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Experiments with Large Enclosed Ecosystems [and Discussion]

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Experiments with large enclosed ecosystems

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Three of the major advantages of enclosure experiments are that they ensure (1) that the same populations are sampled over a long period; (2) that populations of at least three trophic levels are initially enclosed in naturally occurring proportions and that they are self sustaining over a long experimental period; and (3) that replicate enclosed populations can be experimentally manipulated.

There are two disadvantages which must be mentioned. These are (1) that vertical mixing, which may be reduced by as much as an order of magnitude compared to the open sea, will undoubtedly affect the sinking rates of phytoplankton and may influence the structure of the population; and (2) that as a general rule the larger and therefore more expensive the enclosures become, the more difficult it is to run sufficient replicates.

An experiment is described in which 1 μg Hg/l was added to two 95 m^3 bags (3 m diameter by 17 m deep) and the response of the pelagic population monitored over the following 20 days. A further 10 μg Hg/l was then added to each enclosure and the response measured for a further 20 days. The results indicated that:

(i) inorganic mercury added to the water column is very rapidly transformed into 'bound' or 'non-reactive' mercury and that about 25 % of the mercury added was recovered associated with the organic material settling to the bottom of the bags;

(ii) the response of the biological population to 1 μg Hg/l was very limited and in fact a transient reduction in photosynthetic carbon uptake per unit chlorophyll was the only noticeable effect and there were no changes in population size or structure that could be attributed to mercury;

(iii) at 10 μg Hg/l the zooplankton population was reduced markedly and this did produce changes in the structure of both the zooplankton and phytoplankton populations.

These results are similar to the results of a comparable experiment carried out in Vancouver Island (Cepex) and point to the conclusion that the levels of mercury found in surface waters around the coast of the U.K. (0.001–0.022 μg Hg/l) are one or two orders of magnitude below the levels at which a response of the biological population can be demonstrated.

The usefulness of large scale enclosed ecosystems for further pollution research is discussed and it is concluded that those facilities that provided a link between the water column and the sediments would be most useful since they would (1) enable estimates to be made of the flux rates of pollutants from the water column to the sediments; and (2) allow experiments to be carried out with the pollutant in contact with sediment in its natural form.

Introduction

A major problem in marine ecosystem research is inability to sample the same water mass for a prolonged period and hence to measure with any degree of certainty the dynamics of events both within and, more especially, between trophic levels. It is possible to follow plankton patches over a long period as did Cushing (1963) with *Calanus* but here, due to the interaction between the vertical migration of the copepods and the inherent natural patchiness of the pelagic ecosystem (see Riley 1976; Enright 1977) it is highly unlikely that the copepods were in the same water mass throughout the exercise. The international Flex 76 exercise in the North Sea was a recent attempt to circumvent the problem. However, this necessitated the cooperation

of several nations with many research vessels and even aircraft to effect a synoptic study. The alternative is to use numerical modelling techniques and indeed there has been a recent growth of effort in this approach. However, no matter how realistic the model, it requires verification by field observation and, if possible, by experimental manipulation. Hence, with the latter need most specifically in mind, the technique of isolating water masses containing natural populations has been developed. This technique is not new; indeed on a very small scale the laboratory chemostat comes into the definition, but essentially what we are considering is an isolated self-sustaining natural water mass containing several trophic levels. The first large container in the sea was developed by Strickland and coworkers in the early 1960s (Strickland & Terhune 1961; McAllister *et al.* 1961; Antia *et al.* 1963), and about 1970 there was a great increase in activity not only in marine but also in freshwater systems (Lack & Lund 1974).

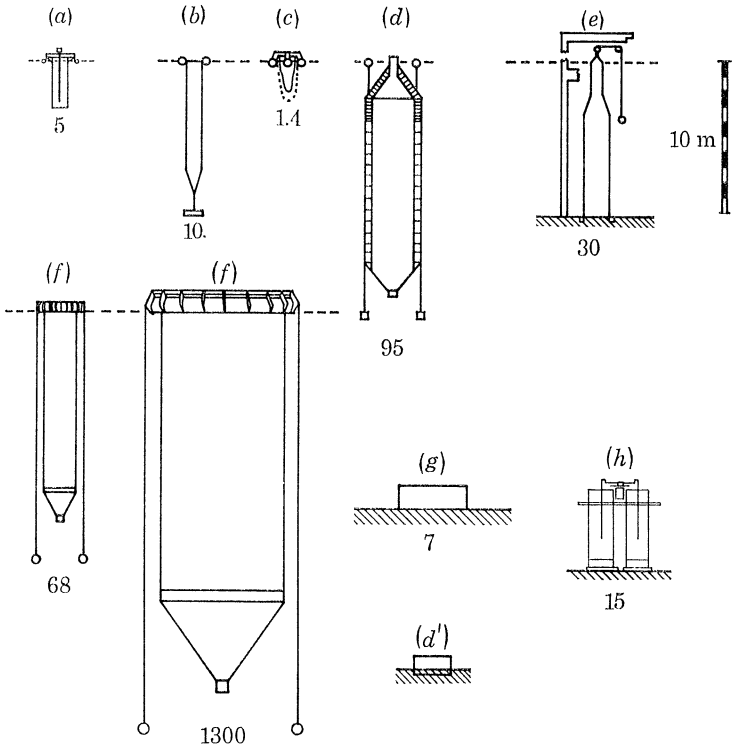

FIGURE 1. Marine enclosure designs, capacity indicated in cubic metres, after Zeitzschel (1978). (*a*) Brockmann *et al.* (1974), Helgoland; (*b*) Berland *et al.* (1975), Marseilles; (*c*) Kuiper (1977), Den Helder, Netherlands; (*d*) Gamble *et al.* (1977), L. Ewe, Scotland (*d'*, benthic chamber); (*e*) von Bodungen *et al.* (1976), Kiel; (*f*) Menzel & Case (1977), Cepex, British Columbia; (*g*) Saward *et al.* (1974), L. Ewe; (*h*) Pilson *et al.* (1977) M.E.R.L., Narragansett Bay, Rhode Island.

In most of the marine experiments the initiating stimulus was the potential of the enclosure technique for the investigation of the effects of pollutants. Several reviews have already been published. Reeve *et al.* (1976) have paid particular attention to zooplankton in enclosure pollution experiments while Menzel & Case (1977) and Menzel (1977) have discussed the U.S. I.D.O.E.-funded Controlled Environment Pollution Experiment (Cepex) on the sublethal effects of copper. A virtual preview of the Cepex mercury experiment has been given by Grice *et al.* (1978) while Oviatt *et al.* (1977) have presented a summary of the rationale for enclosure research. However, these reviews are rather specific and, most recently, Zeitzschel (1978) and

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Steele & Menzel (1978) have presented more definitive assessments and conclusions. We will concentrate in this paper on presenting the results of our own experiment on the sublethal effects of mercury and consider the results in relation to those obtained in the Cepex experiment and the current understanding of the effects of mercury in the marine environment. In this way we hope to highlight some of the advantages and disadvantages of using enclosures.

Steele & Menzel (1978) conclude that the three main advantages of enclosures are in ensuring (1) that the same populations are sampled over a long period; (2) that populations of at least three trophic levels are initially enclosed in naturally occurring proportions and that they are self sustaining over a long experimental period; and (3) that replicate enclosed populations can be experimentally manipulated.

Enclosure design, to date, can be divided into three categories; land tanks, in-situ tubes and in-situ bags (figure 1). They have all been produced in a range of sizes and each has its advantages and disadvantages. The most extensive land-based facility is at Narragansett Bay, Rhode Island, where the Marine Ecosystems Research Laboratory (M.E.R.L.) has recently been set up (Pilson *et al.* 1977) and uses of twelve 13 m^3 capacity tanks. These have elaborate temperature controls and stirrers and, being free standing, can be used to study the interaction between benthic and pelagic components. The largest land-based tank is probably the single Scripps Institution deep tank which contains 70 m^3 of sea water and has been used in the study of population interactions (Strickland *et al.* 1969; Mullin & Evans 1974).

Land-based tanks, though probably the most controllable, have severe limitations as to cost, size and naturalness although, as Oviatt *et al.* (1977) point out, 'One of the prevailing myths in marine ecology is that the goal of microcosm (i.e. enclosure) study is to develop an exact replica in miniature of some particular natural system. Such a goal is neither attainable nor necessary.' The obvious alternative therefore has been to develop the in-situ enclosure from a flexible and, usually, transparent material. The principal physical constraint on design is the activity at the water surface which, for the most part, has meant that marine experiments have been carried out in inshore environments and particularly in sheltered fjordic inlets. It has not been possible to enclose an offshore ecosystem but, since a main objective has been pollution research, the neritic ecosystems that have been studied are more representative of potentially polluted environments.

The in-situ tube is probably the ideal enclosure for studying pelagic–benthic interactions. However, in the marine situation tidal incursions make the design of such enclosures very difficult. They have been used most successfully in fresh water (Lack & Lund 1974) where 18000 m^3 butyl rubber impoundments have been deployed in an English Lake District tarn for several years. To date they have been used for the study of phytoplankton populations under natural conditions. In the tubular enclosure used in the Kiel Bight, where the tidal incursion is only 0.5 m (von Bodungen *et al.* 1976; Smetacek *et al.* 1976), the problem has been that, owing to salinity changes in the surrounding water column, water exchange occurs through the sandy sediment into which the enclosure is embedded.

The most popular marine enclosure type is the floating in-situ bag (see Zeitzschel 1978). Since the intention is to isolate the entrapped water column, floating enclosures have been used most frequently in pollution experiments. Lacaze (1971, 1974) has used 0.6 m^3 enclosures for investigating oil pollution effects on phytoplankton while Berland *et al.* (1975) have studied eutrophication in 10 m^3 structures. However, the most extensive pollution investigations have been carried out by the Cepex group (see *Bull. mar. Sci.* **27**(1) (1977) and *Mar. Sci. Commun.*,

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3(4) (1977)) and by ourselves (Gamble *et al.* 1977; Davies *et al.* 1978) particularly on the effects of Cu, Hg and hydrocarbons. It should be noted that the conical bases of these, the largest, marine enclosures, permit the collection of sedimented material from the trapped water column.

Before discussing the details of our Hg experiment, two problems of microcosm research must be mentioned, namely mixing within and replicability of enclosures. The use of enclosures in ecosystem research has been criticized by Verduin (1969) and Boyce (1974) in that there is a much reduced component of vertical mixing when compared with the outside sea. The M.E.R.L. microcosms and the small floating tanks of Brockman *et al.* (1974) are, as with the original design of Strickland & Terhune (1961), fitted with mechanical mixing devices but such mechanisms have not been incorporated into the larger enclosures used by Cepex and ourselves. Steele *et al.* (1977) have conducted dye studies of vertical mixing rates on both the Cepex and Scottish enclosures and have shown that eddy diffusivity is reduced by at least an order of magnitude. Such a reduction will undoubtedly affect the sinking rate of the phytoplankton and, indeed, a large phytoplankton fallout is often an initial feature of the enclosure process. However our experience, particularly in 1974 and 1977, has been that populations of larger diatoms can sustain themselves in the upper levels of the entrapped water column for several weeks (Gamble *et al.* 1977).

Replicability is more of a problem of experimental interpretation rather than one of enclosure design. There is much natural variance, both in space and time, in the parameters being measured and the need for larger size in order to minimize wall effects makes the provision of replicates more difficult and costly. Lacaze (1971) for instance used fifteen 0.6 m^3 containers, the land-based M.E.R.L. project has been developed around twelve 13 m^3 microcosms while only three 1300 m^3 enclosures were depolyed by Cepex. Oviatt *et al.* (1977) have demonstrated the use of multivariate techniques on the early results of the M.E.R.L. experiments; they showed differences between treatment and controls which have not been explained.

Replication in the smaller Cepex enclosures (68 m^3) was discussed by Takahashi *et al.* (1976) who pointed out that, whereas during an experiment changes within containers were of several orders of magnitude, the degree of replication between the chambers generally differed by a factor of less than two. One of the consequences of the larger container is that the chance of significant patchiness of distribution increases. Indeed, this has been the experience with the 1300 m^3 Cepex enclosures (Grice *et al.* 1977). However, Lawson & Grice (1977) have shown that the 95 % confidence limits for sampling zooplankton in 68 m^3 enclosures were similar to those from field data and concluded that a two- to threefold change in population estimates would be sufficient to detect pollution effects on density.

Methods

The mercury experiment was carried out in Loch Ewe, on the west coast of Scotland, in the autumn of 1976. Three bags were used, identical in design (figure 2) to those described by Gamble *et al.* (1977) except that they were made of nylon mesh reinforced polyvinyl chloride (PVC) instead of polythene and that the bottom bucket for collecting settlement material was replaced by a cone narrowing to a $\frac{3}{4}$ inch (20 mm) i.d. hose connected to a diaphragm pump on the support raft. The bags were 3 m in diameter and 17 m deep, with a conical top and bottom, and contained 95 m^3.

PVC proved to be much stronger than polythene and did not have any apparent toxic

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effects. Laboratory tests which simulated the conditions of the field experiment (48 h pre-experiment soaking with equivalent material surface area:water volume ratio) showed that there was no effect on heterotrophic production, on phytoplankton chlorophyll content or on ^{14}C fixation. Tests with the copepod *Acartia clausi* also revealed no marked difference between PVC and controls in terms of mortality and egg production, although the latter measurements were somewhat erratic.

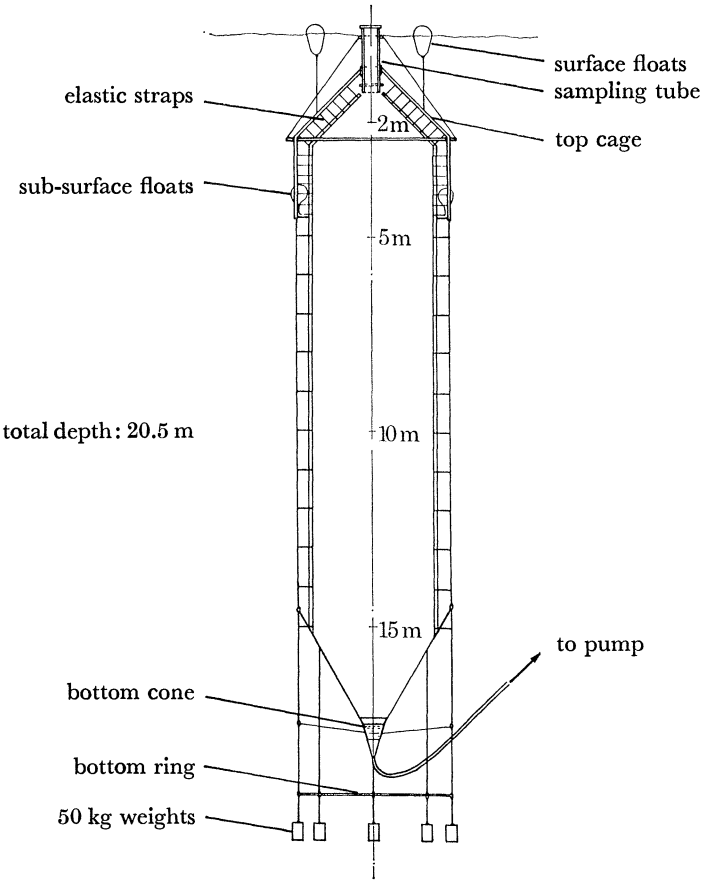

FIGURE 2. Detailed features of enclosure used in the 1976 L. Ewe mercury experiment.

The experiment started on 22 August when the three bags were filled simultaneously by a diaphragm pump. The operation took about 23 h during which the inlet pipe was gradually raised from 15 m to 5 m. All three bags were left to equilibrate for about a week before mercury was added to two of the bags to a nominal level of 1 μg Hg/l. After 20 days a further dose was added to increase the concentration to 10 μg Hg/l. Sampling continued for a further 20 days. The control (A) and one mercury bag (C) had nutrient pumped in between 0 and 10 m depth (nutrient mixture 10:10:1, molar proportions of KNO_3:$Na_2SiO_3 \cdot 5H_2O$:KH_2PO_4) at a concentration three times that of the other mercury bag (D). The addition to higher bags was equivalent to 15 mM N, 15 mM Si and 1.5 mM P per day.

The sampling programme (table 1) was the same in all three bags and the sea except that depths greater than 15 m were sampled in the sea. Most of the sampling and analytical methods are as described in Gamble *et al.* (1977) and Davies *et al.* (1978). All water samples were collected through a 25 mm diameter black PVC pipe with the use of a diaphragm pump housed on the

support raft. This system had been tested against Van Dorn water bottle samples and no difference noted in primary production or chlorophyll. Primary particulate and soluble production was measured as in 1975 (Davies *et al.* 1978). Heterotrophic activity in the bags was measured at five depths by using a method similar to that devised by Williams & Askew (1968) in which an amount of ^{14}C-labelled substrate, reckoned to be small in relation to the total pool size of the naturally occurring substrate, was incubated with filtered (250 μm) sea water for 4 h. The ^{14}C incorporated into the microbial population and the ^{14}C respired were measured after filtering through a 0.45 μm membrane filter (Davies *et al.* 1978). At approximately weekly intervals, plate counts, with the use of nutrient agar medium, were carried out on water from three depths in each bag and outside. Zooplankton samples were collected every four days with a 68 μm mesh net of 45 cm mouth diameter hauled vertically from 15 m in the bags and from 25 m in the sea.

TABLE 1. ENCLOSURE SAMPLING SCHEDULE

parameter	frequency per week	sampling depths/m
salinity, temperature	3	$2\frac{1}{2}$, 5, $7\frac{1}{2}$, 10, $12\frac{1}{2}$, 15
PO_4^{3-}, SiO_3^{2-}	1	$2\frac{1}{2}$, 5, $7\frac{1}{2}$, 10, $12\frac{1}{2}$, 15
NO_3^-	2	$2\frac{1}{2}$, 5, $7\frac{1}{2}$, 10, $12\frac{1}{2}$, 15
chlorophyll *a*	6	$2\frac{1}{2}$, 5, $7\frac{1}{2}$, 10, $12\frac{1}{2}$, 15
heterotrophic uptake	1	$2\frac{1}{2}$, 5, $7\frac{1}{2}$, 10, $12\frac{1}{2}$, 15
^{14}C fixation, particulate and soluble	1	$2\frac{1}{2}$, 5, $7\frac{1}{2}$, 10, 15
particulate carbon and nitrogen	1	5, 10, 15
phytoplankton	1	5, 10, 15
zooplankton	every 4 days	vertical haul from 15
light	1	$2\frac{1}{2}$, 5, $7\frac{1}{2}$, 10, $12\frac{1}{2}$, 15

TABLE 2. IN-SITU LIGHT READINGS IN THE SEA AND AN ENCLOSURE

(Values are expressed as percentage of surface quanta; means ± s.d. of six weekly readings.)

depth/m	sea	bag A
$2\frac{1}{2}$	34.9 ± 6.0	15.4 ± 2.6
5	21.2 ± 4.1	9.4 ± 1.8
$7\frac{1}{2}$	11.5 ± 3.1	4.9 ± 1.4
10	6.7 ± 2.6	3.4 ± 0.9
$12\frac{1}{2}$	3.9 ± 1.8	2.1 ± 0.8
15	1.9 ± 0.7	0.9 ± 0.3

The material settling out from the bottom of the bags was pumped to the surface and collected each day. It was allowed to settle overnight and the supernatant liquid siphoned off until the volume was reduced to a few hundred millilitres. Sub-samples were taken and the remainder was kept for concurrent in-situ experiments being carried out on the benthic mud of L. Ewe.

Light measurements were made once weekly with a Li-Cor underwater quanta meter measuring in the range 400–700 nm. The values obtained (table 2) show that light levels in the bags were 45 % lower than those in the open water. Comparative readings could not be taken at 1 m depth because of the closed top design of the bags.

The bags were dosed with mercury by using the injection techniques described for copper by Topping & Windom (1977). Samples of water (1 l) were collected at daily intervals from 2.5, 5.0, 7.5, 10.0, 12.5, 15.0 m depths and analysed for total and reactive mercury. For total mercury analysis (Topping 1977) the samples were pretreated with a solution of potassium permanganate/sulphuric acid (20 ml) to oxidize the organic matter and the mercury was blown

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off with 'scrubbed' air into an absorption tube containing 20 ml of permanganate–sulphuric acid mixture. These solutions were then transferred to a Dreschel bottle and analysed by flameless atomic absorption. 'Reactive' mercury was taken to be that fraction of the Hg released on addition of $SnCl_2$ solution. Samples of settlement material were also collected daily and a suitable aliquot taken for analysis. These were centrifuged before filtration through a Whatman GF/C paper. The paper and precipitate were then analysed for mercury with the use of a dry combustion – flameless atomic absorption technique. Oxidation was assisted by passing oxygen over the top of the silica boat while it was heated to 1000 °C and the volatized mercury was trapped in 20 ml of permanganate–sulphuric acid solution. This solution was then analysed as described above. Calibration was done by using standards made up to 20 ml of $KMnO_4$–H_2SO_4 (Topping & Pirie 1972).

Results and discussion

The results of the autumn 1976 Loch Ewe mercury experiment are considered in relation to those from a very similar experiment carried out in spring 1976 in Saanich Inlet (Cepex), Vancouver Island, in which mercury was added to larger enclosures, but with an equivalent trophic structure, at 1 and 5 μg Hg/l. The detailed results of the Cepex experiments are contained in *Marine Science Communications*, vol. 3, part 4 (1977).

The salinity data, integrated over the water column, show that the Loch Ewe bags did not leak to any appreciable extent over the course of the experiment. This is substantiated in two other ways, first by the increase in numbers of larvae of benthic invertebrates in the outer water and their exclusion from the enclosed water and, secondly, by the fact that it was possible to account for all the mercury added to bags C and D.

Addition of mercury

The levels of mercury measured in the bags showed that for the first addition (1 μg Hg/l), total mercury fell from its initial level of about 0.9 μg/l by about 6 % per day until it levelled off at about 0.2 μg/l (figure 3). The levels of 'reactive' or non-bound mercury fell very rapidly over the first few days so that after the second day less than 25 % was in the ionic form (Topping 1977). Of the mercury that was lost from the water column about 25 % was recovered associated with the settlement material and there was a close relationship between the amount of mercury and the amount of carbon in the settlement material each day. The mean value was 3000 μg Hg/g carbon in the settlement. The remainder of the mercury lost from the water column could be accounted for in the significant losses to the walls of the bags and small losses to the atmosphere and through the walls of the bag.

The second (10 μg Hg/l) mercury addition was made on day 31 of the experiment when there was very little primary production in the water column so that there was a correspondingly low rate of settlement of material from the bags. This, coupled with the fact that there was so little particulate matter in the water column, meant that the ratio of 'reactive' to total mercury was far higher than after the first (1 μg/l) addition.

The mercury added to the Cepex enclosures had a longer half life, both as total mercury and 'dissolved or reactive' mercury, than in the L. Ewe experiment. On first sight this is puzzling since the chlorophyll levels in the Cepex bags were higher (*ca.* 1 μg Chl *a*/l) than in the L. Ewe bags (0.25–1 μg/l) and yet the 'reactive' or ionic mercury was mopped up more rapidly in L. Ewe than in Saanich Inlet. Furthermore, the rate of removal of mercury from

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the water column in L. Ewe was about twice (6 % per day) that in Saanich Inlet. A possible reason could be the different ratios of volume to wall area of the two enclosures (0.8 for L. Ewe and 2.5 for Cepex) since in L. Ewe only about 25–30 % of the mercury was recovered with the settlement material and most of the rest was adsorbed to the walls of the enclosure, whereas in the larger Cepex enclosure 'the bulk of the mercury lost from the water column' (Takahashi *et al.* 1977) was accounted for in the settlement material, although no figures are given.

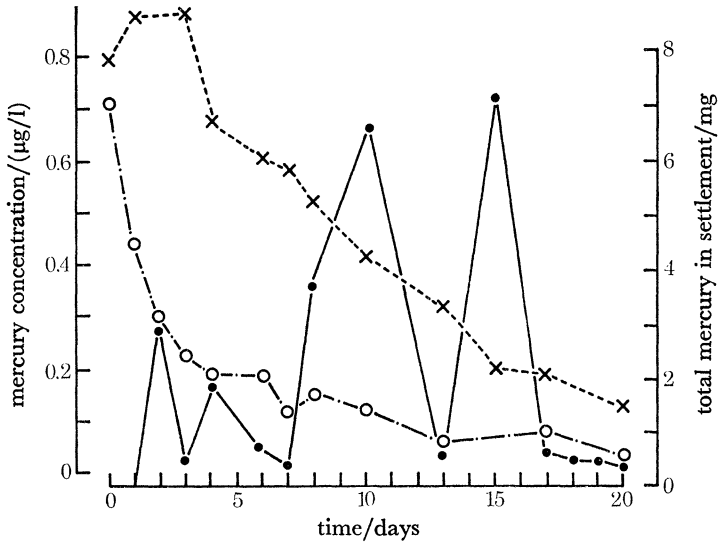

FIGURE 3. Levels of total (×) and reactive (○) mercury in one enclosure following the addition of a nominal 1 μg Hg/l. Total mercury recovered in bag settlement material is also shown (•).

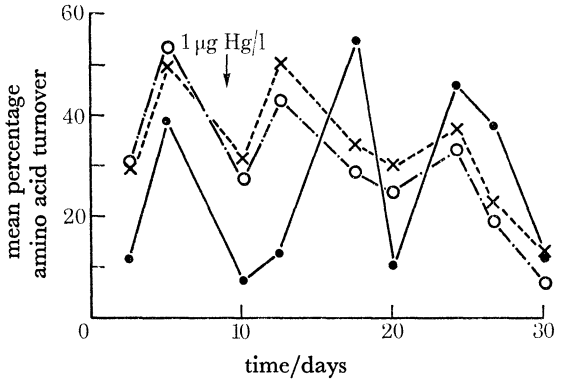

FIGURE 4. The turnover rates (uptake and mineralization) of a ^{14}C-labelled amino acid mixture averaged over three depths in the enclosures: •, bag A; ×, bag C; ○, bag D.

Biological responses to mercury

Microbial populations

The activity of the microorganisms in the water column, as measured by ^{14}C-amino acid uptake and mineralization, showed little response to the added mercury (figure 4). In contrast, the microbial population activity in the Cepex experiment (Azam *et al.* 1977), measured by [^{14}C]glucose and [^{3}H]glucose uptake, decreased by two orders of magnitude immediately after the addition of both 1 and 5 μg Hg/l and had recovered four days later. In the Cepex experiment there was also a concomitant change in the bacterial viable plate counts, again

a change of several orders of magnitude, which recovered after four days, but no such change was apparent from the viable plate counting done on the L. Ewe bags.

Phytoplankton

The phytoplankton populations, as depicted by the Coulter counter, were very consistent in the bags throughout the experiment (figure 5). No major blooms of diatoms above 10 μm apparent diameter occurred, while only small size peaks below 10 μm were evident. The situation in the surrounding sea was much more variable, although the initial size profiles for the sea and the bags on day 4 (26 August) were similar in both size distributions and total particulate volume (t.p.v.). This difference between the sea and the enclosures further demonstrates that the bags did not leak significantly.

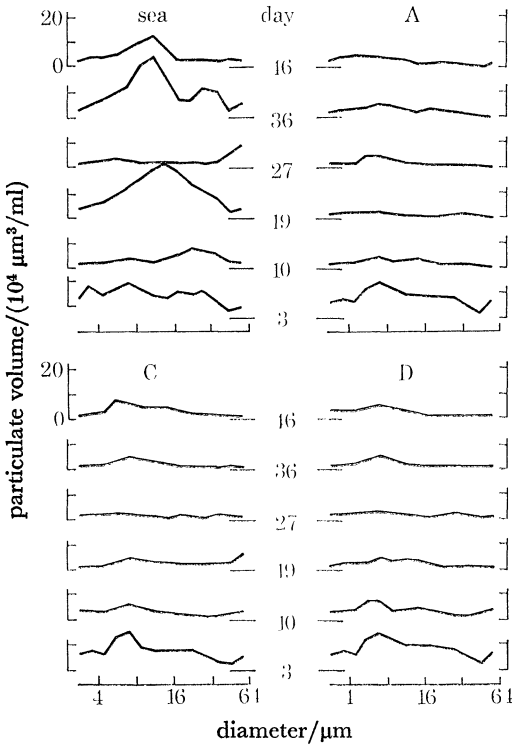

FIGURE 5. Selected particle size spectra of suspended particulates measured with a T_A II Coulter counter fitted with a 140 μm aperture.

Selected samples were analysed by Utermöhl's (1958) sedimentation method for phytoplankton throughout the experimental period. The initial populations in all three bags and the sea were similar and were characterized particularly by both low numbers and few species. Nanoplankton flagellates between 2 and 10 μm were fairly numerous while the dominant diatom species present in the bags were *Rhizosolenia fragilissima* Bergen, *Cylindrotheca closterium* (Ehrenberg) Reiman & Lewin and *Nitzschia delicatissima* Cleve. The same species were dominant in the loch but in addition *Cerataulina pelagica* (Cleve) Hendey was present in moderate numbers.

After 10–14 days, diatom numbers decreased in the bags with the small *C. closterium* being the only obvious survivor. In contrast the sea samples showed an increase in diatom numbers and the appearance of additional species such as *Rhizosolenia delicatuca* Cleve, *Leptocylindricus*

danicus Cleve, *Eucampia zoodiacus* Ehrenberg and *Chaetoceros spp*. During this period small phytoplankton did not show any consistent increase. Towards the end of the experiment, in October (40–50 days), *Skeletonema costatum* (Greville) dominated the sea phytoplankton populations while the situation in the bags remained much the same although, in bag C in particular, the small diatoms *C. closterium* and *N. delicatissima* were slightly more evident.

There was no evidence of any direct effect of mercury on the enclosed phytoplankton population structure even at the higher concentration level. The rapid decline in the larger diatoms after filling and the predominance of smaller cells (after 12–14 days, 80 % by volume of the suspended particulates were below 20 μm diameter) was a consistent feature in polluted and control bags alike. This situation was unaffected by addition of mercury.

There was a similar decline in the larger diatom population after capture of the water column in the Cepex bags (Thomas *et al.* 1977) and the dominant algae, which had been *Chaetoceros* spp., were replaced by an unidentified microflagellate. There seemed to be no species composition shift due to mercury until late in the experiment when there was a dinoflagellate bloom in the 5 μg Hg/l bag followed by a centric diatom bloom. This change in population structure was attributed to reduced grazing in the 5 μg/l bag rather than to a direct effect of mercury on phytoplankton. Phytoplankton diversity indices calculated for the Cepex bags showed that diversity declined in all bags initially and was very similar in all bags until the centric diatom bloom occurred in the 5 μg/l bag.

The low populations of phytoplankton in the three bags (total particulate volume (t.p.v.) = 0.5×10^6 μm³/ml) were somewhat unexpected, particularly when compared with the results of a previous spring-time experiment, t.p.v. < 6×10^6 μm³/ml (Gamble *et al.* 1977), and our latest experiment in Autumn 1977. Simple laboratory experiments, together with our recent (1977) experiences, lead us to believe that the low enclosed populations were not a direct effect of enclosure, i.e. the bags were not toxic to phytoplankton. Furthermore we feel that they were not due to excessive microbial competition for nutrient since microbial counts inside and outside the bags were similar. Our only conclusions are that the low populations of phytoplankton were maintained by grazing pressure from the zooplankton in conjunction with the metabolic control exerted by the reduced bag light régime (table 2).

Total phytoplankton productivity, both soluble and particulate, was not affected by mercury at 1 μg/l although in general the amount of production in bag D was lower than in A and C, presumably a response to the lower rate of nutrient addition. At 10 μg Hg/l there was a significant reduction in C and D which recovered within a week in bag C but remained low in D. The nutrient (figure 6) and chlorophyll (figure 7) data support the idea that phytoplankton productivity was not affected, directly or indirectly, by mercury at 1 μg/l. The levels of nutrients in bags A and C were similar throughout and all three bags show a similar depletion in inorganic nitrogen 5–7 days after enclosure.

This combination of low nutrients for the first few days after enclosure, coupled with the high copepod grazing rate, was not conducive to diatom growth and they were replaced by smaller flagellates. The chlorophyll profiles show that between days 10 and 30 there was a greater rate if increase in chlorophyll in bag A than in bag C although the levels of nitrate in the two bags were very similar. After the addition of 10 μg Hg/l to bag C, when the grazing pressure was removed, the rate of algal growth in C was much higher than in A (days 31–48) and in bag C the small diatoms *C. closterium* and *N. delicatissima* were more evident. This is similar to the effect of reduced grazing noted earlier in the 5 μg Hg/l Cepex bags.

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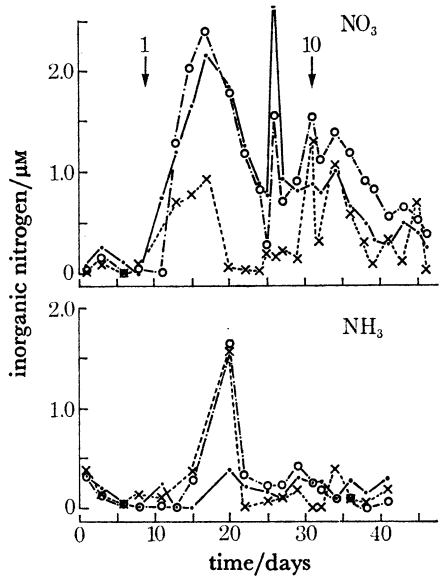

FIGURE 6. The levels of nitrate and ammonia averaged over the whole enclosure volume: •, bag A; ○, bag C ×, bag D. The arrows indicate the addition of 1 and 10 µg Hg/l.

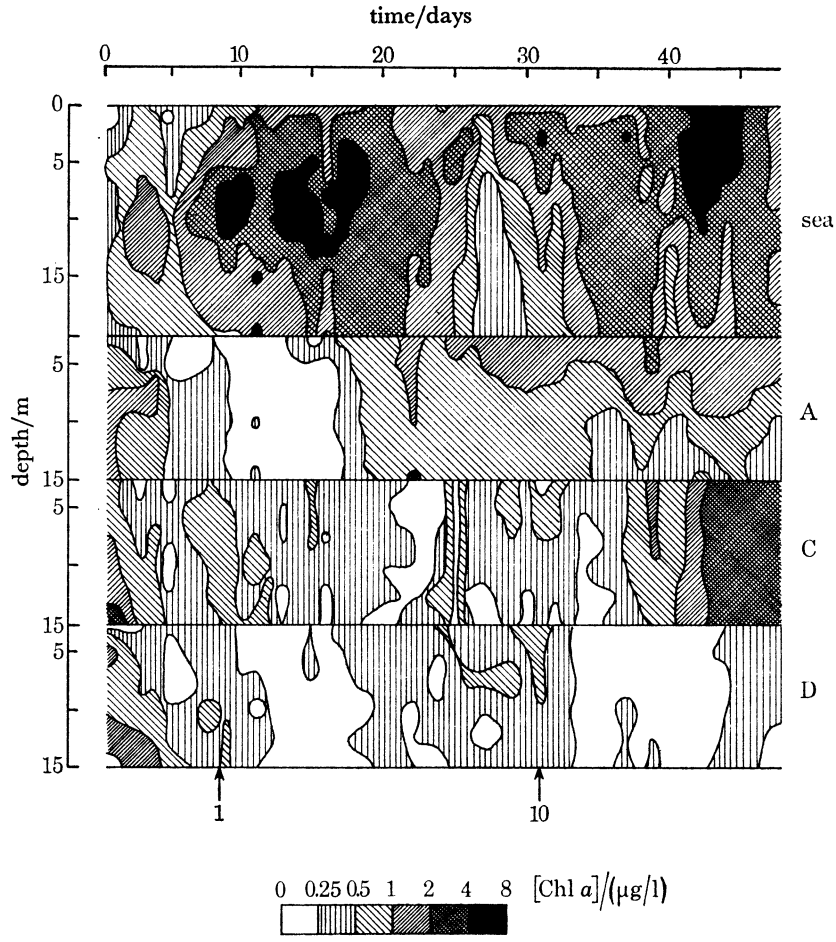

FIGURE 7. Levels of chlorophyll in the enclosures and the sea. Arrows indicate additions of mercury.

Some indications of the physiological state of the phytoplankton may be gained from the specific rate measurement of [^{14}C]Na_2CO_3 uptake per unit of chlorophyll *a*. The in-situ 24 h incubations (all incubated at the appropriate depth in the sea to overcome possible differences in light levels in the bags) showed that there was a substantial (> 50 %) decrease in the rate of ^{14}C-carbonate uptake per unit chlorophyll immediately after the addition of 1 µg Hg/l to the bags (figure 8). Recovery was very rapid, of the same time scale as the disappearance of 'reactive' mercury, and two days after the addition of mercury the rate was no different from the control. The levels of ^{14}C fixation measured in all bags at the time of the addition of 10 µg Hg/l were so low that the in-situ incubations showed little difference. However, ^{14}C uptake measurements made in a light incubator (which gave higher ^{14}C uptake because of the higher light levels) showed that photosynthesis per unit chlorophyll *a* in bags C and D (which had received 10 µg Hg/l) were reduced to one-fifth of the control immediately after the addition but had almost recovered by the ninth day after addition. These results are very similar to those obtained from Cepex experiments in which 1 µg Hg/l was found to reduce photosynthesis per unit chlorophyll by about 50 % with recovery before the next measurement, i.e. within five days.

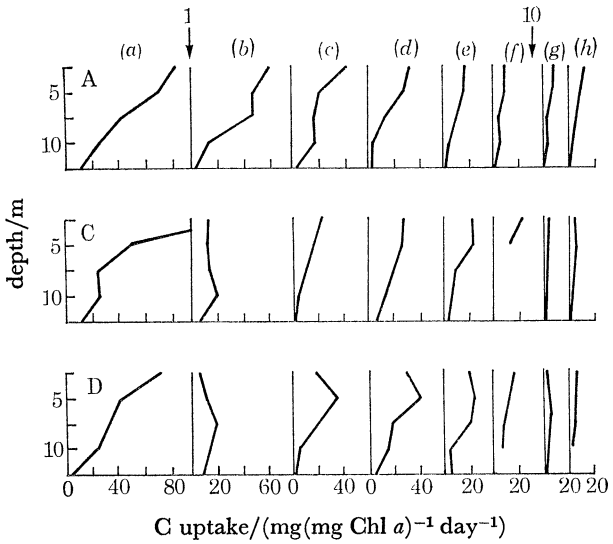

FIGURE 8. Uptake of [^{14}C]$NaHCO_3$ per unit chlorophyll shown as depth profiles for the three bags. (*a*) Days 3–4; (*b*) days 10–11; (*c*) days 12–13; (*d*) days 16–17; (*e*) days 24–25; (*f*) days 30–31; (*g*) days 37–38; (*h*) days 42–43. Arrows indicate addition of mercury.

Zooplankton

Zooplankton populations were sampled in the bags from 15 m, and in the sea from 25 m, by using a simple, vertically hauled 68 µm mesh net. The net mouth diameter, which was delimited by the sampling tube of the bags, was 39 cm and each 15 m tow filtered 0.81 % of the enclosed water column (Gamble *et al.* 1977).

The enclosed populations were characteristic of shallow Scottish waters and the composition in Autumn 1976 differed only in proportions and dominance of component species when compared with that described for Spring 1974 (Gamble *et al.* 1977). The chief difference between the bags and the sea in 1976 (figure 9) was that the enclosed populations of *Oithona* sp. and of the larvae of benthic invertebrates rapidly declined in all the bags whereas they remained dominant in the sea. One would expect meroplanktonic organisms to decline in enclosures as

they metamorphosed, but the fall in *Oithona* numbers is less explicable, particularly when compared with the consistent population size of calanoid copepods. A similar decline in the proportion of *Oithona* in the zooplankton populations in the Cepex bags was noted but not explained.

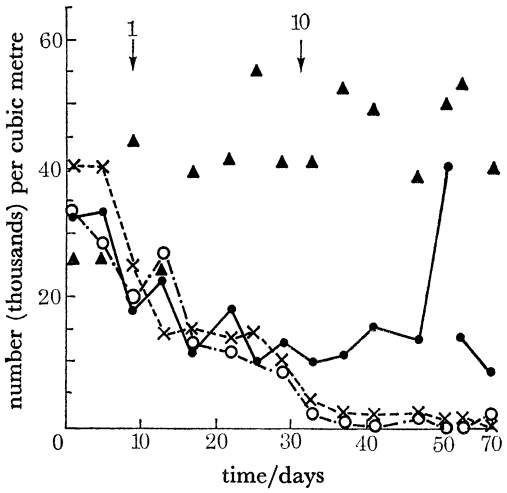

FIGURE 9. Total number of zooplankton: ▲, sea; ● bag A, ○, bag C; ×, bag D. Arrows indicate addition of mercury.

The biomass proportions of the five major herbivorous components are shown in figure 9. Carbon values are averaged from Kraneis & Marten's (1975) values for calanoid copepods (1.95 μg carbon per individual), *Oithona* (0.95 μg), copepod nauplii (0.04 μg) and benthic larvae (3.67 μg). The larvacean value (2.0 μg) was taken from Paffenhöfer (1973). With the decline of dominance of *Oithona* and of the benthic larvae the composition of the bag zooplankton rapidly diverged from that of the surrounding sea. However, all three bags remained relatively similar until the 10 μg Hg/l dose was added. Afterwards there was a rapid decline in the zooplankton numbers of the polluted bags (C and D) with a tenfold decrease in biomass (figure 10). However, the population composition also altered in the polluted bags compared with the controls. Apparently the calanoid copepods and *Oithona* were more affected than the benthic larvae and larvaceans. While the former did not increase over this period their population proportion did; the latter, whose numbers fluctuated during the experiment, were immediately affected by the increased dose of Hg but subsequently showed some recovery during the remaining month of the experiment. Larvaceans have a short generation time (8–12 days at 13 °C (Paffenhöfer 1973)) and could easily be reproducing again once over the initial shock of mercury addition. Calanoid copepods, on the other hand, have a longer generation time of about 30 days at this temperature (Landry 1975; Harris & Paffenhöfer 1976; Paffenhöfer & Harris 1976) and will be starting to produce overwintering eggs at that time of year. It is possible therefore that there were some differential effects of pollutant addition on the enclosed zooplankton at this high concentration.

In the Cepex experiment (Beers *et al.* 1977) there were some notable exceptions to the general decline in micro- and meso-zooplankton in the 5 μg Hg/l bag. For example a sudden increase in numbers of larvaceans occurred during the middle period of the experiment and it was noted that this coincided with sharply decreasing abundance of microflagellates and that the larvaceans, presumably capable of utilizing these small plant cells, were at a maximum when the

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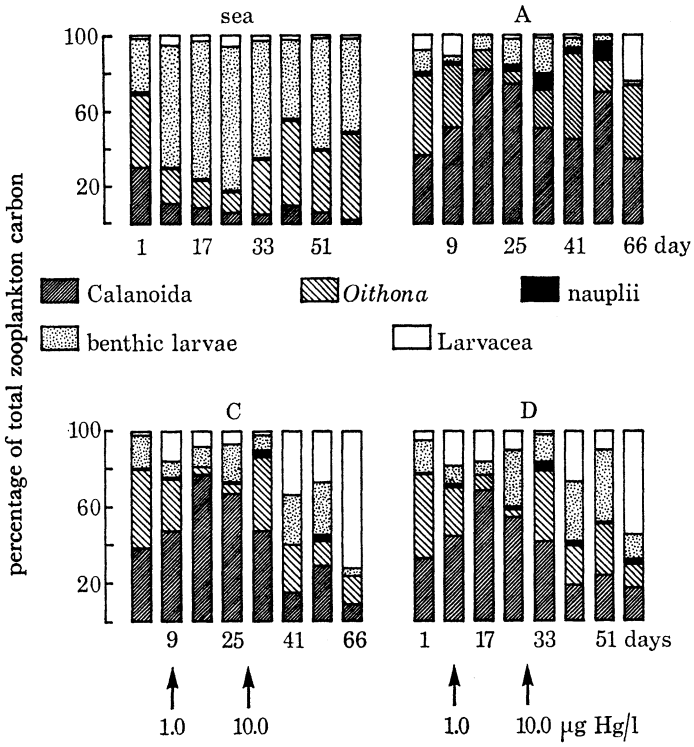

FIGURE 10. Biomass proportions of the major components of herbivorous zooplankton.

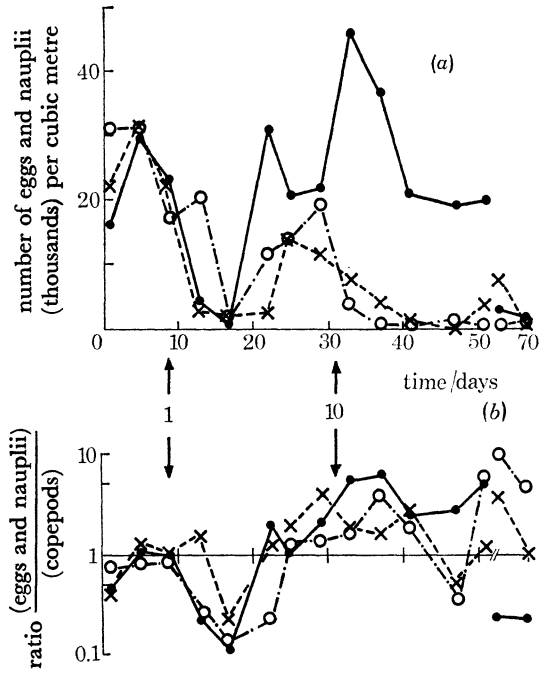

FIGURE 11. (*a*) Numbers of copepod eggs and nauplii; (*b*) ratio of eggs and nauplii to copepod number. •, Bag A; ○, bag C; ×, bag D. Arrows indicate additions of mercury.

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microflagellates were at a minimum. There was no evidence of this inverse relation in the L. Ewe bags.

Egg production is one function which is possibly affected by sublethal levels of pollutants (Reeve *et al.* 1976). Figure 11*a* illustrates the total eggs, egg sacs and copepod nauplii counted in the samples, the production of which followed a cyclical pattern during the experiment.

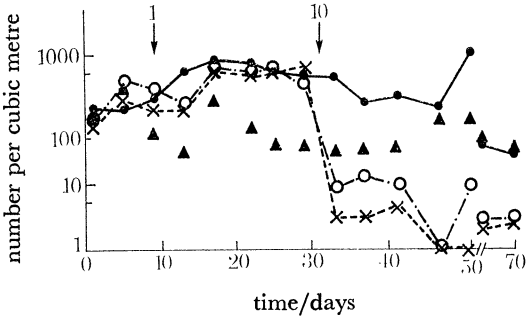

FIGURE 12. Number of planktonic predators, mostly cnidarians: ▲, sea; •, bag A; ○, bag C; ×, bag D. Arrows indicate additions of mercury.

Obviously the initial measurements depended on the state of the enclosed populations but after 10 days both control and polluted bags declined to a level that was only exceeded when the population was reduced after increased Hg addition on day 31. After this initial trough there was an increase in production of eggs and nauplii particularly in bag A, the control. This might be construed as indicating an effect of the 1 μg Hg/l addition but consideration of Figure 11*b*, which relates egg and naupliar numbers to total copepod populations, would seem to disprove this assumption. Furthermore, the effect of the 10 μg Hg/l addition, which was so lethal to large numbers of individuals, did not appear to affect greatly the reproductive performance of the survivors. In fact, on the last two sampling occasions, days 66 and 70, there were signs of greatly enhanced production when compared with the control. Interestingly, eggs and nauplii produced at this stage of the experiment could most probably have come from copepods born in an environment containing excessive mercury since, as just noted, the generation time at this temperature is about 30 days.

The only organisms to increase steadily during the first 20 days of the experiment were the planktonic predators (figure 12). Dominant species in this autumn experiment were anthomedusae, particularly the small *Lizzia blondia* (Forbes) and the larger *Sarsia gemmifera* (Forbes) and *S. prolifera* (Forbes), a feature which contrasted with the dominating ctenophores of the 1974 spring experiments (Gamble *et al.* 1977). These medusae reproduce by a sexual budding and, in the absence of larger predators in the bags, such as scyphomedusae and fish, have potential for increase. Perhaps decline of the *Oithona* populations in the bags was due to the increased abundance of these predators. As with the mesoplanktonic herbivores, the 1 μg Hg/l had little discernible effect but, again, increased addition of Hg reduced the populations by over two orders of magnitude, although there was a suggestion of recovery at the end of the experimental period.

The ingestion rates of calanoid copepods feeding in water taken from their own site of origin were compared at approximately weekly intervals from day 4 until the time of the 10 μg addition, day 31. After this the copepod populations in bags C and D became too sparse for collection of live material. The techniques followed were identical to those described in Gamble

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et al. (1977) except that on three occasions (days 16, 29 and 32) the water taken from the bags was enriched with a culture of phytoplankton obtained by enhancing natural sea water with F_2 culture medium (Guillard & Ryther 1962). The ingestion rates obtained over this period (figure 13) are most easily described by a linear regression of ingestion rate on food concentration but there is no evidence of any difference between copepods taken from the polluted bags (C & D) when compared with the controls (A and the sea). Equal numbers of points of both categories lie to either side of the regression line at both the low, naturally occurring particulate levels (total particulate volume $< 1 \times 10^6$ μm³/μl) and the enriched higher levels.

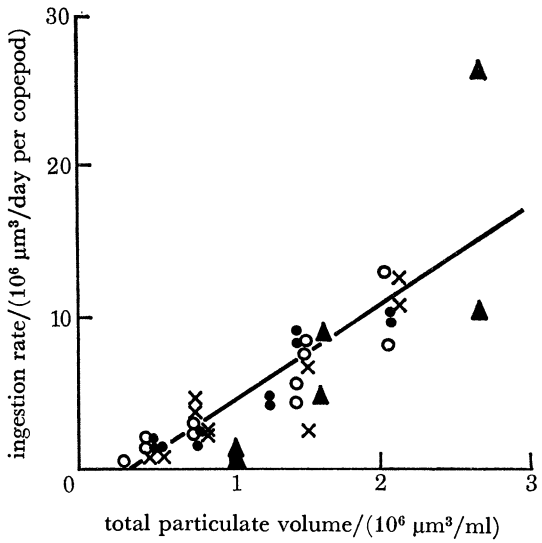

FIGURE 13. Relation between herbivorous copepod ingestion rate and total particulate volume of suspended food particles: ▲, sea; ●, bag A; ○, bag C; ×, bag D.

In the Cepex experiments, faecal pellet production was used as a measure of *Calanus* feeding rate (Beers *et al.* 1978). *Calanus* taken from the bags, maintained in their own bag water and offered uncontaminated food, showed lower faecal pellet production in the 5 μg Hg/l bag water when compared with the control. Animals from the 5 μg Hg/l bag that were maintained in clean water in the laboratory very quickly recovered their feeding ability.

CONCLUSIONS

The effect of mercury in the marine environment

In most respects there was a remarkable similarity in the way the two enclosed ecosystems, L. Ewe and Cepex, reacted to the addition of mercury at 1 μg/l and to the higher dose of 5 or 10 μg/l. In both systems there was a general decline in *Oithona* population as a result of enclosure, an increase in the proportion of larvaceans in the zooplankton and diatoms in the phytoplankton as a response to the higher mercury doses. At the level of 1 μg Hg/l there was a comparable transient reduction in photosynthesis per unit chlorophyll in both systems. Except for the microbial population, in which it was difficult to demonstrate a significant response at Loch Ewe, there were no other marked changes in rate processes or population structure. Thus these two comparable experiments have demonstrated that for mercury added at 1 μg/l these enclosed ecosystems are robust enough to recover from the observed short-term effects (on

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microbial population and photosynthesis) and that no longer term sublethal changes occurred. However, another interpretation of these results could be that the systems studied in these experiments were too insensitive to show such changes. Experience would suggest that this is not so since it has been demonstrated (Gamble *et al.* 1977) that differences in nutrient addition or predator numbers produce changes in phytoplankton species composition and, consequently, their size structure in such experimental systems. The experiments have therefore helped to justify the extrapolation of conclusions based upon laboratory scale experiments to field situations.

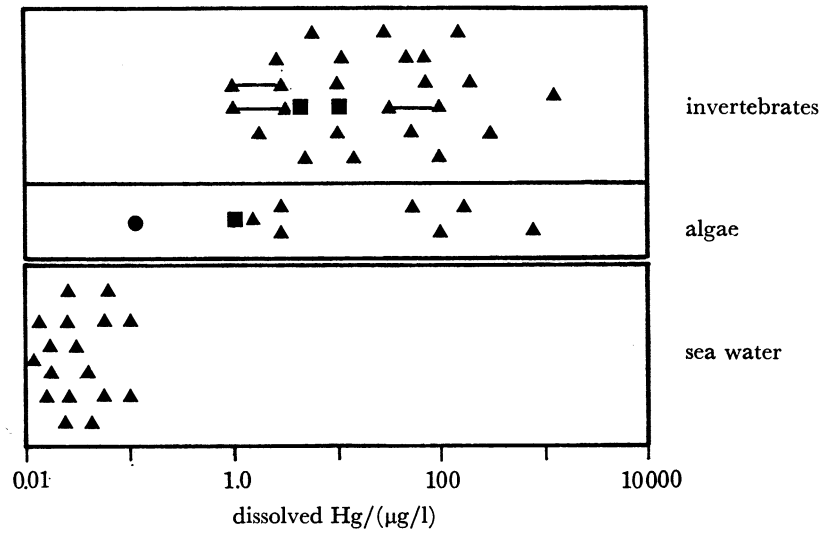

FIGURE 14. The maximum sensitivities of bioassay techniques to mercuric chloride (▲) taken from the literature compared with the levels of total dissolved mercury found in sea water. The data presented in this paper for phytoplankton and zooplankton response to added mercury are shown (■). The data of Saward *et al.* (1974), where there was a response of the algal population to continuous addition of mercuric chloride at 0.1 μg Hg/l over a 200 day experiment, are also shown (●). Figure adapted from Stebbing (1976).

When the results of these experiments are considered in relation to the many other experiments that have been carried out by using mercury (figure 14, after Stebbing (1976)) it is striking that with one exception there would appear to be a barrier at about 1 μg Hg/l beyond which no effect has been demonstrated. The exception was the use of large tanks containing a simple benthos-based food chain of algae, the bivalve *Tellina tenuis* and O-group plaice (*Pleuronectes platessa*) (Saward *et al.* 1974) which was used to test the effect of mercury and to study accumulation of mercury up the food chain at nominal levels of 0.1, 1 and 10 μg Hg/l. In these experiments small but significant enhancements of photosynthesis per unit chlorophyll at 0.1 and 1.0 μg Hg/l were found although chlorophyll *a* was lower in all dosed tanks compared with the controls. However, in these experiments there was a continuous exchange of one-third of the water volume per day and a comparable daily addition of mercury to keep the concentration at the desired level. The organisms in the tanks were therefore exposed to new doses of 'ionic or reactive' mercury each day for about 200 days. This could be considered as a simulation of a substratum in an estuary in the vicinity of an outfall which might receive pulses of pollutant with each tidal excursion.

The levels of mercury which are usually reported in surface waters around the United Kingdom range from 0.001 to 0.022 μg/l (Baker 1977). Thus, except in the case of the long-term exposure experiment (Saward *et al.* 1974), the levels of mercury in open waters would

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appear to be one, if not two, orders of magnitude below the levels at which effects have been demonstrated. This, we feel, is an important observation since before experiments of this type were carried out it was felt that 'important and long term effects of pollution are those which may influence the population structure of marine ecosystems' (Menzel & Case 1977). In other words it was felt that low levels of pollutants, much below the levels where one can demonstrate a lethal effect, may, without altering the total productivity of the ecosystem, change the species and size structure of the phytoplankton and zooplankton populations and ultimately affect fishery resources. But, possibly, the evidence to date suggests that the opposite may be true and that the whole system is actually more robust than isolated individuals taken from it and that it was only at the unnaturally high (5 or 10 μg/l) levels of mercury, when the zooplankton populations were severely reduced, that the structure of the zooplankton and consequently of the phytoplankton populations changed.

The rôle of large-scale ecosystems in pollution research

The question which remains to be answered is whether large scale enclosed ecosystems have a further rôle to play in pollution research and in what direction such experiments should develop. For heavy metal studies the results from these mercury experiments and the previous copper experiments (*Bull. mar. Sci.* **27**(1), 1977) suggest that, for the immediate future, research effort should be changed from 'effects' studies and diverge for a while into two different areas. There is a necessity to do more basic chemistry on the fate and state of heavy metals in sea water. Large scale ecosystems of all types, pelagic, benthos-based, etc. should be used to study the rates of transport of metals from the water column into the sediments and the chemical state of the metals in the water column and the sediment. At the same time an effort should be made to gain more understanding of the very complex biology of these enclosed ecosystems. It is only when this has been achieved and more is known about the speciation of metals in sea water that we should look again, at lower dose levels, at the sublethal effects of toxic metals on enclosed ecosystems.

In the wider field of pollution research, large-scale ecosystem experiments have an important rôle to play in studying the link between the water column and the sediment. Most of the evidence to date (Takahashi *et al.* 1977; Topping 1977) suggests that pollutants are fairly rapidly lost from the water column and bag experiments have proved very useful in quantifying this process and in studying both the method of transport and the chemical form of the pollutant as it falls out from the water column. In this way one of the major rôles of pelagic ecosystems may be to provide information on the rates and chemical nature of the material leaving the water column.

Two areas of pollution research where these large scale ecosystems are proving valuable are in assessing the effects of oily water discharges in the marine environment and in predicting the effects of sewage dumping in coastal areas. With reference to an oil discharge, as well as observing effects on marine organisms, enclosures should help to answer such questions as what quantity actually enters the water column and what components are degraded in the column. Furthermore it should be possible to estimate the nature and quantity of oil-derived materials reaching the sediments. For sewage dumping, the major part of the work is centred on the sediments since the particulate sludge rapidly passes through the water column to the seabed.

Recent work we have been carrying out at L. Ewe (Davies *et al.* 1978), together with that being

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currently undertaken by the M.E.R.L. group at Narragansett Bay (Pilson *et al.* 1977) illustrates the way in which this link between the water column and the benthos is being investigated. At L. Ewe, hydrocarbons have been added to enclosures at 100–200 µg/l and their effects on the food chain and pathways of decay in the water column followed over a three month period. Material was collected at the bottom of the enclosures, a small aliquot analysed and the remainder added to in-situ 2.5 m diameter open chambers (figure 1) deployed in 30 m of water to isolate experimentally areas of a soft mud bottom. Decay processes of the oil derivatives in contact with the sediment were followed and accumulations of oil hydrocarbons in the benthic organisms were monitored. Similar experiments are being carried out at Narragansett Bay with oil hydrocarbons but in this situation the sediment is incorporated within the free-standing tanks (figure 1). Such a design necessitates the provision of a water mixing system designed to simulate the turbulence levels measured in the surrounding bay water.

In conclusion we feel that large scale ecosystems have already played a valuable part in pollution research in that they have to some extent justified the use of laboratory data in assessing field situations. They have also proved to be very useful in quantifying the flux of pollutants into the biota and from water column to the sediments. In the immediate future, pelagic enclosed ecosystems will also play an important part in assessing the effects of oily water discharges on pelagic ecosystems, but the greatest scope for future work will be provided by facilities that link the pelagic and benthic systems.

References (Davies & Gamble)

Antia, N. J., McAllister, C. D., Parsons, T. R., Stephens, K. & Strickland, J. D. H. 1963 Further measurements of primary production using a large volume plastic sphere. *Limnol. Oceanogr.* **8**, 166–183.

Azam, F., Vaccaro, R. F., Gillespie, P. A., Moussalli, E. I. & Hodson, R. E. 1977 Controlled ecosystem pollution experiment: effect of mercury on enclosed water columns. II. Marine bacterioplankton. *Mar. Sci. Commun.* **3**, 313–329.

Baker, C. W. 1977 Mercury in surface waters of seas around the United Kingdom. *Nature, Lond.* **270**, 230–232.

Beers, J. R., Reeve, M. R. & Grice, G. D. 1977 Controlled ecosystem pollution experiment: effect of mercury on enclosed water columns. IV. Zooplankton population dynamics and production. *Mar. Sci. Commun.* **3**, 355–394.

Berland, B. R., Ronin, D. J. & Maestrini, S. Y. 1975 Isolement in situe d'eau de mer naturelle dan des enceintes de grand volume. Application a l'étude d'une eutrophisation intérêt et prospective. *C.N.E.X.O.-Rapp. scient. et Tech.* **21**, 2–18.

von Bodungen, B., von Brockel, K., Smetacek, J. & Zeitzschel, B. 1976 The plankton tower. I. A structure to study water/sediment interactions in enclosed water columns. *Mar. Biol.* **34**, 369–372.

Boyce, F. M. 1974 Mixing within experimental enclosures: a cautionary note on the limnocoral. *J. Fish. Res. Bd Can.* **31**, 1400–1405.

Brockmann, U. H., Eberlein, K., Junge, H. D., Trageser, H. & Trahms, K. T. 1974 Einefache Folientanks zur Planktonuntersuchung in-situ. *Mar. Biol.* **24**, 163–166.

Cushing, D. H. 1963 Studies on a *Calanus* patch. V. The production cruises in 1954: summary and conclusions. *J. mar. biol. Ass. U.K.* **43**, 387–9.

Davies, J. M., Hay, S. J., Hardy, R., Whittle, K., Murray, J. & Mackie, P. 1978 Experimental studies using large scale enclosed ecosystems to study oil hydrocarbons in marine food chains. In *Research perspectives on the impact of petroleum on northern marine environments* (ed. D. C. Malins). New York: Academic Press. (In the press.)

Enright, J. T. 1977 Diurnal vertical migration: Adaptive significance and timing. Part 1. Selective advantage: a metabolic model. *Limnol. Oceanogr.* **22**, 856–872.

Gamble, J. C., Davies, J. M. & Steele, J. H. 1977 Loch Ewe Bag Experiment, 1974. *Bull. mar. Sci.* **27**, 146–175.

Grice, G. D., Reeve, M. R., Koeller, P. & Menzel, D. W. 1977 The use of large volume, transport, enclosed sea-surface water columns in the study of stress on plankton ecosystems. *Helgoländer wiss. Meeresunters.* **30**, 118–133.

Guillard, R. R. L. & Ryther, J. H. 1962 Studies of marine planktonic diatoms. 1. *Cyclotella nana* Hustedt & *Detanula confervacea* (Cleve) Gran. *Can. J. Microbiol.* **8**, 229–239.

Harris, R. P. & Paffenhöfer, G.-A. 1976 Feeding, growth and reproduction of the marine planktonic copepod *Temora longicornis* Muller. *J. mar. biol. Ass. U.K.* **56**, 675–691.

Kraneis, W. & Martens, P. 1975 Ecological studies on the plankton in the Kiel Bight. II. Zooplankton. *Merentutkimuslait. Julk.* no. 239; 187–194.

Kuiper, J. 1977 Development of North Sea coastal plankton communities in separate plastic bags under identical conditions. *Mar. Biol.* **44**, 97–108.

Lacaze, J.-C. 1971 Utilisation d'un dispositif expérimental simple pour l'étude de la pollution des eaux in situ. Effets comparés de trois agents émulsionants antipétrole. *Tethys* **3**, 705–716.

Lacaze, J.-C. 1974 Ecotoxicology of crude oils and the use of experimental marine ecosystems. *Mar. Pollut. Bull.* **5**, 153–156.

Lack, T. J. & Lund, J. W. G. 1974 Observations and experiments on the phytoplankton of Blelham Tarn, English Lake District. *Freshwat. Biol.* **4**, 399-415.

Landry, M. R. 1975 The relationship between temperature and the development of life stages of the marine copepod *Acartia clausii* Giesbr. *Limnol. Oceanogr.* **20**, 854–857.

Lawson, T. J. & Grice, G. D. 1977 Zooplankton sampling variability; controlled ecosystem pollution experiment. *Bull. mar. Sci.* **27**, 80–84.

McAllister, C. D., Parsons, T. R., Stephens, K. & Strickland, J. D. H. 1961 Measurements of primary production in coastal sea water using a large-volume plastic sphere. *Limnol. Oceanogr.* **6**, 237–258.

Menzel, D. W. 1977 Summary of experimental results: controlled ecosystem pollution experiment. *Bull. mar. sci.* **27**, 142–145.

Menzel, D. W. & Case, J. 1977 Concept and design: controlled ecosystem pollution experiment. *Bull. mar. Sci.* **27**, 1-7.

Mullin, M. M. & Evans, P. M. 1974 The use of a deep tank in plankton ecology. 2. Efficiency of a planktonic food chain. *Limnol. Oceanogr.* **19**, 902–911.

Oviatt, C. A., Perez, K. & Nixon, S. W. 1977 Multivariate analysis of experimental marine ecosystems. *Helgoländer wiss. Meeresunters.* **30**, 30–46.

Paffenhöfer, G.-A. 1973 The cultivation of a appendicularian through numerous generations. *Mar. Biol.* **22**, 183–185.

Paffenhöfer, G.-A. & Harris, R. P. 1976 Feeding, growth and reproduction of the marine planktonic copepod *Pseudocalanus elongatus* Boeck. *J. mar. biol. Ass. U.K.* **56**, 327-344.

Pilson, M. E. Q., Vargo, G. A., Gearing, P. & Gearing, J. N. 1977 The Marine Ecosystem Research Laboratory: a facility for the investigation of effects and fates of pollutants. *Proc. 2nd National Conf. Interagency Energy/Environment R. & D. Program.* Washington, D.C.

Reeve, M. R., Grice, G. D., Gibson, V. R., Walter, M. A., Darcy, K. & Ikeda, T. 1976 A controlled environmental pollution experiment (CEPEX) and its usefulness in the study of larger marine zooplankton under toxic stress. In *Effects of pollutants on aquatic organisms* (Society for Experimental Biology Seminar, series 2), pp. 145–162.

Riley, G. A. 1976 A model of plankton patchiness. *Limnol. Oceanogr.* **21**, 873–880.

Saward, D., Stirling, E. A. & Topping, G. 1974 Experimental studies on the effects of copper and mercury on a marine food chain. Presented at *Nato Science Committee Conference on Eco-toxicity of heavy metals and organohalogen compounds*, Mont Gabriel, Canada.

Smetacek, V., von Bodungen, B., von Bröckel, K. & Zeitzschel, B. 1976 The plankton tower. II. Release of nutrients from sediments due to changes in the density of bottom water. *Mar. Biol.* **34**, 373–378.

Stebbing, A. R. D. 1976 The effects of low metal levels on a cloud hydroid. *J. mar. biol. Ass. U.K.* **56**, 977-994.

Steele, J. H., Farmer, D. M. & Henderson, E. W. 1977 Circulation and temperature structure in large marine enclosures. *J. Fish. Res. Bd Can.* **34**, 1095–1104.

Steele, J. H. & Menzel, D. 1978 The application of plastic enclosures to the study of pelagic marine biota. *Rapp. P.-v. Réun. Comm. perm. int. Explor. Mer* (In the press.)

Strickland, J. D. H., Holm-Hansen, O., Eppley, R. W. & Linn, R. J. 1969 The use of a deep tank in plankton ecology. 1. Studies of the growth and composition of phytoplankton crops at low nutrient levels. *Limnol. Oceanogr.* **14**, 23–24.

Strickland, J. D. H. & Terhune, L. D. B. 1961 The study of in-situ marine photosynthesis using a large plastic bag. *Limnol. Oceanogr.* **6**, 93–96.

Takahashi, M., Thomas, W. H., Seibert, D. L. R., Beers, J., Koeller, P. & Parsons, T. R. 1976 Replication of biological events in enclosed water columns. *Arch. Hydrobiol.* **76**, 5–23.

Takahashi, M., Wallace, G. T., Whitney, F. A. & Menzel, D. W. 1977 Controlled ecosystem pollution experiment: effect of mercury on enclosed water columns. I. Manipulation of experimental enclosures. *Mar. Sci. Commun.* **3**, 291–312.

Thomas, W. H., Seibert, D. L. R. & Takahashi, M. 1977 Controlled ecosystem pollution experiment: effect of mercury on enclosed water columns. III. Phytoplankton population dynamics and production. *Mar. Sci. Commun.* **3**, 331–354.

Topping, G. 1977 Measurement and effects of sub-lethal levels of some heavy metals on marine life in simulated marine ecosystems. *Proc. analyt. Div. chem. Soc.* **14**, 222–224.

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Topping, G. & Pirie, J. M. 1972 Determination of inorganic mercury in natural waters. *Analyt. chim. Acta* **62**, 200–203.
Topping, G. & Windom, H. 1977 Biological transport of copper at Loch Ewe and Saanich Inlet: controlled ecosystem pollution experiment. *Bull. mar. Sci.* **27**, 135–141.
Utermöhl, H. 1958 Zur Vervollkommung der quantitativen phytoplankton – methodik. *Int. Ver. Them. angew. Limnol.* **9**, 1–38.
Verduin, J. 1969 Critique of research methods involving plastic bags in aquatic environments. *Trans. Am. Fish. Soc.* **98**, 355–366.
Williams, P. J. le B. & Askew, C. 1968 A method of measuring the mineralization by micro-organisms of organic compounds in sea-water. *Deep Sea Res.* **15**, 365–375.
Zeitzschel, B. 1978 Controlled environment experiments in pollution studies. Presented at *Oceanology Int. 78, Tech. Sess. B. (Biol. Mar. Tech.)*, pp. 22–52.

Discussion

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1. How was the Hg^{2+} uniformly mixed into the enclosed bag water to give an initial concentration of 1 µg/l in the water without disturbing the bag ecosystem?

2. The experiment described was based on the addition of a single pulse of Hg^{2+} which rapidly became 'bound' and hence 'non-reactive'. This loss of reactive Hg may have been due to a high organic and/or high particulate content of the Loch Ewe water. To relate such an experiment to the normal field situation in the vicinity of a continuous Hg discharge, surely a constant addition of Hg^{2+} to the bag is necessary to keep the 'reactive' level of Hg constant throughout the experiment?

J. M. Davies and J. C. Gamble.

1. The mercury was pumped into the bag with the use of a diffusion ring which was raised from 15 m to 2 m in 0.5 m intervals to give an even distribution of mercury throughout the bag. The first profiles of mercury in the bags showed that this was fairly successful with a concentration range of 0.96–1.24 µg/l (Topping & Windom 1977).

2. Either experiment would have been very interesting. Our reasoning was that a body of water in an estuary such as the Firth of Clyde was unlikely to receive more than one pulse of mercuric chloride as it moved past an outlet because the flushing times of those estuaries can be fairly short.

At our field station in Loch Ewe we have carried out other experiments in tanks which contain an artificial benthos-based food chain (Saward *et al.* 1974) in which the water is changed every day and fresh mercury added. We felt that this represented the type of exposure that the sediment in an area around an outfall might receive and that it was appropriate in this position to concentrate on the benthos rather than the water column where continual dosing of a water mass could only really occur in a landlocked body of water.

J. G. Wilson (*Zoology Department, Trinity College, Dublin, Eire*). With regard to the fact that sampling and measurements are carried out in the centre of the bag, could the authors perhaps say a little more about the 'wall effects', such as colonization by fouling organisms and consequent light attenuation and nutrient loss from the system?

J. M. Davies and J. C. Gamble. Growth on the inside walls of the bags certainly occurs but has never proved to be as much of a problem as one might imagine. We have always been struck by the difference in growth between the outside and inside of the bags because on the outside there is always a great deal of algal growth (which is cleaned off by divers) whereas the inside,

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although slimy, has very little apparent algal growth. We think that there are two possible explanations: (1) that the surface on the inside of the bag is smoother (the bag is deliberately made this way) and thus more difficult to grow on; (2) that there is very much less water movement across the inside walls and thus less transport of nutrients to a sedentary population. As we said, growth is fairly rapid on the outside walls and the bags are regularly cleaned by divers. Light profiles taken over the course of the experiment inside the bags and in the sea show that the light levels in the bags was attenuated to about 55–60 % of those outside but that they did not decrease as the experiment progressed.

The amount of nutrients removed by the fouling population on the inside of the bags would not be significant based upon the relative proportions of pelagic and sessile chlorophyll.

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