Marine Ecosystem Enclosed Experiments

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

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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 Sannich 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 substancias 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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Use of n-(1-14C) Hexadecane to Study the Fate of Dispersed Crude Oil in Marine Enclosed Ecosystems

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The fate of Corexit-dispersed crude oil in an enclosed experimental ecosystem in Xiamen Bay was studied using $n-(1^{-14}C)$ hexadecane as a tracer. The removal of crude oil was a two-step process. The first step, from day 1 to day 7, was a fast process, with a removal rate of 0.37% d⁻¹ characterized by nutrient limitation. The removal half-life was 2 d. The time required for forming organic floc was estimated to be 7 d, a 4-d delay from the bacterial bloom. The second step, from day 7 to day 15, was a slow process with a removal rate of 0.17% d⁻¹. The removal half-life was 4 d. Compared with the first step, dominated by heterotrophic bacteria, the mechanism of oil removal during the second step was much more complicated, probably because of changes in the environmental conditions of the seawater.

The study indicated that residual ${}^{14}C$ of suspended particulate matter, sediment, and dissolved organic carbon amounted to 3.4, 12.4, and 1% of the total tracer added respectively. The release of ${}^{14}CO_2$ was 25% of the added tracer. Considering its escape to the atmosphere and assimilation by phytoplankton, this value should be much higher. The experimental ecosystem, when contaminated with a limit of 15 ppm crude oil with a dispersant, was able to remove the oil by natural processes within 2 weeks.

Recently, studies of transfer processes and the fate of crude oil in the ocean have received considerable attention (Gearing et al. 1979; Lee 1980). In cleaning up Corexit-dispersed oil, biodegradation appears to be more important than abiotic processes (Cretney et al. 1981; Lee et al. 1985). However, some questions remain to be answered. For example, how to monitor the effects of biological processes on the transfer of crude oil? How to estimate the rate of oil removal? How much is primary productivity suppressed by oil pollution? What is the capability of a marine ecosystem in terms of the natural removal of crude oil? The use of radio-isotopic techniques allows scientists to trace the pathways of crude oil by determining degradation products in the experimental ecosystem.

This study forms part of the marine ecosystem enclosed experiments (MEEE) sponsored jointly by Canada and the People's Republic of China.

Methods

Sampling

The experimental site was located at the eastern end of Xiamen Bay where the local current reaches 3 km·h⁻¹. Four double-layered polyethylene bags (2 m in diameter × 6 m long) were filled with seawater. On 23 May 1986, one of the enclosures was injected with 150 g of Shengli crude oil (about 15 mg·L⁻¹), 15 g of Corexit 9527 (about 1.5 mg·L⁻¹) and 3.7 MBq of n-(1-¹⁴C) hexadecane (about 370 Bq·L⁻¹) in the upper 3 m of seawater using a sprayer. Seawater was sampled using a 2.5-L Niskin sampler at 0, 1.5, 2.5, and 4 m depth for tracer analyses.

Analyses

¹⁴C in suspended particulate matter

Suspended particulate matter (SPM) in each 500 mL of seawater sample was filtered onto either a 47-mm Millipore filter (0.45 μ m) or a 47-mm Nuclepore filter (3 μ m or 8 μ m) at a suction pressure of about 34 kPa. The filter and its content were then placed in a vial containing 10 mL of Aquasol. All ¹⁴C samples were counted in a liquid scintillation counter (Packard, Tri-Carb 4640).

¹⁴C in sediment

A 25-mL subsample of the sediment collected was mixed thoroughly. From this, a 1-mL aliquot was withdrawn and placed into a scintillation vial containing 10 mL of Aquasol. It was then counted as described above.

¹⁴CO₂ measurement

A 500-mL gas-washer bottle with stopper was filled with seawater from the Niskin sampler. The bottle was connected with the air-circulating loop shown (Fig. 1). Before turning on the air pump, the sample was acidified with 1 mL of concentrated H₂SO₄. Air was bubbled through the sample solution to purge CO₂ gas into a scintillation vial containing a 5-mL solution of SrCl₂ in NH₄OH (25% w/w). The CO₂ was absorbed over 15 min to form a precipitate of SrCO₃. A pinch clamp was used to control the bubbling rate to avoid "boiling" the sample. The scintillation vial was heated slowly to remove the NH₄OH until 2 mL of solution remained. This was mixed with 10 mL of Aquasol to form a homogeneous gel. The production of ¹⁴CO₂ was computed using the equation

[1] ${}^{14}\text{CO}_2 (\text{dpm} \cdot \text{L}^{-1}) = 2(n_{\text{s}} - n_{\text{b}})/(E_1 E_2)$

where n_s is the counting rate of the sample (cpm), n_b is the counting rate of the blank (cpm), E_1 is the counting efficiency corrected for quenching, and E_2 is the chemical recovery efficiency. (Note: dpm = disintegrations per minute = 60 Bq.)



Fig. 1. Closed air-circulating loop.



Fig. 2. Changes in suspended particulate ¹⁴C in the water column.

¹⁴C in dissolved organic carbon

The filtrate collected from the procedure followed to determine the ¹⁴C in SPM using a 0.45- μ m filter was extracted twice by shaking the sample with CH₂Cl₂ in a 1 000-mL separatory funnel. Volumes of CH₂Cl₂ used were 20 and 10 mL, respectively, with each extraction lasting 5 min. Both extracts were combined into a 50-mL graduated test-tube. The CH₂Cl₂ was evaporated at room temperature to a final volume of about 3-mL by bubbling nitrogen gas through the extract. For counting, 2-mL of the extract was used with 10-mL of Aquasol.

Bacterial productivity

The productivity of bacterioplankton was estimated using the method described by Fuhrman and Azam (1982) and Parsons et al. (1984). In this method, the amount

			Integrated amount			
Day	Date	0	1.5	2.5	4.0	(× 10 ⁶ dpm)
1	23 May	23 047			_	
2	24 May	10 207	9 004	8 939	8 314	114.5
3	25 May	6 702	6 634	7 082	6 776	85.4
4	26 May	4 391	4 455	4 700	4 695	57.3
5	27 May	3 391	3 520	2 775	3 902	42.7
7	29 May	1 080	1 510	1 359	1 653	17.6
9	31 May	884	902	1 135	1 052	12.5
12	3 June	613	601	582		7.5

Table 1. Distribution of suspended particulate matter ${}^{14}C$ (suspended, 0.45- μ m filter retained, dpm·L⁻¹) in the water column.

of (methyl-³H)-thymidine incorporated was converted to the total number of bacteria for determining heterotrophic production.

Results and discussion

Distribution of ¹⁴C in the water column

The vertical distribution of SPM ¹⁴C in the enclosure is shown in Fig. 2. On day 2, the average level of SPM ¹⁴C was 9 100 dpm·L⁻¹. It was higher in the surface but lower in the deeper layer. The concentration decreased rapidly to 4 560 dpm·L⁻¹ on day 4 and to a low of 600 dpm·L⁻¹ on day 12. At the end of the experiment, the integrated amount of SPM ¹⁴C remaining in the water column was 7.5×10^6 dpm, which was only 3.4% of the total tracer added (Table 1). The distribution curve shifted in a well-defined front, indicating a well-mixed condition in the enclosure.



Fig. 3. Changes in ¹⁴C in the water column.

The variability of ¹⁴CO₂ production in seawater is shown in Fig. 3. From day 2 to day 7, the integrated amount of ¹⁴CO₂ increased, reaching a peak of 54.53×10^{6} dpm on day 7, which was 24.6% of the total tracer added (Table 2). After day 7, production of ¹⁴CO₂ decreased and spread evenly throughout the water column in the same way as SPM.

The amount of dissolved organic carbon (DOC) was very low (Table 3), mostly less than 1% of the total tracer. Even though the maximum integrated DOC was 1.56×10^6 dpm on day 5, it represented only 3.5% of the SPM plus DOC in the water. As the sediment samples were collected every day, the values of ¹⁴C in the sediment were the sums of all the daily counts. Counting results of sediment ¹⁴C (Table 4) showed a maximum of 10.84×10^6 dpm on day 7. A total of 27.6×10^6 dpm of sediment ¹⁴C was attained, which was 12.4% of the total tracer added. Thus, 10% of the labeled hexadecane had sunk to the bottom.

Estimation of the crude oil removal rate

The actual production of ${}^{14}CO_2$ might be much higher than the concentration detected as some CO₂ probably escaped to the atmosphere or was assimilated by

			De	epth (m)		Integrated amount	Bacterial productivity $(\times 10^6 \text{ cells})$
Day	Date	0	1.5	2.5	4.0	(× 10 ⁶ dpm)	$L^{-1} \cdot h^{-1}$
1	23 May	_					64
2	29 May	1 162	1 771	1 076	1 282	16.65	318
3	25 May	3 693	2 412	2 296	2 775	35.11	373
4	26 May	2 595	2 585	3 178	2 416	33.85	334
5	27 May	5 854	3 065	2 336	4 795	50.42	309
7	29 May	5 888	4 323	4 623	3 223	54.53	213
9	31 May	2 939	3 980	1 638	1 756	32.40	321
12	3 June	1 721	1 769	6 994	_		224
15	6 June	—	—		—		142

Table 2. Released ${}^{14}CO_2$ (dpm·L⁻¹) and bacterial productivity.

Table 3. Dissolved organic ${}^{14}C$ (dpm·L⁻¹) in the water column.

			Dep	oth (m)	Integrated amount	% of total	% of SPM +	
Day	Date	0	1.5	2.5	4.0	(× 10 ⁶ dmp)	tracer	DOC
2	23 May	164	_	_		_	_	
2	24 May	27	43	16	0	0.27	0.1	0.2
3	25 May	4	0	31	20	0.17	0.1	0.2
4	26 May	35		31	35	0.41	0.2	0.7
5	27 May	92	43	359	4	1.56	0.7	3.5
7	29 May	12	16	4	0	0.10	0.04	0.6
9	31 May	35	50	0	69	0.48	0.2	3.7

Note: SPM, suspended particulate matter; DOC, dissolved organic carbon.

Day	Date	Sediment ^{14}C (× 10 ⁶ dpm)	Integrated amount (× 10 ⁶ dmp)
2	24 May	0.13	0.13
3	25 May	2.20	2.23
4	26 May	7.48	9.81
5	27 May	2.30	12.11
7	29 May	10.84	22.95
9	31 May	3.34	26.29
12	3 June	1.31	27.60

Table 4. Sediment ¹⁴C and its integrated amounts.

phytoplankton. If only measured ¹⁴CO₂ data were used, the removal rate of crude oil from the water column might be underestimated. Similarly, decomposition in the sediment might also have occurred, and the DOC values were too low to be used for evaluation. Thus, the SPM ¹⁴C data might provide a better source for estimating the removal rate. The integrated amount of SPM ¹⁴C varied with time as two exponential functions separated on day 7 (Fig. 4). The whole transfer processes was divided into two steps to obtain the respective removal rates. In practice, the exponential function was expressed as

$$[2] \quad A_t = A_0 \exp(-kt)$$

where A_0 and A_t are the integrated SPM ¹⁴C on day 0 and day *t*, respectively, and *k* is the removal rate. After logarithmic transformation, the following equation, is derived:

[3]
$$\ln(A_t) = \ln(A_0) - kt$$

in which the removal rate can be calculated from the slope of the line (Fig. 5). As a result, the removal rates determined for the two steps are:

$$k_1 = 0.37 \,\mathrm{d}^{-1} (r^2 = 0.99, n = 5)$$

and

$$k_2 = 0.17 \text{ d}^{-1} (r^2 = 0.99, n = 3)$$

Both estimates were within the 95% confidence intervals. The results indicated that there were significant differences in dynamics between the two steps. For oil discharge within a limit of 15 ppm, the removal rate was independent of the initial level of crude oil. The coefficients of correlation (r^2) of equation [3] were both 0.99, suggesting that the SPM ¹⁴C was a good estimator of the rate.

The removal half-life, $T_{0.5}$, referred to the time required for one-half of the n-(1-¹⁴C) hexadecane in SPM to sink to the bottom, escape to the atmosphere, or change to soluble forms. From equation [2], A_t can be set equal to one half of A_0 to obtain

 $[4] \quad T_{0.5} = \ln(2)/k$

which gives $T_{0.5} = 2$ d and $T_{0.5} = 4$ d for the two steps respectively.



Fig. 4. Integrated amounts of suspended particulate matter (SPM) ¹⁴C and ¹⁴CO₂ compared with bacterial productivity.



Fig. 5. Changes in log-transformed ¹⁴C in suspended particulate matter (SPM).

Mechanism of oil-transfer process

As soon as crude oil was added to the enclosed ecosystem, the new carbon source would stimulate the activity of bacteria. Bacterial productivity increased rapidly from 64×10^6 cells·L⁻¹·h⁻¹ on day 1 to 373×10^6 cells·L⁻¹·h⁻¹ on day 3 (Table 2). At the same time, ¹⁴CO₂ production increased from 16.65 × 10⁶ dpm to 35.11×10^6 dpm following the same trend. Although bacterial growth decreased from day 3 to day 7, the production of ¹⁴CO₂ did not slow down at all, reaching a peak of 54.53×10^6 dpm on day 7 (Fig. 4). While SPM ¹⁴C decreased, DOC ¹⁴C increased, reaching its maximum of 1.56×10^6 dpm on day 5, and sediment ¹⁴C reached its maximum of 10.84×10^6 dpm on day 7.

Previous research on the fate of oil in the ocean has focused on the effects of bacterial attachment on organic particulates (Lee 1977). Results from the present study showed that at the 2.5-m layer on day 5, SPM ¹⁴C retained on the 3- μ m filter was about 70% of the total particulate observed (Table 5a). On day 7, the amount of SPM retained was 60% of the total (Table 5b). These observations indicated the formation of organic flocs. The first step was a rapid process. Biodegradation was found to be predominant. Peak ¹⁴CO₂ production appeared 4 d after the bacterial bloom, suggesting that it took only a few days for the bacteria to digest the oil droplets. Similar results were obtained in a study conducted in Canada.

The experimental ecosystem maintained a high level of dissolved silicate throughout the first removal step. This level of dissolved silicate decreased abruptly, however, at the beginning of the second step. There were considerable amounts of nitrate, nitrite, and phosphate during the first 3 d, thereafter, however, these amounts dropped to near background levels (Lin, Y., et al., this volume). Primary productivity decreased temporarily after the addition of crude oil, but increased on day 3, only to decrease later due to nutrient limitation (Chen et al., this volume).

The second removal step was a slow transfer process. On day 9, the size of SPM at the 2.5-m layer was mainly in the range of 0.45--3 μ m, and only 27% of the material was >3 μ m. The integrated amount of ¹⁴CO₂ production on day 9 was only 14.6% of the total tracer added. High concentrations of ammonium (3.9 μ g·L⁻¹) also appeared on day 9 (Lin, Y., et al., this volume) in contrast to other nutrients, suggesting a shift to a more reductive ecosystem. Although salinity remained relatively stable during the first step, it decreased significantly after day 11, perhaps due to changes in environmental conditions. Corresponding with the other peak of

			0.45 μn	1		3 µm		8 µm			
Day	Date	SPM	DOC	Total	SPM	DOC	Total	SPM	DOC	Total	
2	24 May	8 939	16	8 955					_		
3	25 May	7 082	31	7 113							
4	26 May	4 700	31	4 731							
5	27 May	2 775	359	3 134	1 840	811	2 651	435	358	793	
7	29 May	1 359	4	1 363	772	542	1 314	107	176	283	
9	31 May	1 135	0	1 135	305	122	427	187	161	348	
12	3 June	582			154	145	299	159	179	338	

Table 5a. Fractionation of suspended particulate matter ¹⁴C (dpm·L⁻¹) and dissolved organic ¹⁴C (dpm·L⁻¹) at a depth of 2.5 m.

Note: SPM, suspended particulate matter; DOC, dissolved organic carbon.

Table 5b. Normalization of the fraction of suspended particulate matter ^{14}C at a depth of 2.5 m.

Day	Date	0.45 μm	3 µm	8 µm
5	27 May	1	0.66	0.16
7	29 May	1	0.57	0.08
9	31 May	1	0.27	0.16
12	3 June	1	0.26	0.27

bacterial production on day 9, primary production also increased to a second maximum, but then decreased rapidly to lower levels. The process controlling the transfer of oil during the second step might be more complicated. The abnormally high $^{14}CO_2$ in the 2.5-m layer on day 12 (Table 2) probably resulted from decomposition of the oil found on the inner wall of the enclosure or contamination of the sampler.

Conclusions

It took about 2 weeks for the total transfer of crude oil dispersed with Corexit 9527. The whole process could be divided into two steps, each lasting about 1 week. The removal rate during the first step was $0.37 d^{-1}$, with a removal half-life of 2 d. The first step was predominantly controlled by bacterial activity. The time required for the formation of organic flocs was estimated to be about 7 d, a delay of 4 d after the bacterial bloom. The removal rate for the second step was $0.17 d^{-1}$, with a half-life of 4 d. The process for oil transfer during the second step was more complicated, probably because of changes in environmental conditions.

Residues of SPM ¹⁴C, sediment ¹⁴C, and DOC ¹⁴C were 3.4, 12.4, and 1% of the total tracer added respectively. Production of ¹⁴CO₂ should be much higher than the measured 25% of the total tracer. The other "unrecovered" ¹⁴C might be in crude oil that adhered to the inner wall of the enclosure, in some metabolic gases that escaped from the ecosystem, or in biodegradation products that are inextractable by CH₂Cl₂ such as carboxylic acids or polar organic matter.

Up to a limit of 15 ppm of oil addition and in the presence of dispersant, the marine enclosed ecosystem was able to transfer the oil totally by natural processes within 2 weeks.

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