TITLE: Benthic communities in experimental ecosystems and the effects of petroleum hydrocarbons.


SUMMARY

The macrofauna of three oiled and six control experimental ecosystems at the Marine Ecosystems Research Laboratory were followed for one year. Water-accommodated fractions of #2 fuel oil were added biweekly from March 6th to July 6th. The average water column hydrocarbon levels were 91.1 ppb (range: 11-235 ppb).

The simulated chronic oil pollution resulted in a highly significant decline in the number of macrofaunal species and individuals in the experimental tanks compared with controls. The effect was apparent in the most common macrofaunal species, Mediomastus ambiseta, Nucula annulata, and Yoldia limatula and in the less common amphipod species, Ampelisca abdita. The only species to increase significantly in the oiled tanks was the gastropod, Retusa canaliculata.

The results of the 1978 experiment differ from those of the 1977 experiment in that the density of individuals in the control tanks remained high instead of declining during the summer months. We attribute this to the improved seawater delivery system. Significant differences in M. ambiseta densities between control tanks are correlated with differences in suspended particulate matter.
INTRODUCTION

In 1978, nine of the twelve 13 m$^3$ experimental ecosystem tanks at MERL have been used to study the effects of chronic low-level additions of #2 fuel oil on a marine ecosystem. These microcosms allow experimental manipulations and replication not possible in the natural ecosystem, thus retaining some of the analytical advantages of simpler laboratory experiments, while still providing much of the perplexing complexity of natural systems.

Even though only about 7-16% of the oil reached the bottom in an experiment completed in 1977 (Gearing and Gearing, pers. comm.), chronic additions of #2 fuel oil averaging 182 ppb resulted in highly significant reductions in the benthic macrofaunal populations (Grasse et al., 1978). The significant differences between tanks with oil additions and control tanks occurred just before and during a period when the populations in all tanks declined. Several changes in the procedure were initiated in order to maintain more natural population densities throughout the year. This year the seawater delivery system has been improved to allow passage of larvae and more natural concentrations of particulate matter. Also, the procedure for filling the trays at the bottom of the tanks with sediment from Narragansett Bay has been improved to minimize disturbance of the top ten cm of sediment. Finally, the large predators in the tanks have been monitored and in some instances have been trapped.

The 1978 oil experiment differs from the experiment with chronic additions in 1977 in the amount of oil added. The average water column concentration in 1978 has been 91.1 ppb (range: 11-235 ppb) compared with an average of 182 ppb (range: 11-636 ppb) in 1977. The additions lasted for a period of 24 weeks in 1977 as opposed to 17.4 weeks in 1978.

METHODS

To monitor the benthic populations in the tanks ten randomly placed 5 cm$^2$ samples were taken each month. Five samples were taken within equal central and peripheral areas of the tanks to test for effects of proximity to the wall. The coring device consisted of a simple core tube on the end of a long pole. The pole was positioned with a rigid frame which allowed precise positioning with respect to the sides of the tank. The core was gently pushed to a depth of 10 cm and a spring-loaded lid was released to seal the top of the core. The same area was not sampled twice unless several months had elapsed between samples.

In July 1978 a comparison was made between the corer used in the 1977 experiment (surface area 4.155 cm$^2$), and the corer used in the 1978 experiment (surface area 5 cm$^2$). Ten cores were taken with each in tanks 3 and 9. The mean number of species per core, and the mean number of individuals per core were not significantly different between corers. However, the "new" corer (5 cm$^2$) consistently produced samples with a higher density of M. ambiseta, N. annulata and Y. limatula than did the "old" corer.

The Mediomastus-Nucula community in Narragansett Bay where the tank sediments were collected in October, 1977 has been sampled seasonally since the
found and both Bay and tank samples were taken. Figure shows the similarity in species numbers in each of ten cores from the Bay. The mean and approximate 95% confidence limits of the number of species per 35 cm$^2$ are given for June samples from each of three consecutive years. There is a significant increase in species density in 1978 from levels of 16-18 species per core in 1977 and 1976 to about 24 species per core in 1978.

To obtain comparable numbers on species density from the six control tanks we have pooled seven of the five cm$^2$ samples to yield a composite 35 cm$^2$. This procedure may slightly enhance the number of species over the number that would have been obtained from a single 35 cm$^2$ sample. The data show that the six control tanks replicate well and reflect the species density in Narragansett Bay at the time the sediments were collected in October, 1977. The mean density in the tanks in June, 1978 was 14 species per 35 cm$^2$ and the mean density in October, 1977 in the Bay was also 14 species per 35 cm$^2$. Comparison of the tanks in June, 1978 with the tanks in June, 1977 indicates a significant increase in the number of species maintained in the tanks during this year's experiment.

Figure shows the density of individuals from the same set of June samples illustrated in Figure . Density per m$^2$ in Bay samples ranged from 130,000 to 185,000 with a mean of about 150,000 individuals per m$^2$. This was about twice the density in June Bay samples in 1976 and 1977. The density in the tanks was closer to the October, 1977 densities in the Bay when the tanks were filled, than to the June, 1978 Bay density. Even so the densities in the tanks in both 1977 and 1978 were considerably below normal densities in Narragansett Bay. Unlike the June samples in 1977 the reduction in numbers in 1978 was more apparent in species other than Mediomastus ambiseta.

The density of Nucula annulata, illustrated in Figure , followed a pattern similar to that for M. ambiseta and for total individuals. The increase in density in Narragansett Bay occurred between 1976 and 1977 instead of between 1977 and 1978. The seasonal variation in density was not as great, so that the October samples were not significantly different from the June samples in 1977 and 1978. The tanks were not significantly different from each other, and the mean for all six control tanks was significantly different from the densities in Narragansett Bay.

Yoldia limatula showed more seasonal variation in density than N. annulata (Figure ). The difference in density in the tanks between 1977 and 1978 was probably the result of the low densities of Yoldia in the Bay at the time the tanks were filled in October, 1977. Yoldia was more patchily distributed than Nucula within the tanks, and tank 5 showed significantly higher densities than the remaining five control tanks.

**Biomass**

Biomass, measured as decalcified ash-free dry weight, was found to be 13.24 $\pm$ 10.05 g/m$^2$ in 4 control tanks in June, 1978 (tanks 1, 3, 4 and 5) while the mean biomass for 5 Narragansett Bay cores was 10.41 $\pm$ 1.79 g/m$^2$. The biomass of M. ambiseta alone in the tank samples was 0.56 $\pm$ 0.17 g/m$^2$, and in
summer of 1976. Twenty closely-spaced 35 cm$^2$ cores were taken by divers along a single transect each sampling period. The corers consist of clear plastic piston core liners with simple rubber flap valves.

All samples were initially washed through 500 µ, 300 µ, and 38 µ standard soil sieves. All individuals retained by the 300µ and 500 µ screens, except the meiofaunal groups such as nematodes, ostracods and copepods, were identi-
fied to species and counted as part of the macrofauna. The samples were stained with rose bengal and sorted under a microscope.

Biomass (decalcified ash-free dry weight) of macrofaunal individuals from tank and Bay cores has been determined for 4 control tanks and 5 Bay cores from June, 1978.

Traps (Lagardère, 1977) were fabricated from stainless steel, and used for intermittent trapping in the tanks to remove large predators attracted to baits.

From April to November, 1978 the top 4 cm of sediment from two 5 cm$^2$ cores from three control tanks (1, 5 and 8) was returned to the laboratory in Woods Hole and live-sorted for Mediomastus ambiseta, Nucula annulata and Yoldia limatula. The M. ambiseta were maintained in culture in the laboratory under conditions similar to those used for other capitellids (Grassle and Grassle, 1976). Sexually mature M. ambiseta were identified with a binocular microscope: males had white, stellate clusters of sperm visible in dorso-
lateral areas of the more anterior abdominal segments; females had large numbers of eggs with large germinal vesicles, packed into abdominal segments making up about two-thirds the length of the abdomen. The gametes were periodically checked for maturity by stripping eggs and sperm from individual worms, mixing them, and following subsequent development. A planktonic larva is produced which metamorphoses after approximately 5 days at 15°C.

It was also noted that individual M. ambiseta often lacked the long, extensible tail filament that is characteristic of the species. Such individ-
uals had regenerating, foreshortened tail filaments developing on abdomens variously reduced in length. Since nothing is known about the way in which M. ambiseta reproduces, it was thought that the incidence of regenerating tail filaments might reflect a) evidence of dehiscence of abdominal segments, or b) the effects of predation on M. ambiseta which habitually lives in the top 1 cm of sediment, with its tail filament often protruding from a semi-
permanent tube that projects above the surface of the sediment.

M. ambiseta that were live-sorted from the cores were therefore sexed, roughly classified into 3 size categories, and classified as to whether or not they had intact or regenerating tail filaments.

RESULTS

To illustrate the replication in control tanks and compare the communi-
ties in the tanks with those in the Bay we have used the month of June. This is a month when significant differences between oil and control tanks were
the Bay cores was $0.99 \pm 0.28 \text{ g/m}^2$. This suggests that although the number of macrofaunal individuals is lower in the tanks than in the Bay in June, 1978 (Figure 5) the biomasses are not significantly different. The range in biomass in the four tanks was $4.29 - 27.17 \text{ g/m}^2$, the two tanks with the highest biomass having relatively large numbers of *Y. limatula* compared with the two lowest biomass tanks.

Although the biomass analyses are not yet complete, the oiled tank 2 had a significantly lower biomass than the four control tanks for June, 1978.

The chronic additions of #2 fuel oil started in March, 1978 did not result in significant differences in species density until June (Figure 7). The differences were significant at the 95% level for each month following June with the exception of August when the confidence intervals overlapped slightly. This contrasts with the results in 1977 when, although species densities were consistently lower in the oiled tanks, significant differences were apparent only in July (Figure 9). Perhaps the most important difference between the 1977 and 1978 experiments is that the species density remained high throughout 1978.

If each tank is considered individually the clearest separation between oiled and control tanks occurred in June during the month of the last oil additions (Figure 8). Even in this month, only some of the differences between individual oiled and control tanks are significant. This is primarily the result of the high variance in number of individuals and species densities in samples as small as 5 cm$^2$. In general, the tanks with the highest density of individuals also had the highest density of species.

By the end of April, 1978 after 8 weeks of oil additions the density of individuals in the oiled and control tanks had diverged significantly (Figure 11). The oiled tanks remained significantly different from the six control tanks for the entire period of oil additions and for the period following the last oil addition on July 6 until at least October, 1978. This result is even clearer than that obtained in 1977 (Figure 12). Although the oil additions were started earlier and the concentrations were higher in 1977, differences between oiled and control tanks significant at the 95% level were not obtained until June. The clearcut difference between oiled and control tanks is at least partly due to the use of six tanks as controls for the oil experiment in 1978. The other important difference between the 1977 and 1978 experiments is that densities of individuals did not show a sharp decline during the summer months. In August the numbers of individuals increased sharply as a result of seasonal recruitment of young individuals.

*M. ambiseta* was the dominant species in almost all samples, forming 36 - 96% of total individuals in the six control tanks ($\bar{x} = 57.1 \pm 5.9\%$) and 22 - 77% of total individuals in the oiled tanks ($\bar{x} = 60.3 \pm 4.5\%$). The density of *M. ambiseta* in the 1978 oil experiment (Figure 13) therefore followed the pattern of the total number of individuals shown in Figure 5 although, without the individuals of other species, the differences between oiled and control tanks did not become significant until May.
Differences in density of the second most common species, *Nucula annulata*, between oiled and control tanks were not significant until August and October following the period of chronic oil additions (Figure 9). The control tanks did not show the sharp increase as a result of new recruits, and the subsequent decline that was evident in the tanks in 1977 (Figure 10). The relatively stable densities of *N. annulata* are more like the usual situation in Narragansett Bay.

Differences in density of *Yoldia limatula* between oiled and control tanks were significant in June through October, 1978 (Figure 11). The densities of *Y. limatula* were generally much lower in the tanks in the 1978 experiment than in the 1977 experiment (Figure 12 and Figure 13) and reflect the low density of *Y. limatula* in the Bay in October, 1977 when the tanks were filled, and relatively low recruitment in 1978.

As the water temperature in the tanks began to increase during the spring and summer months the control tanks began to diverge from one another (Figures 14 and 15). The differences between the six control tanks were most obvious in the late summer and early fall months. This was primarily the result of differences in spawning success between the *Mediomastus ambiseta* populations in the tanks. During this period tanks 5 and 6 had consistently very high densities of *M. ambiseta* whereas tanks 1 and 3 had consistently low numbers. Tank 4 was consistently nearer to the high end of the spectrum and tank 8 was nearer the low end. These differences are correlated with differences in the amount of suspended material present in the tanks during the period from April to September (C. Hunt, pers. comm.). The rank order of mean suspended load in the water column for the period from April to September was tank 5, tank 6, tank 4, tank 8, tank 3, tank 1. The rank order of *M. ambiseta* abundance was 5, 6, 4, 8, 1, 3 in August and 6, 5, 4, 8, 3, 1 in September and October. The probability of obtaining this similarity in rank correlation of *M. ambiseta* abundance and suspended load by chance is .01 (Spearman rank correlation).

Table 1 shows the number of individual *M. ambiseta* live-sorted from tanks 1, 5 and 8 from April to November, the percent sexually mature, the percent of large individuals, the percent of postlarval and small individuals, and the percent with regenerating tail filaments.

Tank 5 is one of the control tanks which developed a large *M. ambiseta* population, while tanks 1 and 8 never did (Figure 16). An examination of the data in Table 1 and Figure 16 indicates that while there may have been some early recruitment in tank 8 (April-May), the large recruitment of small individuals in July and August in Tank 5 did not occur in the other two tanks. That this was not due to an absence of sexually mature individuals in tanks 1 and 8 is attested by the continuing high percentages of large, mature individuals in these tanks lasting through August.

An examination of the *M. ambiseta* meiofaunal and macrofaunal data for 1977 (Figure 17, Grassle et al., 1978 and Rudnick, Frithsen & Elmgren, pers. comm.) indicates that recruitment in that year began in April.
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Observations on the number of individual M. ambiseta with regenerating tail filaments appears to be unrelated to reproductive activity or success, and may indeed reflect the activity of specific predators.
The species most sensitive to the chronic additions of oil were the amphipods, particularly *Ampelisca abdita* (Figure 7). These species were present in low numbers in all tanks in February. By May the amphipod species other than *A. abdita* began to increase in the control tanks. From June through October amphipods were present in most of the control tanks but absent in the oiled tanks except for two occurrences of single individuals that may have migrated into the tanks through the seawater delivery system. A similar pattern occurred in the 1977 experiment except that the densities of amphipods in the control tanks was never more than a very few individuals.

There was one species that reached significantly greater densities in the oiled tanks than in the control tanks. The small gastropod, *Retusa canaliculata*, showed an increase in the oiled tanks following the last addition of oil at the beginning of July (Figure 6). The 1977 experiment indicated that chronic oil additions may favor increases in Foraminifera. At least some species of *Retusa* are known to eat Foraminifera (Burn and Bell, 1974a, b).

We are continuing to analyze the data obtained in the 1977 experiment. Figure 6 uses the normalized expected species shared method or NESS (Grassle and Smith, 1976) to compare faunal similarity of the community in control tank 5 with that in another control tank (8) and in each of the three oiled tanks (2, 7 and 9). While the similarity of the two control tanks remained as high as .95, the similarity of the oiled tanks to control tank 5 declined steadily to as low as .65. The % similarity measure is relatively insensitive to rare species and does not show the decline in similarity of oiled and control tanks (Figure 7) (Smith, Kravitz and Grassle, in press).

Trapping of predators in all nine experimental tanks (Table 2) has indicated that there may be fewer large macrofaunal predators in the tanks in the 1978 experiment than in the 1977 experiment. This is somewhat confirmed by a limited number of visual observations, but an accurate determination of the abundance of larger animals cannot be made until all the sediments are sieved at the end of the experiment. An examination of the numbers of predators trapped indicates that there were no differences between the calibration tanks (3, 4 and 6) and the remaining control tanks (1, 5 and 8), and that the density of macrofaunal individuals in the summer of 1978 does not correlate with the number of predators.

**DISCUSSION**

Improvements in the seawater delivery system have allowed us to maintain more natural systems through the summer months. The new pulse flow system both increases the immigration of larvae and maintains more natural inputs of suspended particulate matter. The correlation of density of individuals with the amount of suspended particulate matter in the tanks further suggests that the influx of particulate matter is an important determinant of benthic production. An experiment to determine the effects of added organic matter on the benthic populations is planned for the early spring of 1979.

The 1978 oil experiment, with chronic oil additions that were lower and of shorter duration than those used in 1977, has also shown that the oil
TABLE 2. NUMBERS OF PREDATORS TAKEN IN TRAPS IN NINE EXPERIMENTAL TANKS.

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produces significant changes in the macrofauna: the number of species were significantly lower in the oiled tanks in June through October, the number of individuals were significantly lower in the oiled tanks from April through October. The dominant species Mediostomus ambiseta was significantly less abundant in the oiled tanks in April through October; Nucula annulata showed a significantly lower abundance in the oiled tanks only in August and October; Yoldia limatula was significantly less abundant in the oiled tanks in June through October. Striking differences between the amphipod species abundance in oiled and control tanks were seen as early as May, and the recruitment of Ampelisca abdita which took place in the control tanks in June, July and August, was completely absent in the oiled tanks. The gastropod Retusa canaliculata increased sharply in the oiled tanks, with a peak in the population in August. We attribute this to an increase in the prey of R. canaliculata, probably in the Foraminifera, since this element of the meiofauna was shown to increase in the oiled tanks in 1977, and is known to be the chief prey of other species of Retusa.

The hypothesized interactions between different components of the MERL ecosystems, for example, between suspended particulate load and abundance of individuals in the spring and summer months of 1978, or between increased populations of meiofaunal (Foraminifera) and macrofaunal elements (Retusa canaliculata) in the oiled tanks (Elmgren et al., in press), could not have been predicted from a knowledge of the biology of the individual species involved. Such interactions emphasize the utility of the ecosystem approach and have suggested further experiments to improve our understanding of the systems.
REFERENCES


Figure 1. Number of species in ten Bay cores (35 cm$^2$ samples) and in six control tanks in June, 1978. The means and 95% confidence intervals for June, 1976, 1977, and 1978, and October, 1977 Bay cores are shown; the means and 95% confidence intervals for June, 1977 and 1978 tank samples are shown for comparison. Sediment for the 1978 experiment was collected from the Bay in October, 1977.
Figure 4. Number of individuals per m$^2$ within each of ten Bay samples and six control tanks in June, 1978. The means and 95% confidence intervals of June 1976, 1977 and 1978, and October, 1977 Bay samples are shown; the means and 95% confidence intervals for June, 1977 and 1978 tank samples are shown for comparison. The sediment for the 1978 experiment was collected from the Bay in October, 1977.
Figure 1. Total number of *Nucula annulata* per m² in each of 10 June, 1978 Bay samples and their mean and 95% confidence interval, and the mean and 95% confidence interval of *Nucula annulata* per m² in each of the six control tanks in June, 1978. The means and 95% confidence intervals of June, 1977 tank and June, 1976 and 1977 and October, 1977 Bay samples are also shown for comparison. The sediment for the 1978 experiment was collected from the Bay in October, 1977.
Figure 1. Total number of *Yoldia limatula* per m² in each of 10 June, 1978 Bay samples and their mean and 95% confidence intervals, and the mean and 95% confidence interval of *Yoldia limatula* per m² in each of the six control tanks in June, 1978. The means and 95% confidence intervals of June, 1977 tank and June, 1976 and 1977 and October, 1977 Bay samples are also shown for comparison. The sediment for the 1978 experiment was collected from the Bay in October, 1977.
Figure 5. Mean and 95% confidence interval of number of species per 5 cm² sample in control and oiled tanks, 1978 experiment.
Figure. Mean and 95% confidence interval of species per sample in control and oiled tanks in the 1977 (4.155 cm² sample) and 1978 (5 cm² sample) experiments.
Figure 1. Mean number of species per 5 cm² sample and 95% confidence interval in individual oiled (2, 7 and 9) and control (1, 3, 4, 5, 6 and 8) tanks.
Figure 1. Mean and 95% confidence interval of the total number of individuals per 5 cm² sample in control and oiled tanks, 1978 experiments.
Figure. Mean and 95% confidence interval of total number of individuals per sample in control and oiled tanks in the 1977 (4.155 cm² sample) and 1978 (5 cm² sample) experiments.
**Mediomastus ambiseta**

Figure. Mean and 95% confidence interval of the number of *Mediomastus ambiseta* per 5 cm² sample in the control and oiled tanks, 1978 experiment.
Figure 1. Mean and 95% confidence interval of the number of *Nucula annulata* per 5 cm² sample in control and oiled tanks, 1978 experiment.
Figure 1. Mean and 95% confidence interval of the number of Nucula annulata per sample in control and oiled tanks in the 1977 (4.155 cm² sample) and 1978 (5 cm² sample) experiments.
Figure 1. Mean and 95% confidence interval of the number of *Yoldia limatula* per sample in control and oiled tanks in the 1977 (4.155 cm² sample) and 1978 (5 cm² sample) experiments.
Figure 1: Mean and 95% confidence interval of the total number of individuals per 5 cm² sample within each of the experimental oiled (2, 7 and 9) and control (1, 5, 8, 3, 4 and 6) tanks, 1978 experiment.
Figure 15. Mean and 95% confidence interval of *Mediomastus ambiseta* per 5 cm² sample within each of the experimental oiled (2, 7 and 9) and control (1, 5, 8, 3, 4 and 6) tanks, 1978 experiment.
Figure 1. Total number of Amphipods and Ampelisca abdita component per 10 samples (50 cm²) within each of the control (1, 5, 8, 3, 4, and 6) and experimental oiled (2, 7, and 9) tanks, 1978 experiment.
Figure 1: Mean and 95% confidence interval per 5 cm² sample of Retusa canaliculata within each of the control (1, 5, 8, 3, 4, and 6) and experimental oiled tanks (2, 7, and 9), 1978 experiment.
Figure 1. Comparison of faunal similarity between tank 5 (control) and tanks 8 (control) and 2, 7, and 9 (oiled) using NESS (Grasse and Smith, 1976), 1977 experiment.
Figure. Comparison of faunal similarity between tank 5 (control) and tanks 8 (control), and 2, 7, and 9 (oiled) using percent similarity (Smith, Kravitz and Grassle, in press).