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Field bioassays for early detection of chronic impacts of chemical wastes upon marine organisms

W. E. Pequegnat[1] & T. A. Wastler[2]

[1]*TerEco Corporation, P.O. Box 2848, College Station; Texas 77840, USA*
and
[2]*Marine Protection Branch, U.S. Environmental Protection Agency; Washington, D.C., USA*

ABSTRACT: A major problem facing those who must assess the environmental effects of the disposal in the ocean of industrial and municipal wastes, including dredged materials, is determining whether given wastes elicit chronic deteriorative responses in important species of organisms. The full importance of such low-level, nonlethal effects is not known, but it is suspected that repeated excitations may result in ecosystem changes as important as those caused by more easily determinable acute effects. Such considerations are important to the marine environment, where dumped pollutants may be quickly diluted to legal nonlethal concentrations, but may still bring forth cumulative chronic response patterns. One objective of this study has been to develop a field method of assessing the impacts of the disposal of various industrial and municipal wastes. The measure of the impact is not mortality measured against time, but the increase or decrease in activity of certain metabolic enzymes that signal whether an organism is under stress from a class of wastes. Also, by analysing tissues of test and indigenous species for the accumulation of metals, PCBs, and high molecular weight hydrocarbons as well as for the enzyme activity, one gains an insight into the actual effect, if any, of the accumulation upon the whole organism. The test organisms are exposed for selected periods of time in the field in devices called Biotal Ocean Monitors (BOMs); they are then assayed for enzyme induction. At present the following enzymes are used: mitochondrial ATPase, which responds particularly to excess biphenyls in the environment; catalase II, it is dissolved in the cytosol and responds to excesses of toxic metals; and cytochrome P-420 and P-450, which respond to cyclic and long-chain hydrocarbons. The applicability of the adenylate energy charge system to this problem is also studied.

INTRODUCTION

Regulatory call for bioassays

In the United States during the past two years, laboratory bioassays have assumed a major role in regulating the disposal of varied wastes into marine waterways. The regulating agent is the Environmental Protection Agency (EPA), which is directed to protect the integrity of U. S. ocean waters in Section 102 of Public Law 92–532, the Marine Protection, Research, and Sanctuaries Act of 1972 (the Act). Accordingly, EPA issued the final version of regulations and criteria for ocean dumping in the Federal Register, Vol. 42, No. 7, 11 January 1977. Section 227.27(b) of the regulations calls for bioassays of most waste materials prior to issuance of a dumping permit. In May 1976,

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EPA published a manual which was revised in 1978 (U. S. EPA, 1978) detailing methods for bioassaying in the laboratory wastes other than dredged materials prior to issuance of a dumping permit. In July 1977 a manual describing techniques for bioassaying dredged materials was published jointly by EPA and the Corps of Engineers (U. S. EPA & U. S. Army Corps of Engineers, 1977).

In most environmental work we are not employing standard physiological bioassays that have been used with great success in medical and related research for years. Characteristically in physiological bioassays one is testing known quantities of a given drug or toxicant under carefully controlled conditions. In environmental work one utilizes what we call ecological bioassays in which the exact composition of the waste being assayed is not known. For example, it is not feasible to determine the exact composition of dredged material from a polluted harbor. Moreover, if its composition were known, the impacts of the mixture of materials would be aggravated by synergisms and augmentations. Hence, one must predict from the percent of mortality found in the ecological bioassay what impacts, if any, the material will have upon the receiving environment if permission is given to dump it at a designated dumpsite.

Validity of bioassay results

Some difficulties have been encountered by various laboratories in attempting to carry out these bioassays. This has been particularly true of so-called solid phase bioassays of dredged materials. Partly this has been related to the particular species of organisms used in the tests. For example, the small estuarine mysid *Mysidopsis bahia* was recommended in the manual for use in dredged material bioassays, but it is seldom used today because it proved to be vulnerable to the high level of turbidity in solid phase bioassays. Certainly there is as yet no unanimity of opinion as to the most appropriate species for these tests. Other difficulties with survival have been related to the fact that most laboratories were equipped to work only with static rather than flow-through systems. Granted that flow-through systems may be superior to static systems and that other technical problems can be overcome, there are still more fundamental doubts that the ecological bioassays carried out in the laboratory are measuring what we need to know about dumping practices. Except in a few ideally situated laboratories, it is unlikely that laboratory tests can simulate field conditions. Yet major decisions on dumping, involving in some case hundreds of thousands of dollars, have to be made from less than ideal information.

Potential of field bioassays

At present, mortality is the end-point of most ecological bioassays conducted in the U. S. laboratories. In fact, this is mandated in the manual (U. S. EPA & U. S. Army Corps of Engineers, 1977). Those who question whether this is the proper gauge of the acceptability of materials for dumping or of the way in which the dumping is carried out cite the real possibility that some wastes approved for dumping may elicit chronic deteriorative responses in important species of organisms. We are uncertain as to the ultimate importance of such low-level, nonlethal effects on the biota of the receiving ecosystem, including man. Still such possibilities are important to those managers of the marine environment who feel that even though dumped pollutants may be diluted to

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legal, nonlethal concentrations in prescribed time periods they are still capable of evoking cumulative, chronic response patterns in important organisms. Three technical changes in the bioassay routine discussed here can overcome several of the present problems with laboratory bioassays. These are, first, that the bioassays be conducted in the field at the dumpsite during actual dumping operations; second, that indigenous species be exposed alongside of usual bioassay species; and, third, that metabolic enzyme activity be utilized to detect the potential of a waste to elicit chronic responses in test organisms.

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Study objectives

Stimulated by the above considerations, the principal objective of the present study has been to develop an early warning system to reveal when organisms are being seriously stressed by particular wastes and disposal practices well before fatality occurs or is inevitable. Chronic effects cannot be gauged by mortality unless tests are carried out for such long periods that costs become a controlling factor. Thus, if chronic effects impinge seriously upon human welfare, it is to our advantage to develop in situ tests that can be carried out in a short time, and in which the severity of the impact is measured by changes in the induction of selected metabolic enzymes that will signal that test organisms are under stress as compared with controls. Since most enzyme levels cannot be measured in the field, the method that TerEco Corporation has developed over the past five years is actually a field/laboratory technique.

MATERIALS AND METHODS

Development of Biotal Ocean Monitors

During the course of monitoring the physical impacts of the ocean incineration of organochlorine wastes by the incinerator ship M/T Vulcanus in 1974 and 1975 (Wastler et al., 1975), TerEco Corporation saw the need for a free-floating device in which organisms could be held in contact with the ambient environment containing waste products long enough for the possible development of subtle metabolic responses that would foreshadow chronic impacts. Since incineration was carried out in deep water, it was concluded that only organisms living in the water column would be impacted by unburned organochlorines carried to the sea surface in the plume of stack gases. Hence the floating monitoring laboratories that TerEco designed were called Pelagic Biotal Ocean Monitors or P-BOMs. It is to be emphasized that the mesh bags of the P-BOMs permit rapid exchange of water in both directions across the walls of the bag. This is unlike the bags used in the CEPEX program where the walls were impervious to water exchange. By placing dye inside the P-BOM bag, we determined that under even moderate sea conditions exchange between inside-the-bag and ambient waters took place immediately and was complete in no more than 1 to 2 min. When it was evident that the pelagic units formed the basis for a viable monitoring program, work was started in 1976 on a very different type of BOM that could be deployed to monitor high-density wastes such as dredged material that would tend to impact benthic organisms. These units were named Benthic Biotal Ocean Monitors or B-BOMs (Fig. 2). Both units are not

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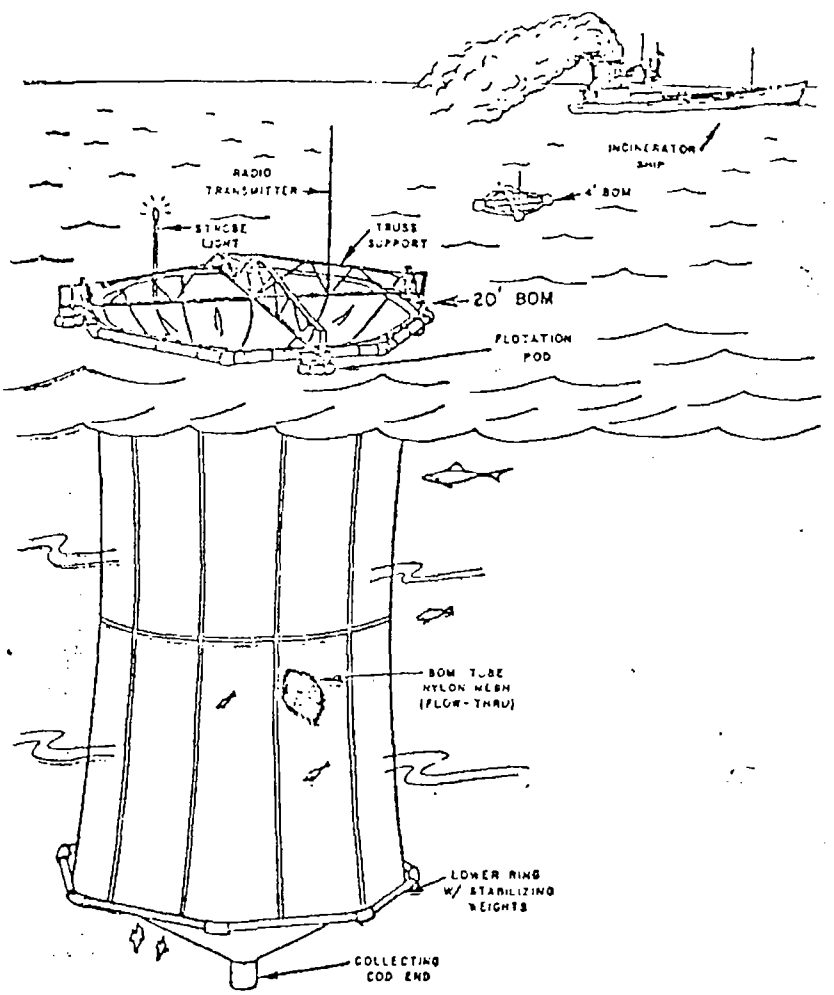

Fig. 1. Pelagic Biotal Ocean Monitors (P-BOMs) of two sizes (4 and 20 feet in diameter) deployed to monitor the incineration of toxic organochlorine residues of the manufacture of polyvinylchloride and other compounds. The BOMs, both controlled and exposed, contain a variety of organisms that are analysed after the 5–7 day exposure

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only equipped to hold a wide range of species, but are also designed to capture and hold indigenous species (Pequegnat et al., 1978, 1979).

Field activities

The field tests during which organisms were exposed to polluted dredged material and sewage sludge were conducted in the northwestern Atlantic Ocean off the coasts of New York and New Jersey in the New York Bight (Fig. 3). The test organisms in the

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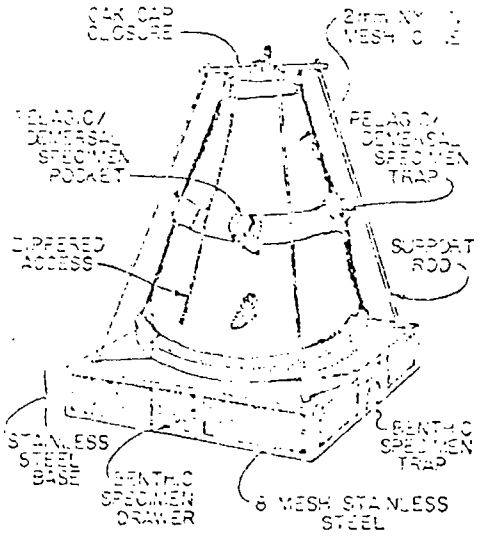

Fig. 2. Schematic of a Benthic Biotal Ocean Monitor (B-BOM). The stainless steel mesh base is 4 feet on edge and the nylon mesh cone is 6 feet tall. The device is designed to hold a variety of organisms in steel drawers, nylon pockets, and free spaces. It is effective in trapping and holding apart various indigenous species

pelagic units included both shellfish and finfish. The master cylinder of the P-BOM is made of monofilament nylon woven into a 2-mm mesh. Smaller organisms, such as grass shrimp and commercial shrimp, are housed in small-mesh nets hung in the master cylinder. Larger fish are free to swim in the large-mesh cylinder. All tests require the launching of a control BOM at an appropriate station outside the polluted zone.

After launching the BOM alongside the ship, each compartment is loaded with sufficient organisms to provide a statistically adequate sample for analysis. Furthermore as each species is introduced into the BOM, a sample is immediately sacrificed for the laboratory analysis of the pretest condition of the organisms. When loaded the test P-BOM is placed where it will be exposed to the pollutant involved and then, depending on the objective of the experiment, either set adrift or anchored in a place where it will receive further exposure. The control is placed in a position that will remain free of the pollutants for the 5–10 days of the monitoring. When set adrift, the P-BOMs are equipped with radio beacons for easy retrieval.

Loading of the benthic units is done either alongside the ship or, in shallow water, by divers after it has been placed on the bottom. The bottom of the B-BOMs is made of stainless steel mesh (2-mm) allowing the floor to be covered with a veneer of sediment. The drawers in the benthic units are loaded with polychaetes, bivalves, and grass shrimp, whereas the nylon upper compartment contains finfish. The traps in the [illegible] generally capture diverse species of fish, crabs, lobsters, and starfish, all of which can be analysed for metals and hydrocarbons to ascertain what materials are being accumulated in their tissues.

Upon retrieval of both the P-BOMs and the B-BOMs, the organisms are prepared immediately for laboratory analysis. In some cases the whole organism is quick-frozen in

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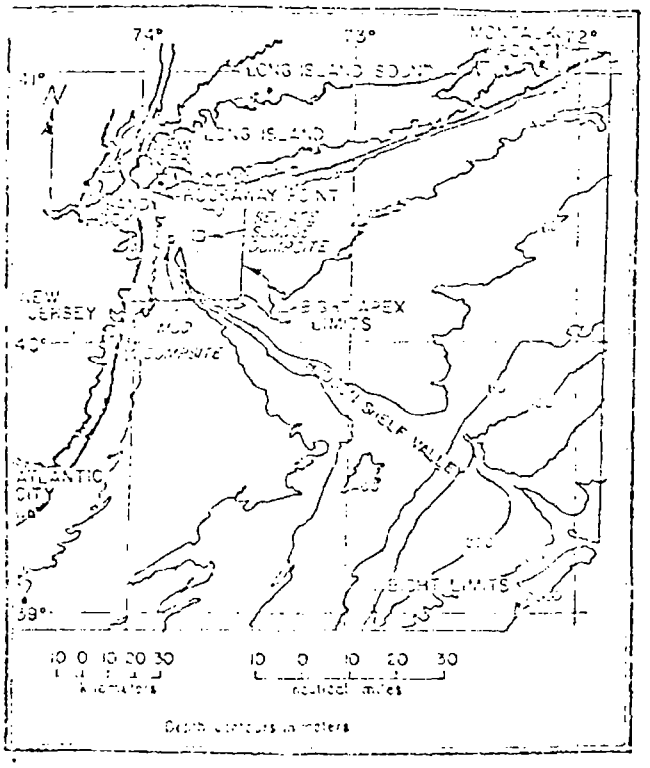

Fig. 3. Bathymetric map of the New York Bight showing the location of the Mud Dumpsite and Sewage Sludge Dumpsite where both P-BOMs and B-BOMs were used in monitoring impacts

liquid nitrogen, whereas in others the liver is removed and frozen separately for enzyme analysis and other tissues prepared for trace metal or hydrocarbon analyses. By comparing the concentration of, say, toxic metals such as cadmium in the tissues of individuals of a species living (a) at the dumpsite, (b) held at the dumpsite in BOMs, and (c) individuals of the same species at the control site, one can determine whether or not bioaccumulation of critical compounds is taking place in key species. We know that bioaccumulation is a normal function, but what is not so easy to determine is whether or not the accumulation is stressing the organism. In other words, we are lacking a calibrator; the metabolic enzyme approach promises to fill this need.

Laboratory analyses

The initial laboratory analyses of organisms exposed to organochlorine wastes in P-BOMs attempted to discern the presence of organochlorine wastes in exposed organisms. Later histological studies of various tissues of exposed and control organisms were added to the routine, but both endeavors required longer periods of exposure than were deemed practicable for monitoring work. Therefore, more subtle means of detecting stress early on in the exposure were sought. As a result, intitial tests were run on a suite

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of metabolic enzymes that it was anticipated would respond to particular classes of chemicals. The rationale for this search was simply that any living cell maintains itself by extracting energy from its environment and transforming it into chemical energy to drive biosynthetic reactions and many other energy-requiring reactions of which the various specialized cells of the body are capable (Atkinson, 1969). All of these activities are mediated through enzymes that are quite functionally specific. It is well known that changes occur at various points in the enzyme systems when an organism is under environmental stress (Bend & Hook, 1977). The initial response may be either an increase or decrease of enzyme activity in cells, say, of the liver (Chambers & Yarbrough, 1976). It should be pointed out, however, that the methods of analysis followed in our work measure enzyme activity, not actual concentrations. In some instances there is undoubtedly a direct proportion between activity and concentration, but this is beyond the scope of the present investigation. Eventually there may be a failure of the cell function catalysed by the particular enzyme under study; prior to this the organism is likely to exhibit a chronic nonlethal response. Thus, the disposal of toxic chemical wastes in the aquatic environment, which is often periodic, will serve as the stimulus for chronic stresss responses. Between disposals the organism may be able to rid itself of sufficient toxic material to exhibit only nonlethal responses. Even so, its subnormal response is signalling that if it is subjected to an intensification of the disposal activities, by increasing amounts per dump or by shortening the time between dumps, acute and lethal responses may be evoked (Pequegnat, 1978).

Enzymes selected for monitoring

After testing a dozen or so enzymes, TerEco selected three for field work. At present we are using ATPase, which is found in cell mitochondria and responds particularly well to excess biphenyls in the environment; catalase, which is dissolved in the cytosol and responds to excesses of toxic metals; and cytochromes P-420/450, which respond to metals but particularly to cyclic and long-chain hydrocarbons. In addition, TerEco has employed the adenylate energy charge system in a few field tests. The advantage of this latter technique, which involves analysis of ATP, ADP, and AMP, is that a complicated set of enzyme reactions is reduced to a single parameter that relates all control mechanisms to the energy level of the cell (Atkinson, 1969), which is then expressed as the following ratio:

$$\text{E. C.} = \frac{\text{ATP} + \frac{1}{2}\text{ ADP (adenosindiphosphate)}}{\text{ATP} + \text{ADP} + \text{adenosinmonophosphate (AMP)}}$$

The energy charge of a healthy cell centers around 0.85. Only at and above this level can growth and reproduction occur. Viability is maintained between 0.8 and 0.5, but death occurs at levels below 0.5. Chlorinated hydrocarbons and some metals act as inhibitors of the electron transport system enzymes with a consequent lowering of the energy charge ratio.

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RESULTS

ATPase analyses

During the summer of 1978 tests of the effectiveness of ATPase as an indicator of stress were run on fish (*Fundulus grandis*) kept in B-BOMs at the Mud Dumpsite in the New York Bight (Fig. 3). During this period dredged material taken from polluted New York Harbor was being dumped three times daily throughout the seven days of the test. B-BOMs were placed upstream of the dumpsite (control), in the site, and one mile downstream of it in the extended impact zone (Fig. 4). The results are shown in Table 1. The method employed to estimate ATPase activity was derived from Pullman et al. (1960) and Fritz & Harick (1966). As indicated above, only mitochondrial ATPase activity is being measured. The differences among the three sites analysed in Table 1 are significant at the 95 % confidence limit.

Tests of sediments showed the presence of PCBs in the dumpsite and well downstream of it. This is reflected in the lower ATPase values in fish held in the dumpsite and downstream BOMs as compared with the upstream site. The differences in ATPase activity among the three sites are significant at the 95 % confidence limit (P = .05).

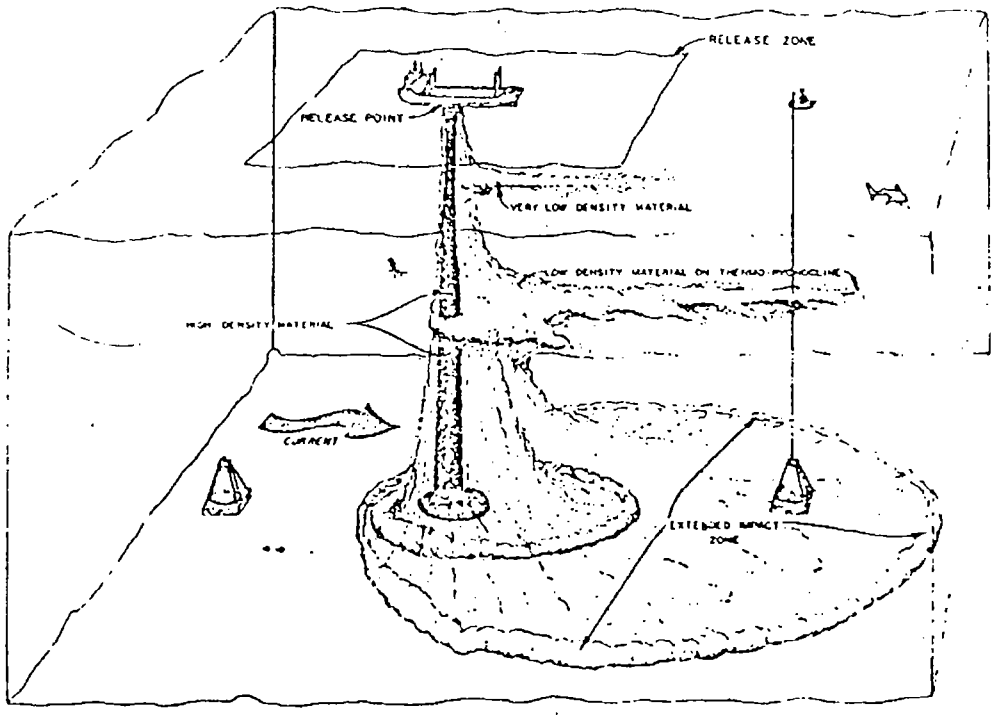

Fig. 4. Conceptualization of the fate of dredged material released from a hopper dredge and the placement of control and test B-BOMs to monitor potential impacts

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Table 1. ATPase levels in *Fundulus grandis* liver after 7 days exposure in B-BOMs in the New York Mud Dumpsite*

Location	No. of fish analyzed	ATPase units** x 10^{-4}/mg protein	Standard deviation · 10^{-4}
B-BOM upstream of Dumpsite	33	12.400	.0004
B-BOM in Dumpsite	35	4.000	.0002
1 Mile downstream of Dumpsite	32	9.100	.0003

* In this and the following tables, analysis of variance was used to evaluate the data
** Bergmeyer Unit for ATPase = amount of enzyme needed to decompose 1 g of NADH (Nicotinamide adenine dinucleotide reduced form) in 1 min

Cytochrome P-420 and P-450

Values for this enzyme are expressed in terms of n Mole P-450 per mg of liver protein. Again tests (~~cats~~) were run at the New York Mud Dumpsite, the Sewage Sludge Dumpsite, and at the control. At the latter dumpsite sewage sludge is dumped by barge several times a day. B-BOMs and P-BOMs were used in the sites and at the control site well east of dumpsites. The results of this summer 1978 test are shown in Table 2. The cytochrome P-450 levels of the *Fundulus grandis* individuals exposed in P-BOMs are significantly higher than levels of benthic fish in B-BOMs at both the Control and Mud Dumpsite (MD) ($P = .05$), whereas at the Sewage Sludge Site (SSS) the two values are relatively close ($P = .10$). When compared to the control site values, the SSS fish show similar P-450 values at the pelagic level, whereas benthic levels are significantly higher ($P = .05$). Pelagic fish at MD show the highest P-450 levels, whereas benthic fish show levels close to those of the control site benthic fish. Cytochrome P-420 levels are significantly higher in pelagic fish than benthics at both the SSS and MD, whereas at the control site the two values are relatively close. Moreover, we note that P-420 levels in pelagic fish at both dumpsites are greater than levels at the control. Since cytochrome P-

Table 2

Table 2. Cytochrome P-450 values from *Fundulus grandis* livers exposed at New York Bight Dumpsites and control (July–August 1978)

Site & BOM type	No. of livers analyzed	nanomoles P-450 + 420 per mg liver protein x · 10^{-2}	Standard deviation
Control Site			
P-BOM	45	0.63	0.10
B-BOM	45	0.35	0.08
Sewage Sludge Site			
P-BOM	45	1.10	0.16
B-BOM	45	0.68	0.09
Mud Dumpsite			
P-BOM	45	2.36	0.70
B-BOM	45	0.34	0.16

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420 is a solubilized form of P-450, the high P-420 levels in the pelagic fish of the dumpsites is perhaps indicative of even stronger induction of the P-450 system at these two sites where experimental fish were undoubtedly exposed to relatively high levels of petroleum hydrocarbons due to heavy ship traffic.

Catalase

Tests for catalase change in the killifish (*Fundulus grandis*) when exposed to results of the dumping of sewage sludge and dredged material were also carried out in the New York Bight in July-August 1978. The results are shown in Table 3.
The lower values of catalase in fish at the sewage and mud dumpsites compared to the upstream control site reflect stresses on the organism in response to excess toxic metals in these disposal environments.

Table 3. Catalase values from *Fundulus grandis* livers exposed at New York Bight Dumpsites and control (July–August 1978)

Site & BOM type	No. of livers analyzed	Catalase units* x 10^{-4}/mg protein	Standard deviation
Control Site			
P-BOM	45	1.20**	004
B-BOM	45	1.80	.003
Sewage Sludge Site			
P-BOM	45	0.9	.0009
B-BOM	45	1.4	.002
Mud Dumpsite			
P-BOM	45	0.5	.0002
B-BOM	45	0.9	.0001

* Bergmeyer Unit = amount of enzyme needed to catalyze the breakdown of 1 g of H_2O_2 per min
** The differences between the P-BOM of control and mud dumpsite are significant (P=.01). The difference betwen the B-BOM of control and sewage site is significant (P=.10) as is the difference between control and mud site (P=.01)

Adenylate energy charge

The following data are the first field data that TerEco has derived from application of an energy charge analysis. They were obtained from the 1978 New York Bight study using the grass shrimp, *Palaemonetes pugio* as the test organism. Data are insufficient to permit drawing firm conclusions, but the following observations can be made from examination of Table 4.
First, all energy charge values are 0.67 or above. Life can be sustained between 0.5 and 0.8, but only at 0.85 can growth and reproduction occur. All of the smaller shrimp (< 200 mg) averaged an E. C. of 0.85 or above, but the larger shrimp did not. In fact, in all cases the older shrimp had low E. C. values, but not to the point of lethality. The B-BOM values are uniformly lower than those from P-BOMs. In addition to pollutant effects, the lower

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Table 4. Adenylate energy charge P-BOM and B-BOM samples New York Bight Control, Sewage Sludge, and Mud Site. Test organism: *Palaemonetes pugio* (July–August 1978; average 40 shrimps' BOM)

Site & BOM type	Population segment wet weight (mg)	Mean energy charge
Control Site		
P-BOM	< 200	.85 ± .06
	> 200	.70 ± .11
B-BOM	< 200	.77 ± .12
	> 200	.69 ± .11
Sewage Sludge		
P-BOM	< 200	.85 ± .05
	> 200	.67 ± .04
B-BOM	< 200	.80 ± .04
	> 200	.73 ± .05
Mud Dumpsite		
P-BOM	< 200	.87 ± .04
	> 200	.72 ± .05
B-BOM	< 200	.77 ± .01
	> 200	.68 ± .08

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Table 5. Enzyme levels (means and standard deviations) in *Fundulus* liver tissue. Fish exposed in Biotal Ocean Monitors off Louisiana 1979. 15 fish were analyzed at each Station

Station No.	Cytochrome P-450 + 420 nanomoles P-450/mg protein	Catalase units* x 10^{-2}/mg protein+	ATPase units** x 10^{-4}/mg protein++
1	.1202 ± .0557	2.951 ± .010	7.666 ± .0002
2	.1168 ± .0732	3.371 ± .015	7.901 ± .0005
3	.1504 ± .0731	2.255 ± .009	4.573 ± .0002
4	.1464 ± .0671	3.588 ± .011	9.106 ± .0006
5	.1142 ± .0459	3.559 ± .015	7.725 ± .0004
6	.2072 ± .0540	4.120 ± .017	7.34 ± .0001
7	.2047 ± .0969	2.813 ± .008	6.151 ± .0002
8B	.1520 ± .0346	3.891 ± .018	5.045 ± .0002
8P	.2688 ± .1358	2.297 ± .007	4.243 ± .0002
Mean of means	.1600 .0700	3.210 .010	6.640 .0003

* Bergmeyer Unit (Catalase) = amount of enzyme needed to catalyze the breakdown of 1 g of H_2O_2 per min

** Bermeyer Unit (ATPase) = amount of enzyme needed to catalyze the breakdown of 1 g of NADH (Nicotinamide adenine dinucleotide reduced form) per min

\+ Standard deviation x 10^{-2}

\+\+ Standard deviation x 10^{-4}

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Table 6. Adenylate energy charge ratios for whole grass shrimp *Palaemonetes pugio* and commercial shrimp (Abdominal Muscle) exposed in Biotal Ocean Monitors for seven days (LOOP 1, September 1979)

Station No.	Grass shrimp (avg. 40 animals/station) Mean ratio	Standard deviation
1 (B-BOM)	.75	.12
2 (B-BOM)	.73	.12
3 (P-BOM, diffuser)	.72	.07
4 (B-BOM)	.86	.08
5 (B-BOM)	.82	.12
6 (B-BOM)	.79	.11
7 (B-BOM)	.84	.13
8 (B-BOM) Controls	.73	.17
8 (P-BOM)	.77	.12
Reference control	.77	.12
Stations as above	commercial shrimp (avg. 10 animals/station)	
1	.81	.08
2	.85	.14
3 (no sample)		
4	.83	.09
5	.83	.10
6	.87	.01
7	.78	.11
(B-BOM only)	.77	.07
Reference control	.77	.13

B-BOM values may als be related to a factor of depth at and below the thermocline where temperature is depressed with increasing depth.

In September 1979 the enzyme levels in *Fundulus grandis* and the energy charge (E. C.) were analysed in grass shrimp (*Palaemonetes pugio*) and the commercially important brown shrimp (*Penaeus aztecus*) held in BOMs for seven days at eight stations in an area of relatively unpolluted waters off the Louisiana coast. This baseline study is sponsored by Louisiana Offshore Oil Platform, Inc. (LOOP) and is related to the planned discharge (in 1980) of large quantities of concentrated brine (265 ppt) into coastal waters when the deep water port is operational. The results for the enzymes are shown in Table 5 and for E. C. in Table 6. The enzyme and E. C. results are comparable to those obtained in the New York Bight but do reflect a cleaner environment. Note also in Table 6, which is based on the same data, that the E. C. of the penaeid shrimp (commercial species), which were captured in the vicinity of the stations and were adapted to the environment, averaged higher than that of the grass shrimp. which is characteristically an estuarine organism.

DISCUSSION

Catalase and ATPase

The uses of catalase and ATPase as measures of response to excess toxic metals and biphenyls in the environment are sufficiently straightforward as to need no further

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discussion at this time. However, we do plan to maintain them in our Biotal Ocean Monitoring System along with E. C. and cytochrome P-450, which are just now coming into more general use.

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P-450 MFO system

Evidence is accumulating that the P-450 mixed function oxidase (mfo) system can be a useful indicator of the sublethal effects of petroleum hydrocarbon pollution on a variety of marine organisms (Stegemann, 1978). If so, it will become an important monitor of environmental impacts. Buhler (1966) provided the basis for this by demonstrating that detoxification of organic xenobiotics in fish can be related hepatic metabolic activity. Since then evidence has accumulated that the cytochrome P-450 dependent mfo enzyme system is the mechanism by which a variety of environmental pollutants (especially petroleum hydrocarbons and PCBs) are detoxified in the livers of fish. Components of the cytochrome P-450 linked mfo system are bound to the membranes of endoplasmic reticulum with highest levels in the liver. When analysed spectrophotometrically, the cytochrome P-450 hemoproteins in a CO-bound reduced state display a Soret absorption maximum peak near 450 nm, while the solubilized P-420 exhibits a Soret peak at 420 nm (Omura & Sato, 1964).

The biotransformation of sublethal concentrations of environmental pollutants by the mfo system is interesting due to the apparent inducibility of the system. Lidman et al. (1976) and Statham et al. (1978) demonstrated induction of increased P-450 and mfo enzyme levels in rainbow trout upon exposure to PCBs and polycyclic aromatic hydrocarbons, respectively. In a study of the estuarine fish *Fundulus heteroclitus*, Burns (1976) found induction of P-450 in laboratory fish exposed to phenylbutazone and some evidence of elevated P-450 levels in fish from polluted natural environments relative to populations from nonpolluted areas. Of particular interest to us is that Yarbrough-& Chambers (1977) showed induction of the mfo system in mullet exposed to crude oil extract for only four days, and Stegeman (1978), monitoring P-450 in natural fish from an oil-contaminated marsh relative to fish from unpolluted environments, found significantly higher P-450 levels in polluted fish. Results of the present enzyme analyses suggest that biphenyls average higher in the sediments of the Mud Dumpsite than off the Louisiana coast. It appears that trace metals in sediments of the mud and sewage dumpsites in the New York Bight are having greater impact on benthic organisms than off Louisiana. More surprising, however, is the observation based on cytochrome P-450 levels that petroleum hydrocarbons are a more significant impact in the New York Bight than off Louisiana where offshore oil platforms abound.

Adenylate energy charge

Few reports exist on the application of the adenylate energy charge system (E. C.) to pollution studies. But the generality of its potential use should make it a very sensitive and useful assay for low-level or chronic responses to toxicants. Brezonik et al. (1975) discuss the rapid response of ATP to additions of toxic substances and suggest the use of ATP in toxicity studies; hence the potential for pollution studies of certain types is well founded. The rapid drop in E. C. ratios noted by Chapman et al. (1971) as a response to oxidative phosphorylation inhibitors would suggest the use of this assay for chlorinated

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hydrocarbons or PCBs, since Pardini (1971) notes that their toxic effect is observed most readily as inhibition of electron transport system enzymes. Since a ratio is dimensionless, a direct comparision can be made from baseline to test to control conditions and from one collecting station to another without the ancillary sample series required were one to use ATP values alone. Accordingly, the advantages of its use would be its sensitivity for assaying detrimental effects of low levels of toxicants and the simplification of field sampling logistics.

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