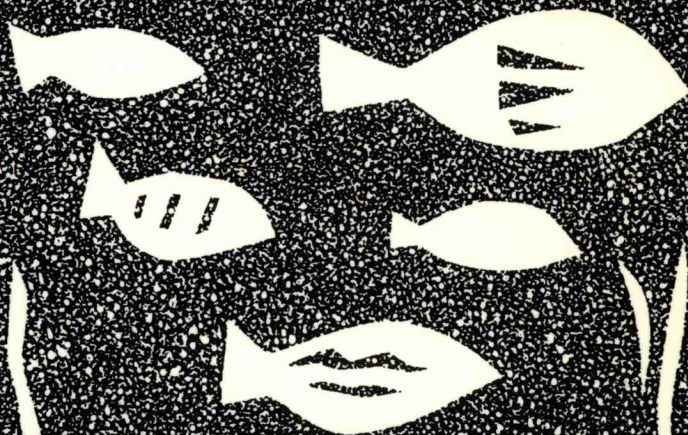
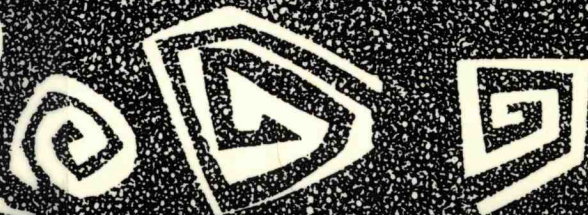


Marine Ecosystem Enclosed Experiments

Proceedings of a symposium held
in Beijing, People's Republic
of China, 9-14 May 1987



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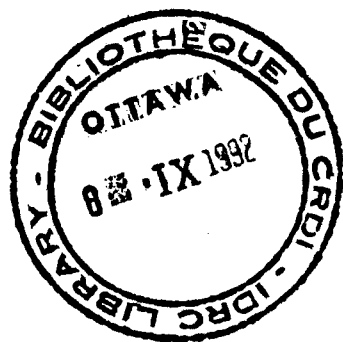


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Editor: C.S. Wong and P.J. Harrison



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Abstract

This symposium on marine ecosystem enclosed experiments (MEEE) consists of nine review papers that describe various types of ecosystem enclosures and a series of papers resulting from enclosure experiments in Xiamen, People's Republic of China, and Saanich Inlet, BC, Canada. The reviews on types of enclosures include benthic enclosures for rocky and sandy shores and the effects of pollutants (primarily hydrocarbons) on bacteria, macroalgae, and invertebrates. The pelagic enclosures were used to study the control of phytoplankton blooms, the uptake and release of dissolved organic substances, and the effects of pesticides on freshwater ecosystems.

Six enclosure experiments were conducted in China and Canada from 1986–87. Some of these experiments examined the effects of contaminated sediments, primarily heavy metals, on bacteria, phytoplankton, and zooplankton and the pathways and fates of these heavy metals in the seawater. Other experiments studied the chemistry and biological effects of chemically dispersed oil.

Résumé

Ce compte rendu du symposium sur les expériences faites en écosystèmes marins comprend neuf communications qui décrivent les écosystèmes retenus et les expériences faites à Xiamen en République populaire de Chine et à Saanich Inlet, C.-B., au Canada. Les communications portent, notamment, sur les écosystèmes benthiques des littoraux rocheux et sablonneux et sur les effets des polluants (surtout les hydrocarbures) sur les bactéries, les grandes algues et les invertébrés. Les expériences sur le contrôle des brutales pullulations ("blooms") du phytoplancton furent menées dans les écosystèmes pélagiques, ainsi que l'absorption et le dégagement des substances organiques dissoutes et les effets des pesticides sur les écosystèmes d'eau douce.

Six expériences ont été faites en Chine et au Canada entre 1983 et 1987. Certaines ont porté sur les effets des sédiments contaminés, principalement par des métaux lourds, sur les bactéries, le phytoplancton et le zooplancton et sur le cheminement et le sort de ces métaux lourds dans l'eau salée. D'autres expériences portaient sur la chimie et les effets biologiques du pétrole dispersé chimiquement.

Resumen

Este simposio sobre Experimentos Marinos en Ecosistemas Cerrados (MEEE) consistió en nueve trabajos de análisis que describen varios tipos de enclaustramientos ecosistémicos y una serie de trabajos derivados de experimentos con estos enclaustramientos en Xiamen, República Popular de China, y en Saanich Inlet, Canadá. Los estudios incluyen enclaustramientos bentónicos para costas rocosas y arenosas, y los efectos de los contaminantes (fundamentalmente hidrocarburos) sobre bacterias, macroalgas e invertebrados. Los enclaustramientos pelágicos se utilizaron para estudiar el control de la reproducción del fitoplancton, la ingestión y expulsión de sustancias orgánicas disueltas y los efectos de pesticidas en los ecosistemas de agua dulce.

Se realizaron seis experimentos en ecosistemas cerrados en China y Canadá, de 1983 a 1987. Algunos de estos experimentos examinaron los efectos que ejercen los sedimentos contaminados, fundamentalmente los metales pesados, sobre bacterias, fitoplancton y zooplancton, y el ciclo y destino final de estos metales pesados en el agua de mar. Otros experimentos estudiaron los efectos químicos y biológicos de los aceites crudos dispersados por medios químicos.

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Effect of Chemically Dispersed Crude Oil on the Distribution of Primary Microfouling Organisms

Lin Yanshun, Yao Ruimei, and Liang Ziyuan

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The impact of chemically dispersed crude oil on the formation and distribution of subtropical primary microfouling organisms was studied using marine ecosystem enclosures. Direct microscopic observations showed elevated numbers of bacteria in association with dispersed oil droplets. However, dispersed oil appeared to reduce the formation of the microfilm-slime layer and subsequent growth and attachment processes of macrofouling organisms.

Because oil spills influence physical, chemical, and biological processes in marine ecosystems, they are now recognized as a potential environmental hazard. Although the frequency of marine oil spills is increasing with new offshore developments and greater use of marine transport, little information is available on the effects of oil and oil dispersants on primary microfouling organisms. Thus, during a marine ecosystem enclosed experiment (MEEE) designed to evaluate the environmental impact of a crude oil and an oil dispersant on pelagic primary and secondary producers, changes in the distribution of primary microfouling organisms were also monitored.

Materials and methods

Four marine ecosystem enclosures (MEEs) were launched from a catamaran on 21 May 1986 in Xiamen Bay, People's Republic of China (MEEE Group 1986). The enclosed ecosystems were amended with nutrients ($\text{NO}_3:\text{SiO}_4:\text{PO}_4 = 15:10:0.5$) immediately after the launch. MEE-1 was designated as a "control" bag; thus it received no further treatment. Using a diffusion apparatus similar to that described by Topping and Windom (1977), 15 g of Corexit 9527 was added homogeneously to the top 3 m of MEE-2. In addition to the dispersant, the same depth zone of MEE-3 was injected with 3.7 MBq of $n\text{-(1-}^{14}\text{C)}\text{-hexadecane}$ and 150 g of Shengli crude oil. Except for the radioisotope addition, MEE-4 was an experimental replicate of MEE-3.

Glass microscope slides were used as the experimental substrate for micro-

fouling organisms. These slides, mounted in a wooden frame, were suspended at 1-m depth within MEE-1, MEE-2, and MEE-3, and at an "open water" site next to the enclosures. The slides were retrieved over a 20-d period. Upon retrieval, they were immediately refrigerated and transported to the laboratory within 2 h for analysis. Slides for microscopic identification and enumeration of bacteria and diatoms were fixed in 3% formalin.

A microscope fitted with epifluorescence and phase-contrast condensers was used for direct observation of oil droplet distribution. Size and morphology of bacteria were observed with a scanning electron microscope using samples that were desalted, dehydrated in ethanol, dried with Freon-13 at the critical point, and coated with gold.

Results and discussion

Formation and succession of microfouling organisms are influenced by a multitude of environmental factors, e.g., nutrient concentrations, temperature, salinity, contaminants, etc. Thus, it is not surprising that numerous species of microfouling organisms were observed during the experiment. After 1 d of immersion, elevated numbers of bacteria (cocci, bacilli, and vibrio) were found in association with dispersed oil droplets adhering to the surface of slides recovered from MEE-3 and MEE-4. Morphologically, these bacteria did not differ from indigenous microorganisms found on surfaces of other submerged objects recovered from the waters of Xiamen Bay. In addition to bacteria, microfouling organisms, including fungi, yeasts, and diatoms (Table 1), were also observed.

Bacteria, such as *Achromobacterium*, *Pseudomonas*, *Vibrio*, and *Acinebacter*, isolated during the experiment are known to have the potential to degrade hydrocarbons. The ability of yeasts and fungi to use hydrocarbons as sole sources of carbon and energy is a well-documented phenomenon and their isolation from oil-contaminated environments has suggested that they also play an important role in degrading oil spilled in the environment (Nyes et al. 1968; Ahearn and Meyers 1972; Cerniglia and Perry 1973). The primary colonizing species found in this study have frequently been isolated from submerged substrates found elsewhere (Corpe 1972; Walker and Colwell 1976). The predominant microfouling bacteria, *Pseudomonas* spp, are generally reported in experiments using liquid culture medium designed to isolate oil-degrading bacteria (Austin et al. 1977; Riquelme and Garcia-Tello 1986), and are known to have potentially high degradation rates for crude oil and its components (Cundell and Traxler 1973; Walker and Colwell 1976; Austin et al. 1977).

Colonization by filamentous bacteria, diatoms, and barnacles on glass slides recovered from MEE-3 and MEE-4 on day 4 was patchy and incomplete. In comparison with slides immersed in "open water" and in the "control" enclosure, it was evident that bacterial in the treated bags was stimulated by the dispersed oil. Bacteria that have the capacity to produce extracellular polysaccharides are generally the first microfouling organisms to inhabit the surface of submerged substrates, forming a slime layer in a matter of days.

Laboratory experiments with batch cultures have demonstrated that the proportion of bacteria that adhere to the walls of the culture vessel increases as the

Table 1. Bacteria, filamentous fungi, yeasts, and diatoms observed on the surface of glass slides recovered from MEEE enclosures.

Bacteria	
<i>Achromobacter</i> spp	<i>Pseudomonas</i> spp
<i>Acinebacter</i> spp	<i>Vibrio</i> spp
Yeasts	
<i>Candida</i> spp	<i>Cryptococcus</i> spp
<i>Citermyces</i> spp	<i>Schizosaccharomyces</i> spp
Fungi	
<i>Aspergillus</i> spp	<i>Mecelia sterilla</i>
<i>Clodosporium</i> spp	<i>Penicillus</i> spp
<i>Dendryphon</i> spp	<i>Torula</i> spp
Diatoms	
<i>Achnanthes brevipes</i>	<i>Fleueosigma rhombeum</i>
<i>Amphora angusta</i>	<i>Grammatophora marine</i>
<i>Amphora coffaeiformis</i>	<i>Licmophora flabellata</i>
<i>Bacteriastrum</i> spp	<i>Mastogloia fascistriata</i>
<i>Biddulphia mobiliensis</i>	<i>Melosira nummuloides</i>
<i>Biddulphia reticulata</i>	<i>Navicula comcellata</i>
<i>Caloneis formosa</i>	<i>Navicula marine</i>
<i>Cocconeis pellucida</i>	<i>Nitzschia obtusae</i>
<i>Cocconeis heteroidea</i>	<i>Nitzschia paleacea</i>
<i>Cyrisigma balticum</i>	<i>Nitzschia panduriformis</i>
<i>Diploneis bombus</i>	<i>Rhizosolenia styliformis</i>
<i>Diploneis incurvata</i>	<i>Stauroneis constricta</i>
<i>Fleueosigma naviculaeum</i>	<i>Trachyneis aspera</i>

numbers of free bacteria in solution increase. Stimulation of bacterial growth by Corexit-dispersed crude oil has also been demonstrated in marine mesocosm studies conducted by Lee et al. (1985). In the present experiment, between days 2 and 6, enhanced growth of unattached and primary microfouling bacteria due to Corexit-dispersed oil was observed simultaneously on the glass slides. Furthermore, observations of the close association of bacteria and oil droplets suggest a positive chemotactic response by bacteria to the oil or rapid growth by the oil-degrading bacteria, or both.

Nutrient limitation by nitrogen and phosphorus has been recognized as a major factor controlling bacterial growth in the marine environment, especially in situations where carbon is in great excess after an oil spill. Stimulation of bacterial growth in the present experiment may be attributed to nutrient enrichment and dispersive action by Corexit 9527, which decreases clumping of cells, thus maximizing the surface area available for nutrient absorption. Although zooplankton grazing may have a significant effect on the development of microbial microfouling communities, this process was not assessed during the present experiment.

A large number of benthic and attached diatoms frequently found in the waters of Xiamen Bay were observed on the surfaces of the recovered glass slides (Table 2). Although the close association between the distribution of diatoms and

Table 2. Numbers of major macrofouling organisms observed on the surface of glass slides recovered after 12 d of exposure.

Species	Open water	MEE-1	MEE-3	MEE-4
<i>Tubularia</i> spp	4	1	0	0
<i>Oystrea</i> spp	3	1	0	0
<i>Balanus</i> spp	3	1	0	0

oil droplets observed under phase-contrast microscopy in the present experiment could be regarded as a chemotaxic response of diatoms to oil, it could also be attributed to physicochemical interactions between diatom surface polysaccharides and dispersed oil. Results obtained in arctic waters by Hsiao (1978) suggest that oil spills would result in phytoplankton communities dominated by flagellates because of the sensitivity of diatoms to oil. In contrast to these results, stimulatory effects of dispersed crude oil on the growth of bacteria (Fig. 1) and diatoms (Fig. 2) were observed in our experiment. Unlike bacteria (Fig. 1), however, an immediate stimulatory response by diatoms to dispersed oil was not observed on day 1 (Fig. 2).

Previous experiments on the ecology of microfouling organisms in Xiamen Bay demonstrated a two-tier fouling phenomenon in which the organisms form a dis-

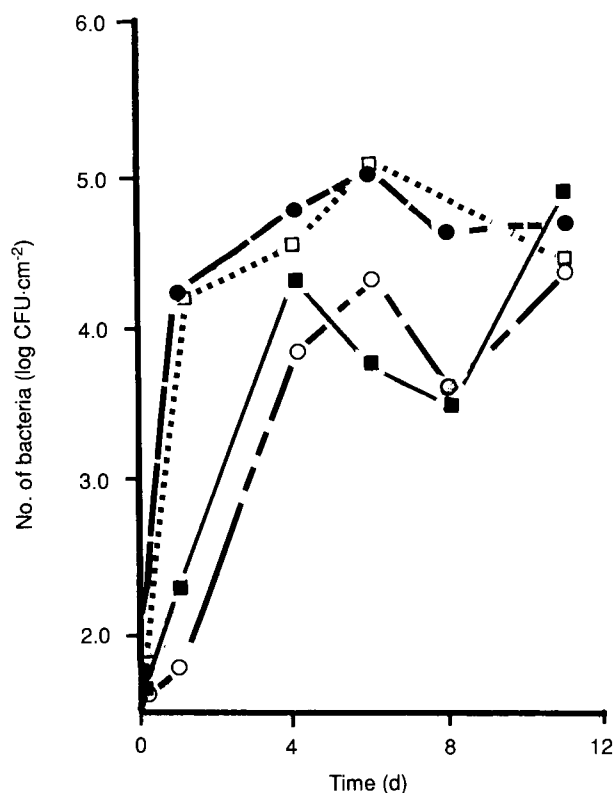


Fig. 1. Heterotrophic bacteria observed on the surface of glass slides recovered from MEE-1, MEE-3, MEE-4, and "open-water."

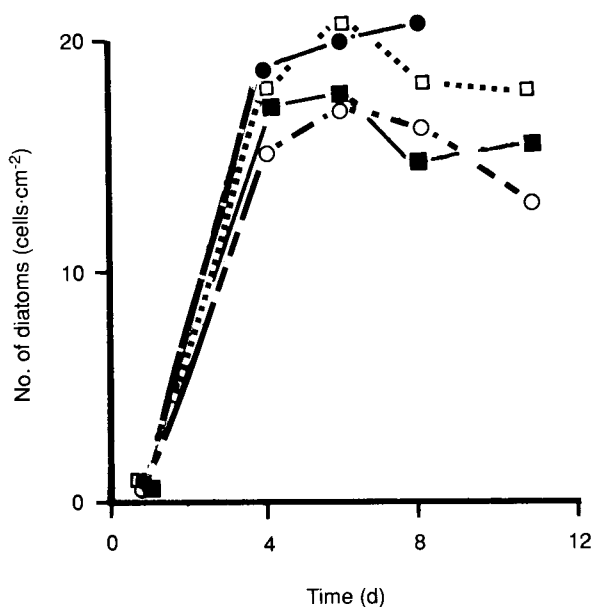


Fig. 2. Diatoms attached to the surface of glass slides recovered from MEE-1, MEE-3, MEE-4, and "open water."

tinct microfilm-slime layer within 7 d. In the present experiment, formation of such a layer was observed on slides immersed in the "open water" after 7 d. Because of seasonal and geographic variations, however, the observed slime layer was not as thick as expected. Microfilm formation was slower on the slides recovered from MEE-3 and MEE-4 compared with that on slides recovered from MEE-1 and the "open water." Extensive development of a slime layer was not observed on slides exposed to dispersed oil because of physiochemical alterations of the surface microlayer or growth inhibition induced by the Corexit-dispersed oil.

Zobell and Allen (1935) noted that bacteria and, to a lesser extent, other microorganisms (fungi, diatoms, etc.) are the organisms that colonize submerged surfaces during the early stage, thereby forming a primary film. They are followed by macroscopic organisms that belong to the primary successional stages. In the present experiment, after 4 d of immersion in "open-water" conditions, macrofouling organisms (predominantly bryozoans) covered 10–15% of the surface area of the glass slides, greatly impeding further development of microfouling films. After 7 d, species found on the slides included barnacles and oysters. The numbers and diversity of macrofouling organisms were much greater on slides immersed in "open water" than those immersed in the "control" enclosure (Table 2). In contrast, larval and adult forms of macrofouling organisms were not observed on the slides suspended in MEE-3 or MEE-4 during the experiment, suggesting that dispersed oil inhibited the growth or attachment mechanism, or both, of the macrofouling organisms.

In many aquatic ecosystems, particularly when the water is low in nutrients, surfaces colonized by microorganisms are sites of relatively intense biological activity. Consortia composed of attached microalgal and bacterial populations have essential roles in aquatic ecosystems, acting as sites of active nutrient regeneration (Loeb

and Rueter 1981; Rueter et al. 1983) and serving as a trophic resource for other organisms (Cattaneo 1983). Attached biofilm communities are also relevant to industry, because microbial fouling is associated with corrosion and reduces the efficiency of industrial processes (Characklis and Cooksey 1983).

With the expected increase in the incidence of oil spills associated with coastal and offshore developments globally, it is evident that further studies on these processes should be conducted.

Acknowledgments

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