Using Bioaccumulation and Growth in Caged Intertidal Oysters to Assess Oil Exposure and Effects in Delaware Bay

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Abstract

As part of a controlled release of oil on an intertidal beach in Delaware Bay, 130 oysters (*Crassostrea virginica*) were transplanted at each of 11 intertidal sites. Oyster survival was over 96 percent. Exposure was characterized by measuring the accumulation of polynuclear aromatic hydrocarbons (PAHs) in oyster tissues on days 0, 1, 8, 15, and 28. Effects were characterized by measuring oyster weights and lengths at the beginning and end of the test to estimate growth. End-of-test (EOT) tissue weights were found to be the most discriminating effects endpoint, and statistically significant differences were found when comparing treatment and control groups of oysters. A statistically significant relationship was also found between Day 1 tissue PAHs and EOT tissue weights. This suggests that bivalve tissue burdens measured after only one day of exposure can be used to predict potentially adverse effects over a longer period of time. This relationship demonstrates potential applications for monitoring oil spills on a real-time basis for both exposure and effects.

1.0   Introduction

In the summer of 1994, EPA conducted an experimental oil spill on the shoreline of Fowler Beach, Delaware Bay to evaluate the effects of various bioremediation techniques (Venosa *et al.*, 1996). A series of laboratory bioassays were also conducted on sediment and pore water sampled at various intervals within the oiled and control plots as part of the assessment (Mearns *et al.*, 1995). These tests included Microtox, sea urchin and grass shrimp embryo tests, and amphipod survival tests. They were primarily intended to evaluate toxicity, under controlled laboratory conditions, of sediment-sorbed oil and pore water samples collected from the oiled plots at various intervals. This would also provide valuable information on toxicity at various stages of the oil degradation process. These traditional approaches provided little information on fate and effects of the applied oil in the water column adjacent to the oiled plots under natural conditions. Therefore, this field bioremediation experiment also provided a unique opportunity to conduct a study to evaluate the use of caged bivalves in the intertidal zone to characterize exposure and effects of the experimental shoreline spill. The intertidal transplant had three objectives: (1) Evaluate the use of transplanted bivalves for monitoring oil spills; (2) Quantify temporal and spatial variability in exposure and effects associated with the oil release; and (3) Assess relationships between PAH tissue burdens and oyster growth.

There is a need to develop and standardize protocols for using caged bivalves as a field bioassay for oil spills because traditional field monitoring and laboratory bioassays do not adequately characterize exposure and associated biological effects. Using transplanted bivalves as *in-situ* bioindicators bridges the gap between lab and field by combining the environmental realism of field monitoring with the experimental control of laboratory bioassays (Figure 1). This concept of experimental control has also been used to justify the use of experimental oil spills (Lindstedt-Siva, 1994). More useful information can be gained by characterizing exposure and effects under realistic field conditions than just by using traditional laboratory bioassays, field monitoring, or even microcosm or mesocosm tests.
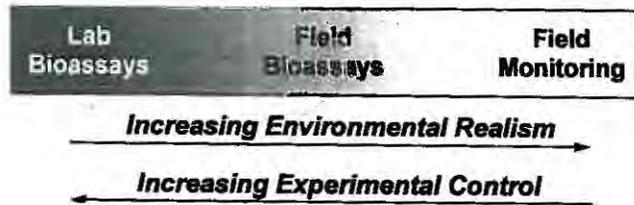
Figure 1. Bridging the Gap between Laboratory Bioassays and Field Monitoring

This field bioassay approach using caged bivalves also facilitates the synoptic measurement of chemical exposure and bioeffects on a *real-time* basis during an oil spill. Integrated sampling over space and time is critical to monitoring oil spills in progress due to the ephemeral nature of spills. Most of the basic methodologies, including exposure and effects assessments, currently exist to conduct in-situ monitoring of oil spills. Although useful relationships have been established for relating exposure, dose, and response, very few studies have utilized these methodologies to provide useful information for either spill response, damage assessment or ecological risk assessment applications. Caged mussels have been used to monitor other chemical releases, such as during dredge disposal operations. This has facilitated near real-time decisions regarding project scope and duration by measuring chemical exposure endpoints and effects endpoints like bioaccumulation and growth or scope-for-growth (Nelson *et al.*, 1987; Nelson, 1991; Nelson and Hansen, 1991). However, emphasis has traditionally been on using bivalves to characterize exposure rather than effects.

Bivalves can be strategically deployed along physical and chemical gradients and in assessment areas where they might not normally settle, either within or outside the intertidal zone (Figure 2). This is particularly important in evaluating exposure and potential effects of oil spills removed from shorelines where it is more difficult to measure natural populations of organisms. Transplant studies conducted with caged animals also facilitate repetitive measurements of the same animals or different groups of the same animals. Repetitive measurements help to identify the fine

structure of temporal and spatial variability (Salazar and Salazar, 1995) and have potential applications for real-time monitoring (Salazar and Chadwick, 1991).

Bivalves are probably the most commonly used *in-situ* bioindicators because they are ubiquitous, sedentary, and responsive to their environment at both micro- and macro-geographical scales and at all levels of biological organization (Green *et al.*, 1985). They integrate biologically available PAHs through filter feeding. Bivalves are also relatively easy to collect and maintain. There is a tremendous amount of background data available based on bioaccumulation in the field and in the laboratory.
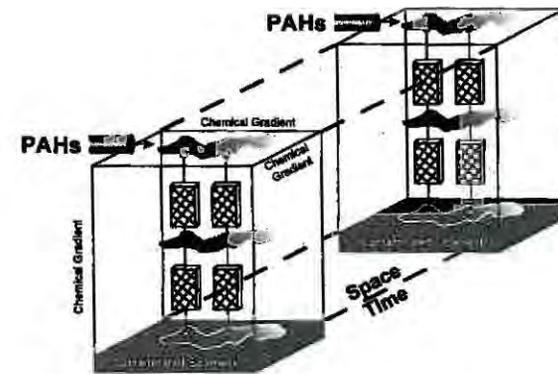
Figure 2. Sampling Space and Time with Caged Bivalves

Measuring bioaccumulation in bivalve tissues provides integrated information about environmental conditions that cannot be defined with chemical measurements of discrete water samples. Bioavailable chemicals like PAHs are accumulated by the tissues of bivalves at concentrations much greater than, and in proportion to concentrations found in the environment. This is why bivalves are commonly used in bioaccumulation studies to estimate exposure and why they can be used to assess oil spills. PAHs have been measured in the tissues of transplanted bivalves when concentrations were below the limits of detection in seawater. Even if PAHs were detectable and bioavailability could be estimated by chemical analysis of water samples, the number of samples that would be necessary to adequately describe exposure to PAHs in the water column would be cost prohibitive. Bivalves have also been shown to be more effective PAH accumulators than lipid bags or other artificial concentration devices (Shigenaka and Henry, 1995). Transplanted mussels were used to show that particulate oil was biologically available well below the surface after the *Exxon Valdez* oil spill (Short and Harris, 1996). This useful information would not have been acquired using indigenous populations because mussels are not naturally found suspended in the water column.

2.0   Materials and Methods

Both mussels (*Mytilus edulis*) and oysters (*Crassostrea virginica*) were transplanted to evaluate exposure to and effects after the release of oil in the intertidal zone. Two null hypotheses were tested: (1) there is no difference in accumulation of total PAHs by oysters between treatment and control sites, and (2) there is no difference in oyster growth between treatment and control sites. Approximately 540 gallons of light crude oil were released by the U.S. EPA on a 400 meter section of beach in a randomized block design (Venosa *et al.*, 1996). The U.S. EPA oil spill experiment was initiated on June 30,1994 and lasted 14 weeks. Although all of this oil was released on day one, chemical measurements demonstrated that some residual oil continued to be released to the environment during the 28-day oyster study (Mearns *et al.*, 1997).

Only the oyster portion of the study will be described here since mussels suffered high mortalities, which were associated with high temperatures outside of their normal range and the high intertidal position. From the randomly assigned oysters, separate groups were deployed for effects (i.e., growth) measurements and exposure (i.e., bioaccumulation) measurements. Disease-free oysters were provided by the State of Delaware. Oysters for the growth study ranged in length from 49 to 104 mm and in weight from 19.4 to 133.1 g-wet (Table 1). Oysters for the bioaccumulation study ranged in length from 63 to 103 mm and in weight from 45.8 to 166 g-wet (Table 2). There was no statistical difference in either oyster length or whole-animal wet-weight among sites at the start of the test ($\alpha = 0.05$) for either the growth or bioaccumulation groups. Oysters were held in coarse mesh plastic sleeves (oyster culch netting) with each oyster confined to an individual compartment. Compartmentalization facilitates paired growth measurements on the same individuals at the beginning and end of the test, reduces variability when compared to unpaired measurements, and increases the statistical power of the test. Growth metrics included whole-animal weights and lengths, tissue weights, and shell weights. Due to the stressful conditions in the intertidal zone in the summer, weight and length increases were extremely small and end-of-test (EOT) tissue weights proved to be the most reliable growth metric. Only EOT tissue weight results will be discussed here.

Oysters were prepared for deployment by randomly assigning individuals to bags. The systematic distribution process described in Salazar and Salazar (1995) was used to minimize differences in oyster lengths and weights at the beginning of the test. Statistical analyses were performed before deployment to ensure that there were differences in oysters weights or lengths before the experiment began. No differences were found. Plastic cable ties were used to separate individuals in the mesh sleeves. Bags of oysters were attached to a PVC frame in the field so that the bags were parallel to the beach; the legs of the PVC frame were driven into the sediment to secure the deployment array in the intertidal zone at approximately +1 meter MLW. This high intertidal position combined with high summer temperatures provided an additional stress to the oysters. Mussels were sorted on the first day, oysters on the second day and both were attached to the PVC frames on the third day.

Table 1. Oyster Metrics on Animals in Growth Experiment

	Control Group 1			Treatment Group					Control Group 2		
	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10	Site 11
Initial Length (mm)											
Min	49	52	50	51	51	50	50	53	50	52	53
Max	88	104	97	96	86	96	91	94	93	92	91
Mean	71.4	73.3	72.4	73.1	70.7	72.5	71.8	73.1	72.5	71.4	71.7
Site Dev	8.6	9.4	8.8	8.7	8.2	8.9	8.5	8.4	8.7	8.6	9.1
N	80	80	80	80	80	80	80	80	80	80	80
EOT Length (mm)											
Min	54	51	52	52	52	55	52	53	50	54	53
Max	92	101	96	96	89	94	91	92	88	94	93
Mean	73.8	75.7	74.0	75.3	72.8	74.1	71.2	73.8	74.1	73.8	72.8
St Dev	8.1	9.0	8.4	8.4	7.6	8.6	8.2	8.3	8.4	7.8	8.6
N	79	77	77	80	77	78	70	76	79	79	79
Initial WA Weight (g-wet)											
Min	22.7	22.9	20.5	24.1	25.0	23.2	19.4	20.4	19.9	27.3	29.9
Max	118.1	133.1	127.4	127.0	107.9	122.1	103.9	124.3	116.9	114.5	107.6
Mean	66.2	69.4	68.2	67.8	64.4	68.7	64.7	69.9	67.0	66.7	65.8
St Dev	20.8	22.3	20.6	20.0	18.3	20.3	19.0	20.7	21.0	18.5	19.9
N	80	80	80	80	80	80	80	80	80	80	80
EOT WA Weight (g-wet)											
Min	24.3	24.3	22.1	24.9	28.2	24.8	19.6	20.7	20.1	29.8	30.6
Max	117.3	132.5	120.5	129.3	107.9	122.1	101.6	122.4	115.7	115.4	108.2
Mean	66.5	69.8	68.0	69.1	65.1	68.8	62.4	69.1	68.0	67.3	66.4
St Dev	20.2	21.4	19.5	19.6	17.6	19.9	18.6	20.1	20.4	17.8	19.7
N	79	77	77	80	77	78	70	76	79	79	79
EOT Tissue Weight (g-wet)											
Min	2.4	2.5	2.6	2.6	2.1	3.1	2.2	1.7	2.0	2.6	2.6
Max	13.0	13.7	8.2	14.2	9.4	10.8	9.5	10.5	9.7	8.9	9.1
Mean	6.7	7.2	5.8	5.8	5.8	6.4	4.9	5.5	5.5	5.4	6.3
St Dev	1.9	2.2	1.3	1.7	1.6	1.7	1.4	1.9	1.7	1.4	1.3
N	79	77	76	80	77	78	70	76	79	79	78
Percent Survival	99%	96%	95%	100%	96%	98%	88%	95%	99%	99%	98%

WA = Whole Animal

Table 2. Oyster Metrics on Animals in Bioaccumulation Experiment

	Control Group 1			Treatment Group					Control Group 2		
	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10	Site 11
Initial Length (mm)											
Min	73	67	67	70	69	71	63	68	68	68	65
Max	96	103	96	96	96	93	97	96	95	98	95
Mean	84.7	83.4	83.9	82.8	84.5	83.1	84.9	82.8	83	84.2	84.4
St Dev	5.4	7.51	6.96	7.3	5.37	6.44	6.36	6.41	7.1	6.91	6.46
N	50	50	50	50	50	50	50	50	50	50	50
Initial WA Weight (g-wet)											
Min	52.8	58.3	49.2	59.2	55.9	51.3	59.3	67.5	45.8	62.3	57.4
Max	150	147	148	160	141	141	160	166	155	153	145
Mean	99.5	95	96.8	98.1	97	95.7	102	102	103	101	103
St Dev	22.4	19.3	21.8	23.3	21.9	18.1	23	21.8	22	20.3	21.4
N	50	50	50	50	50	50	50	50	50	50	50

WA = Whole Animal

A total of 130 oysters (80 for growth measurements and 50 for bioaccumulation measurements) and 150 mussels (100 for growth and 50 for bioaccumulation) were deployed on June 29,1994 at each test site. A total of 280 bivalves were deployed at each of 11 sites along an intertidal transect (Figure 3): five treatment sites in the immediate vicinity of the oil treatment plots, and three control sites on either side of the oil treatment plots. The three control sites toward the head of the bay are designated Control Group 1 (C1), and the three sites toward the mouth of the bay Control Group 2 (C2). Each of the treatment sites was separated by 100 meters.
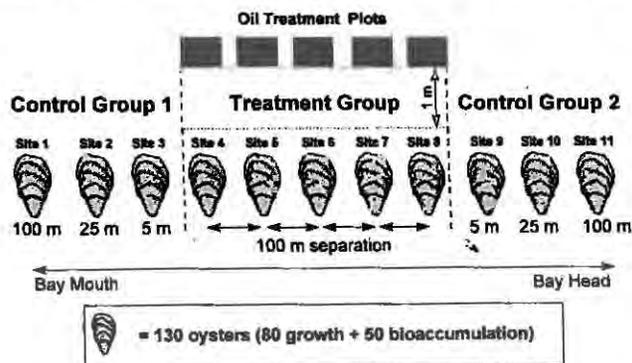
Figure 3. Intertidal Oyster Deployment Configuration

All oysters in the growth group were measured for weights and lengths at the beginning of the test and at the end of the test after 28-days exposure. At the end of the test, the tissues were removed from all surviving individuals and weighed. During the exposure period, subsets of oysters from the bioaccumulation group were sampled on days 1, 8, 15, and 28 to assess the rate of accumulation and depuration of PAHs. Although tissues were analyzed for individual PAH compounds, only total PAHs will be discussed here because the data for individual PAHs are still being analyzed.

Temperature was measured at 6-minute intervals for the Control Site 1 (closest to the bay mouth), Treatment Site 7 (in the middle of the oiled plots), and Control Site 11 (nearest the bay head) throughout the 28-day exposure period. These measurements were made with three *in-situ* monitors that recorded measurements electronically and provided over 6,500 temperature data points. This allowed us to confirm the time of air exposure and water immersion on a daily basis and calculate mean water and air temperatures over the exposure period. Although these data are still being analyzed, there are some generalizations that can be made with regard to possible temperature effects.

3.0 Results

Mean oyster survival was over 96 percent and ranged from 88 to 100 percent at each site. There was some apparent growth, both in terms of length and whole-animal wet-weight, but there were no statistically significant differences among sites in either of these parameters. Significant differences among sites were found for EOT tissue weights. Mean EOT tissue weights for Control Group 1 (C1=6.6 g-wet) were significantly higher (p< 0.001) than either Control Group 2 (C2=5.7 g-wet) or the Treatment Group (TG=5.7 g-wet). However, pooling the two control groups showed that mean EOT tissue weights in the controls (C1+C2=6.1 g-wet) was significantly higher than the Treatment Group (TG=5.7 g-wet). Summary statistics for the oyster metrics are provided in Table 1.

All oysters used in this study accumulated elevated concentrations of PAHs in their tissues when compared to tissue burdens at the start of the test. For the first sampling interval (Day 1), oysters at C1 had significantly less PAHs in their tissues than either C2 or TG (p = 0.0014). The accumulation and depuration of total PAHs in oyster tissues over time is shown in Table 3 and Figure 4. By the second sampling interval (Day 8), there was no difference in the concentration of PAHs in oyster tissues among C1, C2, and TG (p = 0.2845). By the third sampling interval (Day 15), oysters from TG had significantly higher concentrations of PAHs in their tissues than either C1 or C2 (p = 0.0012), and this relationship persisted until the last sampling interval (Day 28) (p = 0.0036). The statistical comparisons of PAHs in oyster tissues are summarized in Table 4. Oysters in the treatment group did not depurate PAHs to pre-exposure levels in 28 days. A statistically significant relationship was shown between total PAHs in oyster tissues at day 1 and end-of-test oyster tissue weights (Figure 5).

Table 3. Total PAHs (ng/g-dry) in Oyster Tissues at Sampling Intervals

		Day 1	Day 8	Day 15	Day 28
C1 Control Group 1	Site 1	18.34	28.47	21.56	2.63
	Site 2	32.18	30.62	32.89	8.29
	Site 3	18.38	37.90	35.52	2.38
TG Treatment Group	Site 4	133.11	53.50	52.71	14.10
	Site 5	131 02	279.57	95.25	29.25
	Site 6	69.82	75.68	84.25	15.46
	Site 7	149.93	95.94	99.49	22.37
	Site 8	124.07	51.01	73.87	15.58
C2 Control Group 2	Site 9	112.13	55.77	31.64	6.27
	Site 10	86.69	40.33	33.81	4.53
	Site 11	78.82	57.34	16.05	4.20

There were no statistically significant differences in temperatures among sites. Except for two occasions during the 28-day exposure period when the water temperature dropped to 17°C and 19°C respectively, water temperatures generally remained above 21°C. Mean exposure temperature (air and water) was about 26.5°C for all sites. On three occasions, air temperature exceeded the limits of the temperature monitor (38°C). Separate measurements on those days revealed air temperatures near 40°C. On three other occasions air temperatures exceeded 35°C.

Table 4. Statistical Comparisons of PAHs in Oyster Tissues at Sampling Intervals

Day 0	Day 1	Day 8	Day 15	Day 28
C1 = C2 = TG	C1 = C2 ≠ TG	C1 = C2 = TG	C1 = C2 ≠ TG	C1 = C2 ≠ TG
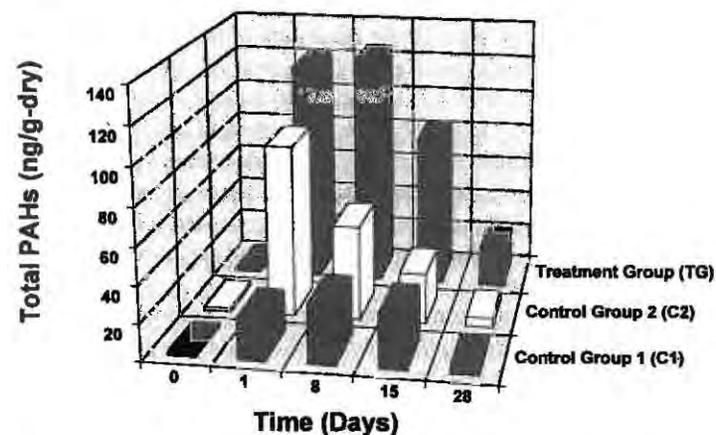
Figure 4. Accumulation/Depuration of Total PAHs in Oyster Tissues Over Time
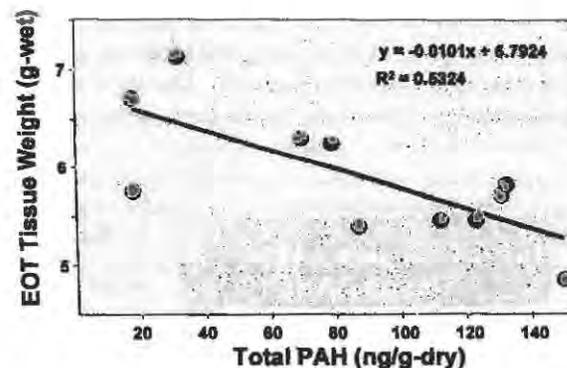
Figure 5. EOT Tissue Weight vs Total PAHs in Oyster Tissues on Day 1

4.0 Discussion

This study provided useful information on rates of accumulation and depuration of PAHs in oysters, the relationship between tissue PAH concentrations and oyster growth, and the effects of temperature on survival and growth of oysters and mussels in Delaware Bay. The relationship established between oyster growth based on EOT tissue weights and tissue PAHs after only one day of exposure suggests that the initial exposure may be associated with subsequent reductions in growth, even after body burdens have declined. It should be added, however, that even though the primary dose of PAHs came as a single pulse at the beginning of the test, some residual PAHs continued to be released from the oiled plots (Mearns *et al.*, 1997). Nevertheless, this study provides evidence that tissue PAH concentrations can be used to predict potential effects, particularly if tissue burdens are measured near their maxima. Further, in the context of oil spill monitoring, it provides some evidence that a short-term exposure and bioaccumulation associated with a tissue dose, can be used to predict subsequent effects. Although this study only provided information on general PAH uptake and depuration, it identified several areas of the *in situ* methodology that would benefit from modification.

The most encouraging result from this study was the relationship between EOT tissue weights and tissue concentrations of total PAHs after only one day of exposure. A number of investigators have emphasized the importance of using chemical tissue burdens to predict potentially adverse effects. McCarty (1991) has suggested the combining exposure and effects endpoints in standard laboratory bioassays, and we have routinely used this approach in our caged bivalve studies (Salazar and Salazar 1995, 1996, 1997a, 1997b, in review) because of the ease of measuring bioaccumulation and growth in caged bivalves.

Donkin *et al.* (1989) not only developed similar relationships for mussel (*Mytilus edulis*) tissue burdens and feeding rates, but they actually measured them. It was as a result of this work and the pioneering work of Long and Chapman (1985) that we developed the exposure-dose-response triad (Figure 6). This approach is consistent with the both damage assessment and risk assessment formats that emphasize a characterization of exposure and effects.
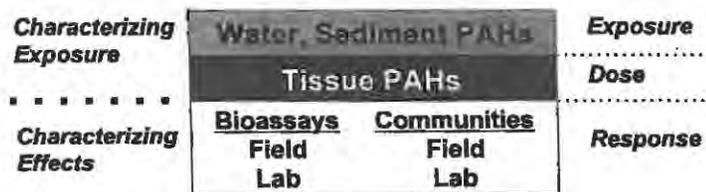
Figure 6. The Exposure-Dose-Response Triad emphasizing Tissue PAH Burdens

In most of our previous studies, multiple measurements of whole-animal wet-weights and lengths have been more discriminating than EOT tissue weights. Generally, two or more measurements on the same individual (paired measurements) reduces the variance when compared to two measurements made on random samples from the same two groups (unpaired measurements), as well as the variance associated with single measurements (Salazar and Salazar, 1995). Recently however, this and other studies have shown that EOT tissue weights can be equally or more discriminating than multiple measurements on the same individual (Salazar and Salazar, in review). This seems to be particularly true when growth rates are small (Salazar *et al.*, 1996; URS, 1994; this study). Furthermore, our most recent analyses suggest that since shell growth and tissue growth are decoupled and occur at different rates and sometimes in a different sequence (Hilbish, 1986), measuring tissue growth can provide a different perspective on different aspects of whole mussel growth (Salazar and Salazar, in review). We have always advocated measuring as many different mussel metrics as possible to provide a more complete assessment of mussel growth.

Results of this study also emphasized the importance of temperature in mussel physiology and conducting pilot studies before transplanting bivalves in a study of this importance. Due to time constraints and the need to work within a pre-defined study design, the bivalve study was conducted during the summer when air and water temperatures were at a maximum. The primary risk in using *Mytilus edulis* were associated with high summer temperatures at the study site near the southern limit of its range on the east coast of North America (Wells and Gray, 1960). Growth reductions have been previously associated with temperatures >20°C (Incze *et al.*, 1980; Almada-Vilella, 1982; Salazar and Salazar, 1996). This situation was exacerbated by transplanting the bivalves high in the intertidal zone so they were close to the oiled plots. While it was predicted that the test animals would be immersed in water 17 to 19 hours per day, it is not clear that this actually happened. It is also possible that warm temperatures >20°C in the holding facilities contributed to pre-deployment stress, decreasing their chance for survival. In a subsequent experiment, mussels from the same collection site were transplanted to the same test site in Delaware Bay without being held or processed in the lab and they suffered high mortalities as well. This study confirms that intertidal transplants add more stress on test animals than animals held subtidally. This must be considered during the study design phase.

Previously we have shown how caged bivalves can be used to demonstrate site-specific differences, short- and long-term trends, temporal and spatial variability, source identification, and dose-response relationships (Salazar and Salazar, 1995). Not all of those capabilities were demonstrated here because of the short test duration. However, this study has shown the versatility of caged bivalves in assessing exposure and effects in a variety of environments. It has also highlighted the ability to use interval sampling on a short time scale to provide information that could be used in

spill response. Repetitive sampling of tissues can also document the duration of the exposure and the duration of the effects. This is a potentially powerful tool for both damage assessments and risk assessments. Widdows and Donkin (1992) have provided a format for using similar approaches to establish cause and effect relationships. Clearly, this single experiment in Delaware Bay has not demonstrated cause and effect since other variables could have contributed to the observed decreases in oyster growth with increasing tissue burdens of total PAHs. Nevertheless, a framework has been established for using this method in assessing oil spills on a real-time basis.

As a result of this and other studies (Salazar *et al.*, 1996; URS, 1994), the significance of using EOT tissue weights to estimate growth and associated effects has become more apparent. We suggest that the standard protocols include measuring as many tissue weights at the start of the test as there are for each replicate during the test. For example, in our most recent studies which contained three replicates of 100 animals each at each site, three replicates of 100 animals each were measured at the start of the test for tissue weights and shell weights in addition to whole-animal wet-weights and lengths. The tissues from these animals were then used to obtain background tissue burdens of the chemicals of concern. This approach provides a better estimate of tissue weights for all individuals at the start of the test. Although it is impossible to obtain true tissue weight changes for deployed individuals, by having a larger number of T_0 tissue weight measurements, it is possible to identify significant increases or decreases in tissue weights which are necessary when evaluating the tissue chemistry data.

5.0 Conclusions

(1) Time scales of exposure and effects monitoring need to be commensurate with those changes that occur naturally in the field and with species selected for the assessment. (2) The exposure-dose-response triad provides a framework for collecting useful information to support spill response, damage assessment, and ecological risk assessment. (3) Caged bivalves are a potentially powerful tool for characterizing exposure and effects under natural conditions within environmentally-relevant time scales.

6.0 Acknowledgments

7.0 References

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