after the addition of oil, showing that they were more sensitive to the oil than the other organisms in the mesocosm. This experiment suggests that oil pollution may have a direct impact on primary productivity in the Changjiang Estuary.

Key Words Changjiang Estuary, mesocosm, marine ecosystem, nutrients, oil pollution

1. Introduction

It is feared that the rapid growth of the Chinese economy will cause an increase of nutrients and/or hazardous chemical loading to the Changjiang Estuary and the East China Sea through the Changjiang River which may have a large effect on the marine environment. Increased nutrient loading or a change in nutrient quality will affect the composition of species, the structure of the food chain, and the elemental cycle in the marine ecosystem.

Concentrations of PO_4^{3-} in the Changjiang Estuary range from 0.2 to 1 μM, that is comparable with that of other large rivers. But inorganic nitrogen is considerably richer (e.g. 20 to 50 μM of NO_3^-) than in the others such as the Amazon1). An explanation for this high N/P ratio in the estuarine water is that a large quantity of excess nitrogen is discharged from the many large cities and agricultural lands in the Changjiang catchment area where less phosphorous fertilizer is used. In addition, phosphates may be adsorbed by suspended particles that sink to the bottom, carrying the phosphates with them2). At present, the Chinese government is strongly promoting increased agricultural production and, in the near future, fertilization will be further increased and conversion to more phosphorous-rich fertilizers will occur. Furthermore, with development of the catchment area for flood control, it is presumed that there will be fewer suspended particles reaching the estuary. These factors may cause an increase in the total amount of nutrient loading and/or a change of nutrient quality (e.g. a decrease in the N/P ratio) in the Changjiang Estuary. In addition to excess nutrient loads, recently, oil pollution by nautical accidents, etc., is becoming a serious problem for marine environments along the Chinese coast. Oil pollution has a large adverse effect not only on the quantity of fishing resources, but also on the value of the seafood in the market.

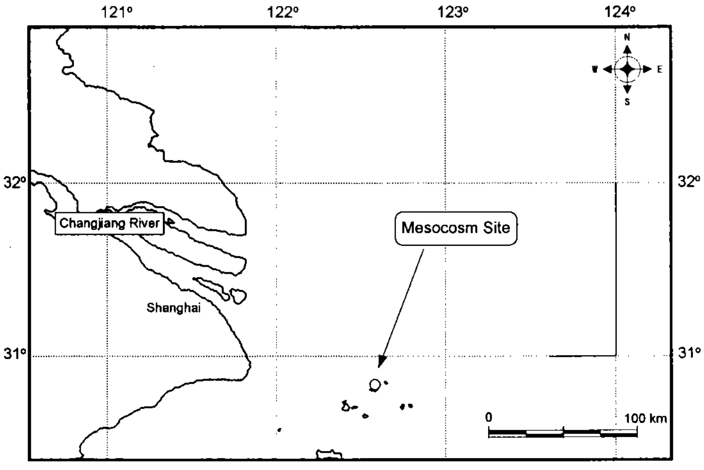

Figure 1. Location of the mesocosm experiments

Therefore, the evaluation and prediction of the effects which anthropogenic pollution loads have on the marine ecosystem in the Changjiang Estuary are urgently required.

The purpose of this study was to simulate and understand how the marine ecosystem and its biological elemental cycle is affected by nutrient loads and the disturbance that will occur in the near future, and how it is affected by the oil contamination that has recently become a serious problem in China. First, a floating mesocosm system was developed to evaluate the effects of artificial loads on the marine ecosystem. Then, using the newly developed mesocosm, the 2 following experiments (A & B) were carried out in the Changjiang Estuary area (near Liuhuashaw – Huanaoshan; about 100 km southeast of Shanghai, Fig. 1) in October 1997 and May 1998.

A) Phosphorous enrichment experiment (Oct. 1997 and May 1998)

The objective of this study was to investigate the effect of a decrease in the N/P ratio resulting from phosphate enrichment in a marine ecosystem, particularly the effect on phytoplankton and predator-prey interactions in the Changjiang Estuary.

B) Fuel oil enrichment experiment (May 1998)

The objective of this study was to investigate the impact of fuel oil on the plankton ecosystem in the Changjiang Estuary, in particular, the influence on primary producers and predator-prey interactions.

2. Development of the floating mesocosm system

Mesocosms are useful tools for investigating the responses of an ecosystem to nutrient and pollution loading. Our research group developed and used a mesocosm in the Seto Inland Sea, Japan, for several years3), 4). It was an enclosure-type system enclosing an area of seafloor and had a vertical circulation system that could provide turbulence to suspend non-motile phytoplankton species. However, the Changjiang estuary has a strong tidal current, higher waves (over 1 m), and a large change in water depth between tidal ebb and flow (a maximum of several meters in maximum); we needed to develop a new mesocosm system that could endure such conditions.

As a system more suited to these conditions, we chose a floating-type mesocosm that we planned to deploy at sites off islands in the Changjiang estuary. The prototype floating mesocosm, equipped with a couple of detachable mixing devices, was designed and made in 1997 (Fig. 2). In the Seto Inland Sea, Japan, we estimated the mixing conditions of the water mass within the mesocosm by using the dye, Rhodamine WT. High wave conditions (30 to 60 cm) resulted in a well-mixed water mass within the mesocosm. The estimated vertical diffusion coefficient was quite large (ca. 11 $cm^2 sec^{-1}$) compared with values previously reported for other mesocosm systems (Fig. 3). These results appeared to be mainly owing to the mixing effect of the heaving and rolling of the mesocosm itself, rather than the mixing devices. It is often insisted that, under calm conditions, a vertical-mixing device of some kind is necessary to suspend non-motile plankton species within the mesocosm water column. However, this investigation suggested that a mixing device would not be always required in sea areas under high wave conditions, such as in the Changjiang estuary where the mean wave height is more than 1 m throughout the year. Based on this result, we prepared a new type of floating mesocosm with no mixing devices for the experiments in the Changjiang Estuary (Fig. 4).

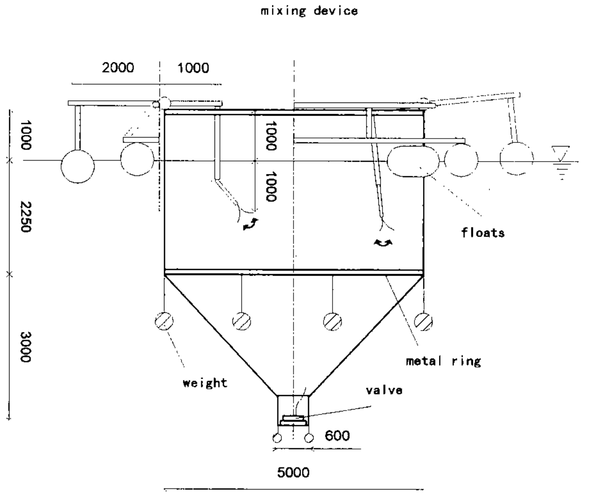

Figure 2. Prototype of floating mesocosm

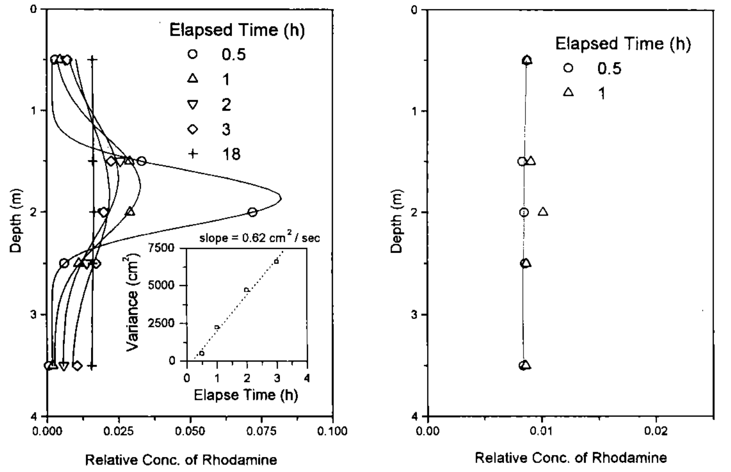

Figure 3. Temporal changes in the vertical profile of Rhodamine concentrations in calm conditions (waves of 0.1 to 0.2 m) left figure, and high wave conditions (0.3 to 0.6 m) right. The small plot within the left figure shows temporal changes in variance of the vertical concentration profile by Gaussian regression. The slope (0.62 $cm^2 sec^{-1}$) means the vertical diffusion coefficient in calm conditions. In high wave conditions, the dye diffused completely within an hour and the slope was more than 11 $cm^2 sec^{-1}$.

3. Phosphorous enrichment experiment

1) Materials and Methods

Two bag-type mesocosm ecosystems (ca 25 m^3) were deployed in the Changjiang Estuary for about one week in autumn (October 1997) and again in spring (May 1998). Phosphate was initially added to one of the mesocosms, lowering the N/P ratio of the contained seawater to about 10. Seawater temperature, salinity, nutrient concentrations, and abundance of phytoplankton, zooplankton, protists and bacterioplankton were monitored daily; in situ incubation experiments with organic or inorganic ^{13}C tracers were also carried out every day to determine the rate of transfer of carbon from bacterial and photosynthetic production to higher trophic organisms.

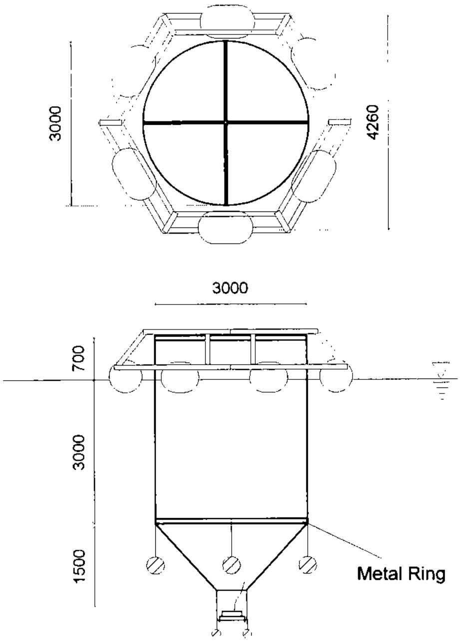

Figure 4. Floating mesocosm in the Changjiang Estuary

2) Results and Discussion

After the phosphate enrichment, blooms of a phytoplanktonic diatom (*Skeletonema costatum*) and a dinoflagellate (*Prorocentrum sp.*) were observed in the autumn and spring mesocosms, respectively (Fig.5). With development of the blooms, nitrate (initial concentration ca 24 and 16 μM, respectively) rapidly decreased together with the added phosphate and became depleted. Silicate concentration fell from ca 40 μM to ca 1 μM in the autumn mesocosm in which the bloom of *S. costatum* was observed and from 29 μM to 20 μM in the spring mesocosm in which *Prorocentrum sp.* was dominant (Fig.5).

The dominant predators that increased in number as a result of the phytoplankton bloom in the autumn mesocosm were largely different from those in the spring mesocosm (Fig.6), implying that the mesocosms had different trophic relationships from each other. In the autumn mesocosm where diatoms were dominant, a rapid increase in appendicularians followed a gradual increase in copepods. Tracer experiments with inorganic ^{13}C showed that the percentages of ^{13}C transferred to >100-μm particles (i.e. the fraction of metazooplankton assemblages) increased in proportion to the increase in metazooplankton abundance, and the values were higher than 10% (Fig.6). Appendicularians are incapable of ingesting large diatoms 5) like *S. costatum*; thus, the high percentage of label transfer was probably mainly due to ingestion of diatoms by copepods. The diet of the appendicularians was likely to have included not only photosynthetic production but also bacteria to a large extent, because appendicularians are able to ingest pico- and nano-sized particles only. The proportions of organic ^{13}C tracer became markedly higher in the 20- to 100-μm and/or >100-μm fractions when appendicularians increased in number. It was also observed that dissolved organic carbon (DOC) concentration, numbers of bacterioplankton and uptake rate of dissolved organic ^{13}C (glucose) increased in the latter part of the autumn mesocosm experiment (Fig.8), implying that organic matter produced mainly by the diatom bloom decomposed comparatively rapidly, leading to an increase in bacterial production, which supported growth of the appendicularians.

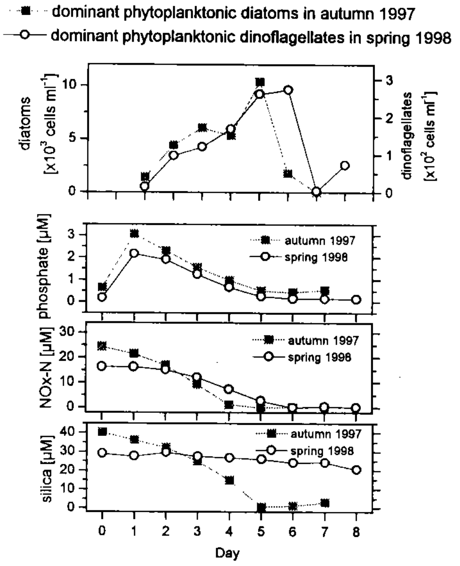

Figure 5. Changes in dominant phytoplankton and nutrients.

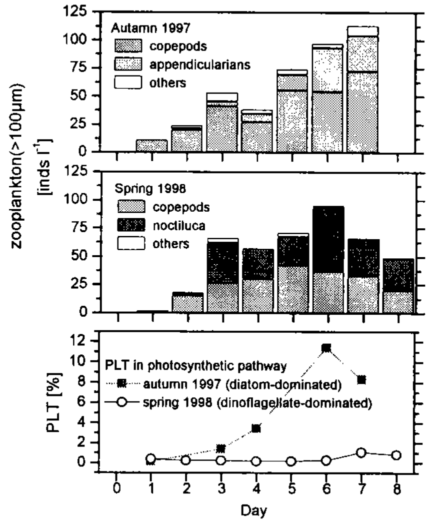

Figure 6. Dominant zooplankton (>100μm) and percentage of ^{13}C label transfer (PLT%)

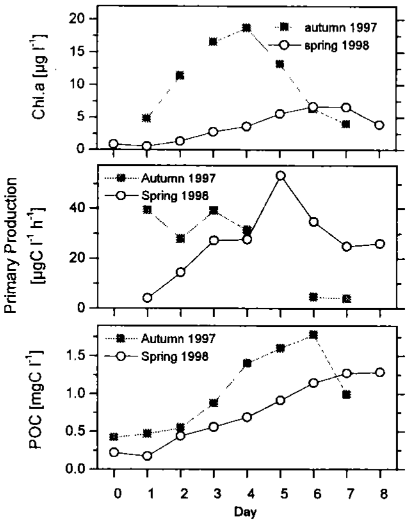

Figure 7. Changes in Chl.*a*, primary production and POC.

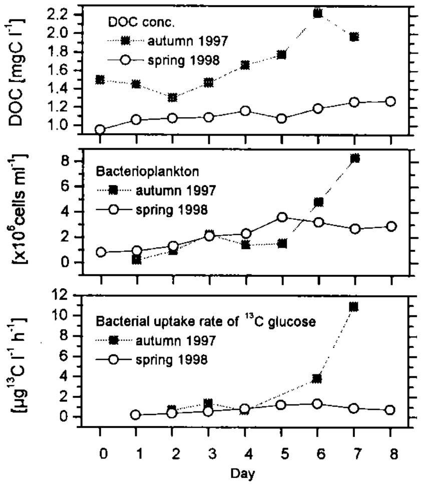

Figure 8. Changes in DOC, bacterioplankton and glucose.

In the spring mesocosm, where the phyto-dinoflagellate *Prorocentrum sp.* was dominant (Fig.5), copepods, a heterotrophic dinoflagellate (*Noctiluca scintillans*) and tintinnid ciliates increased in abundance (Fig.6). Tracer experiments with ^{13}C bicarbonate showed that carbon transfer to >100-μm particles (mainly copepods and *N. scintillans*) was very low (0.7 % maximum) in contrast with that in the autumn mesocosm where diatoms were dominant (Fig.8), suggesting that grazing pressure by copepods and *N. scintillans* on *P. cordatum* was low. The increases in DOC concentration, bacterioplankton numbers and uptake rate of ^{13}C glucose were also smaller than those in the autumn mesocosm. These results indicate that the rate of carbon cycling (e.g. primary production → grazer → decomposition → bacterial production → grazer) was slower in the *Prorocentrum*-dominated ecosystem than in that dominated by diatoms.

3) Conclusions

The results suggest that phytoplankton blooms can easily occur at the projected level of phosphorous increase in the Changjiang Estuary. Such blooms may be not only of diatoms but also of phyto-dinoflagellates, despite high concentrations of silicate in the Estuary. Control of phytoplankton blooms through grazing by metazooplankton may be more effective for diatoms than for phyto-dinoflagellates.

4. Fuel oil enrichment experiment

1) Materials and Methods

Two mesocosm systems (bag type, 25 m^3) were deployed for 7 days in the Estuary, one for oil enrichment (OE-meso) and the other the control (OC-meso). On 26 May 1998 (day 0), we enclosed the two mesocosm systems at almost the same time and added the same concentration of phosphate (ca. 1.5 μM), which was a limiting nutrient. After two days of observing the initial biological conditions in the mesocosms, the water-soluble fraction of diesel oil was added into OE-meso.

Samples were taken every morning for plankton enumeration and chemical analyses such as nutrient concentrations. The concentration and chemical composition of the oil were determined by the TLC-FID method. Photosynthetic production and bacterial activity in glucose uptake were determined by 4-h *in situ* incubation using ^{13}C bicarbonate and D-glucose, respectively. Carbon transfer from autotrophs and bacteria to large grazers was estimated by ^{13}C label transfer to large particles (>100 μm).

To determine the impact of oil addition on the producers more directly, two further batch-incubation experiments (Tests A and B) were performed as follows. Test A was conducted just before oil enrichment into OE-meso (day 2). Seawater samples were taken from both mesocosms and incubated with the ^{13}C-bicarbonate or ^{13}C-D-glucose. After 4 h, the incubated seawater was divided into two 2-L bottles and the water-soluble fraction of the oil was added to one at the same concentration as that in OE-meso. Incubation with and without the oil was continued for 16.5 h. Subsamples were taken after 2, 5.5 and 16.5 h and ^{13}C uptake by the particles was determined.

Test B was conducted on day 4 to examine the growth potential of the phytoplankton in both mesocosms, and consisted of *in situ* incubation with added nutrients (10 μM of nitrate and 1 μM of phosphate) using performed with ^{13}C-bicarbonate. Seawater samples were taken from the two mesocosms; each sample was divided into two 2-L bottles. The nutrients were added to one of the bottles and the incubation continued for 69 h. Subsamples were taken after 6, 21, 45 and 69 h and ^{13}C uptake into the particles was determined.

2) Results and Discussion

The plankton composition in the two mesocosms was quite similar initially: the phyto-dinoflagellate *Prorocentrum cordatum* was the most abundant as phytoplankton, while the heterotrophic dinoflagellate *Noctiluca scintillans* and micro-sized ciliate Tintinnid ciliate were the most abundant as grazers. Initial abundance of the plankton organisms was not the same in the two mesocosms. The density of phyto-dinoflagellates in OC-meso was about double than that in OE-meso. It was found that the rapid decrease of nutrients in OC-meso was due to the higher density of the phyto-dinoflagellates (Fig.9).

After the addition of oil on day 2, the DOC concentration in OE-meso increased from 1.1 mgC.L^{-1} (day 2) to 3.2 mgC.L^{-1} (day 3) and remained at a similar concentration until day 6. The

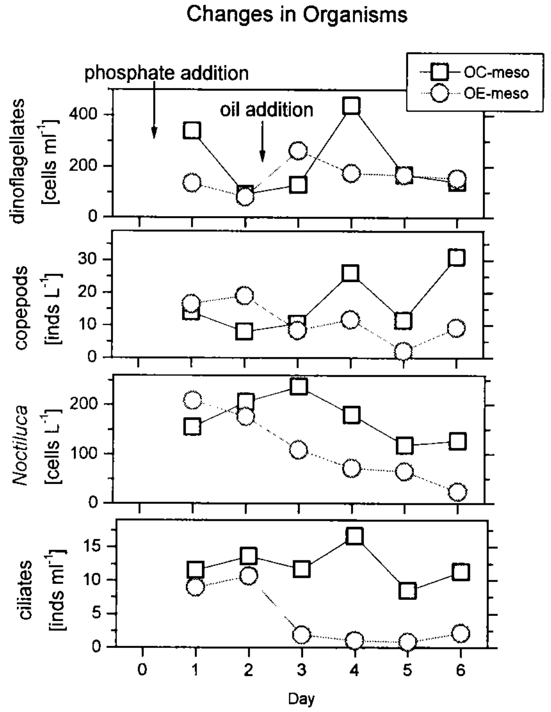

Figure 9. Changes in the abundance of dominant organisms in OC- and OE-meso.

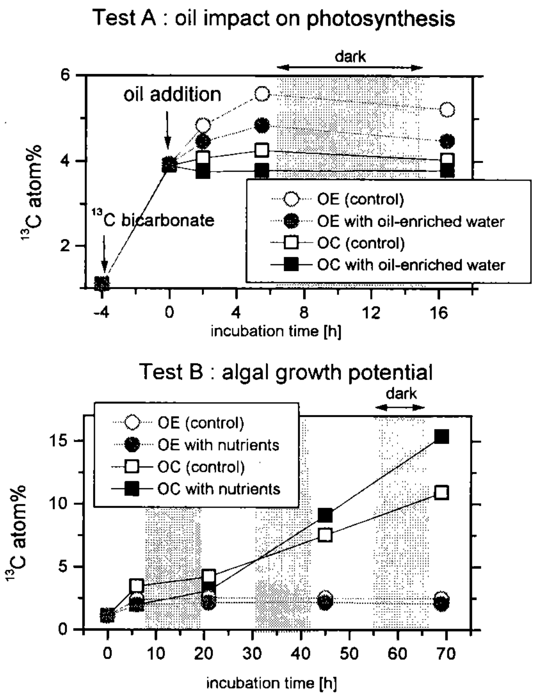

Figure 10. Results of Test A and Test B

TLC-FID measurements showed that water-soluble fraction of the diesel oil was mainly comprised of resin and asphaltene, and the total oil concentration was about 1.6 mg.L^{-1}.

After the addition of oil in OE-meso, the number of *N. scintillans* and nano- and micro-sized Tintinnid ciliate decreased, while the relative abundance of phyto-dinoflagellates, heterotrophic and autotrophic nanoflagellates and copepods remained similar to that in OC-meso (Fig.9). Photosynthetic production per unit Chl.*a* concentration was also similar in the two mesocosms after addition of oil to OE-meso (4.0 to 10.5 μgC.L^{-1}.h^{-1} per Chl.*a* in OC-meso and 5.0 to 8.6 μgC.L^{-1}.h^{-1} per Chl.*a* in OE-meso). Further, the percentages of ^{13}C-label transfer from autotrophs to the >100 μm particles were similar in the two mesocosms throughout the experimental period.

The above observations may imply that the addition of the water-soluble fraction of diesel oil did not influence the plankton community strongly, except for *N. scintillans* and ciliates. However, in Test A, it was found that ^{13}C-bicarbonate uptake decreased significantly after oil addition in both the incubation experiments using seawater taken from OC- and OE-meso, in comparison with ^{13}C uptake without oil addition. In Test B, nutrient addition was more effective for ^{13}C-bicarbonate uptake in the seawater from OC-meso than in the seawater from OE-meso. These experiments suggest that ^{13}C uptake (photosynthetic activity) was clearly influenced by the addition of oil, and that uptake in OC-meso was restricted mainly by nutrient concentration while photosynthetic activity in OE-meso was inhibited by the oil (Fig. 10).

3) Conclusions

The following results were obtained from this study: 1) from the changes in abundance of organisms, the ciliates and *N. scintillans* were more sensitive to the water-soluble fraction of diesel oil than the other organisms; and 2) the inorganic ^{13}C experiments revealed that photosynthetic activity was clearly affected by the addition of the water-soluble fraction of diesel oil in the mesocosm. These results suggest that oil pollution may have a direct impact on primary productivity in the Changjiang Estuary.

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