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IN-SITU BIOASSAYS USING TRANSPLANTED MUSSELS: I. ESTIMATING CHEMICAL EXPOSURE AND BIOEFFECTS WITH BIOACCUMULATION AND GROWTH

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ABSTRACT: Using transplanted mussels as an *in-situ* bioassay to assess marine environmental quality has provided important information on bioavailability of contaminants and associated bioeffects that would not have been available with traditional chemical monitoring, biomonitoring, or laboratory bioassays. It is one of the most promising field bioassay systems because of the relative ease in making synoptic measurements of bioaccumulation and growth to estimate chemical exposure and bioeffects, respectively. *In-situ* field studies that utilize transplanted animals combine the advantages of environmental realism associated with field monitoring and experimental control associated with laboratory testing. Because they are sedentary and concentrate contaminants, resident mussels have been used extensively to estimate exposure by measuring contaminants in their tissues. The use of resident mussels as a response indicator has been very limited because of the difficulties associated with measuring biological processes and defining the exposure period in natural populations. Transplanting caged mussels facilitates measurements of bioeffects and clearly defines the exposure period. Data from a series of mussel (*Mytilus galloprovincialis*) transplants in San Diego Bay, California, demonstrate how this methodology can be used to assess the extent of contamination and associated biological effects. Although more than 18 sites were studied in nine separate transplants between 1987 and 1990, emphasis will be placed on two sites in the Shelter Island Yacht Basin separated by only 3 meters vertical distance. The mussel field bioassay was used to identify the following: (1) site-specific differences, (2) temporal and spatial variability, (3) short-term and long-term trends, (4) potential sources of contamination, and (5) dose-response relationships.

KEYWORDS: mussel transplants, sediments, bioaccumulation, growth, contamination, *in-situ* bioassay, standard protocols

Biological monitoring systems are needed to identify the presence of potentially toxic chemicals, quantify their presence in animal tissues, and provide meaningful measurements of biological effects (Mearns 1985; U.S. Environmental Protection Agency 1988). Although there has been extensive use of filter-feeding bivalves to measure bioaccumulation in both laboratory and field studies, synoptic field measurements of exposure and bioeffects have been extremely limited.

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Transplanted mussels are an effective tool to obtain information about exposure and bioeffects associated with chemical contamination that is not provided by traditional chemical monitoring of water, sediment, and tissue or from laboratory bioassays (Phillips 1980; Martin 1985; Nelson 1990; Widdows and Donkin 1992; Phillips and Rainbow 1993; Salazar and Salazar 1994). Traditional approaches do not adequately measure bioavailability or the potential effects of contaminants. The exposure period is always uncertain in chemical monitoring of resident mussels, there is little experimental control, and bioeffects are difficult to measure. In laboratory bioassays, bioeffects are easily measured but the experimental control may introduce unwanted artifacts and the environmental significance is always questionable. Even the most comprehensive approaches that combine chemical measurements of seawater or sediment with laboratory bioassays and community assemblages (Long and Chapman 1985) should be augmented with field bioassays that include more direct measurements of actual exposure and associated biological effects.

Currently, there are no ASTM protocols for *in-situ* bioassays to estimate exposure or bioeffects. There is a need to standardize protocols for the use of transplanted mussels as indicators of exposure and effects because the few protocols that have been developed differ significantly (Martin 1985; Foe and Knight 1987; Weber 1988; Phillips 1988; Clark 1989; Nelson 1990; Salazar and Salazar 1991). Standardization would help ensure consistent and comparable data and interpretation of the results. *In-situ* bioassays should undergo the same level of scrutiny for standardization as any laboratory bioassay, and biological effects monitoring should be emphasized as much as chemical monitoring.

Because of the relative ease in chemical monitoring of mussel tissues, extensive protocols have been developed for collection, tissue extraction, and subsequent chemical analysis. Since only live animals are collected, maintenance during the exposure period has not been an issue and animal health at the time of collection has generally been ignored. The robust nature of mussels and the emphasis on using mussel tissues for estimates of exposure to contaminants has probably led to the lack of appropriate care by inexperienced investigators using caged mussels as an *in-situ* bioassay.

Established in 1977, the State of California has the longest running Mussel Watch program, but the emphasis has been on measuring bioaccumulation in resident populations and mussel transplants to estimate "hot spots" of contamination (Martin and Severeid 1984; Martin 1985). The National Oceanic and Atmospheric Administration (NOAA) National Mussel Watch Program measures bioaccumulation in mussels and oysters throughout the United States (National Oceanic and Atmospheric Administration 1989). As evidenced by these two major monitoring programs, biological processes are seldom measured in the field, and they are clearly not part of routine monitoring (Roesijadi et al. 1984; Foe and Knight 1987). Few studies have been conducted to validate laboratory bioassay results in the field. Although the methods for conducting *in-situ* exposure and bioeffects studies with mussels are relatively simple, few studies with synoptic measurements have been conducted. Furthermore, problems associated with experimental design and handling have commonly led to spurious data and inappropriate data interpretation (White 1984; Salazar 1992). In advocating the use of biological indicators for environmental monitoring, Phillips (1980) has stressed the potential power of bioindicators, but has also cautioned against the potential pitfalls in data interpretation. There are a number of unexplained inconsistencies in survival, bio-accumulation, and growth of mussels in response to contaminant exposure in the laboratory and the field (Kiorboe et al. 1981; Chapman 1983; Cossa 1989; Salazar 1989; Lobel et al. 1990, 1991, 1992; Salazar 1992).

We have successfully transplanted mussels in San Diego Bay and Puget Sound to assess the extent of contamination and associated bioeffects. In all of our studies, both exposure and bioeffects were measured in the same test animals. Data from mussel transplants in San Diego Bay, California, will be used as a case study to demonstrate how this methodology can be used in an environmental assessment. Although more than 18 sites were studied in nine separate transplants between 1987 and 1990, emphasis will be placed on two sites in the Shelter Island Yacht Basin (the most contaminated area) separated by only 3 meters vertical distance. The Puget Sound data are discussed elsewhere (Salazar et al. this volume) and are used to demonstrate how the protocols presented here can be varied to address different issues, such as sediment effects. This "protocol" paper has three primary objectives: (1) Describe the advantages of using transplanted mussels as *in-situ* indicators of chemical exposure and bioeffects; (2) Present the basic protocols that we found successful; and (3) Present data supporting the utility of the approach.

ADVANTAGES OF *IN-SITU* MUSSEL BIOASSAYS

Advantages of Transplants

Transplanted mussels can be used as an *in-situ* bioassay to obtain information on marine environmental quality that would not be available through routine chemical monitoring, biological monitoring, or laboratory bioassays. The transplant approach combines the experimental control of laboratory testing and the environmental realism of field testing (Green et al. 1985; Salazar and Salazar 1994). There is control of integrated sampling over space and time (Martin 1985) with a clearly defined exposure period. As shown in Fig. 1, caged mussels can be strategically deployed along physical and chemical gradients and at sites in the assessment area where resident mussels would not normally be found; subtidally in the water column and away from the shoreline. Caged mussels can be transplanted near suspected sources of contamination, such as sediment or outfalls, to confirm the relative bioaccumulation and bioeffects associated with those sources. Caged animals can be used to sample an infinite matrix over space and time. Transplants also help avoid some of the factors that add to the variability associated with sampling natural populations that have been detailed by Lobel et al. (1990, 1991, 1992). For example, different populations may be at different tidal heights, have a different size/age structure, and may even be composed of different species.

Transplant studies conducted with caged animals also permit repetitive, non-destructive measurements of individuals to increase the statistical power of the test and the ability to identify site-specific differences. Repetitive measurements during a given bioassay and successive transplant studies in a given area allow estimates of temporal and spatial variability. Caging facilitates tracking individuals and making measurements of bioeffects over time to identify short- and long-term trends. Lastly, serial, sequential studies permit quantification of dose-response relationships over a variety of environmentally realistic test conditions. This can be used as a first-order-approximation to predict environmental risk.

Several investigators have used differences in accumulation of contaminants in transplanted mussels for source identification. Sediments were shown to be the source of dichloro diphenyl trichloroethane (DDT) and polychlorinated biphenyls (PCB) contamination by measuring tissues of mussels transplanted at various depths from the surface to the bottom. The concentration of contaminants in tissues decreased with distance from the bottom (Young et al. 1976). Tissues from mussels nearest the bottom had concentrations 10 times higher than

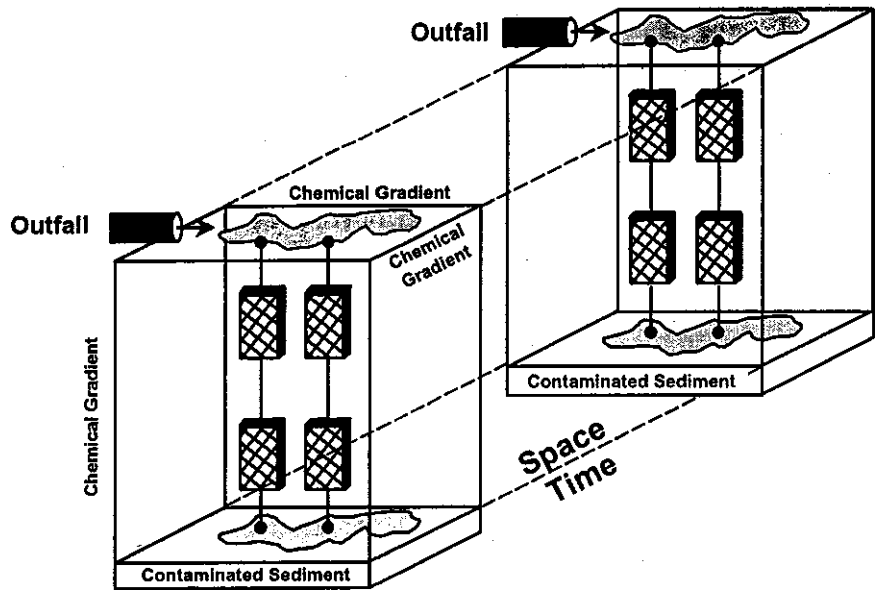

FIG. 1--Diagram of environmental sampling over space and time using *in-situ* transplants of caged mussels along gradients of chemical contamination. Two suspected sources (outfall and sediment), two sites, two depths, and two sampling intervals are shown.

those nearest the surface. In a related study, it was shown that resident mussel populations in the area also reflected temporal and spatial changes in the ambient concentrations of DDT and PCB. There was no correlation with depth for mercury in tissues of mussels transplanted at various depths in the same assessment area (Eganhouse and Young 1978). These data suggest that, in contrast to DDT and PCBs, mercury associated with sediments was not leaching to the water column and becoming bioavailable. As part of the environmental assessment for the *Exxon Valdez* oil spill, transplanted mussels were used to show that particulate oil in subsurface waters was bioavailable and bioavailability decreased with water depth (Short and Rounds 1993). In a related *Exxon Valdez* study, Shigenaka and Henry (1993) showed that the concentration of oil in tissues from transplanted mussels was 100 times greater than sediment concentrations and two times higher than oil accumulated by lipid bags. These results suggest that although the semi-permeable membrane devices may be more convenient and provide more experimental control, they are not a perfect surrogate for live mussel bioaccumulation and they cannot predict bioeffects. In comprehensive reciprocal transplant experiments, Roesijadi et al. (1984) and Widdows et al. (1990) were able to identify sources of contamination and show rates of accumulation and depuration for several different contaminants.

Advantages of Mussels

Mussels are particularly well-suited to the transplant methodology because they are sedentary; their hard shells make them easy to collect, handle, cage, and measure. They survive well under most environmental conditions. Mussels are probably the most common *in-situ* bioindicator for marine environments because they are ubiquitous, sedentary, and responsive to their environment at both the micro- and macro-

geographical scales and at all levels of biological organization (Green et al. 1985). They integrate bioavailable contaminants at concentrations that can be orders of magnitude above those found in other environmental compartments (water or sediment). There is a tremendous amount of background material available based on bioaccumulation in the field (State of California 1988; National Oceanic and Atmospheric Administration 1989) and in the laboratory (Bayne et al. 1985; Widdows and Donkin 1992). Other advantages of using mussels as *in-situ* bioindicators have been documented elsewhere (Goldberg et al. 1978; Phillips 1980; Farrington et al. 1987; Phillips and Rainbow 1993).

Although the use of mussels as a bioindicator is sometimes criticized for relative insensitivity to contaminants, their ability to survive under sub-optimal conditions is a strength of the approach (Phillips and Rainbow 1993). We have refined measurement protocols for a sublethal response (growth) to increase the sensitivity and the discriminating power of the bioassay. Transplants could be used as an exposure system to facilitate measuring any clinical biomarker of environmental effects (McCarty 1991). This includes the most sensitive biomarkers that are being developed to assess effects at the cellular and the molecular level of organization. Almost any hypothesis regarding bioaccumulation and bioeffects relationships over space and time is testable using the transplant approach with mussels.

Advantages of Bioaccumulation

Mussels have been used extensively as indicators of exposure for several reasons: (1) they are capable of concentrating contaminants in their tissues at concentrations that are frequently orders of magnitude above those found in seawater or sediment, (2) they provide integrated information about environmental conditions and the bioavailability of contaminants that cannot be defined with chemical measurements of discrete water or sediment samples, and (3) they can provide a more direct link between exposure and bioeffects. This is why bioaccumulation is used to estimate exposure. Tissue concentrations of contaminants better represent *actual* exposure whereas concentrations of contaminants in environmental media (e.g., water and sediments) only represent *apparent exposure*. Actual exposure can lead to biological effects. Even if contaminants were detectable and bioavailability could be estimated by chemical analysis of water samples, the number of samples that would be necessary to adequately describe the ephemeral nature of contaminants in the water column would be cost-prohibitive. In a recent study where tributyltin (TBT) could not be detected in sediment at many locations in the assessment area, TBT was measured in all natural populations of oysters (Espourteille et al. 1993). Similarly, PCBs and polycyclic aromatic hydrocarbons (PAHs) were measured in tissues of transplanted mussels when concentrations were below the limits of detection in seawater (Green et al. 1986; Short and Rounds 1993).

Advantages of Growth

Growth, a commonly used indicator of biological effects, is a sensitive and environmentally realistic biological response because it is a sublethal effect that shows a quantifiable dose-response relationship. It is a biological response that represents the integration of all internal biological processes and has been identified as a significant effect to be measured in environmental assessments (Bayne et al. 1985; Widdows and Donkin 1992). It also integrates total environmental exposure and can be related to adverse effects at the population level (Bayne et al. 1985). Reductions in growth are easily quantified and correlated with adverse environmental effects. However, since both natural and pollution-related stresses have been shown to reduce mussel growth rates, cause-and-effect is not easily proven

(Widdows and Donkin 1992). Repetitive measurements and the experimental control associated with the transplant methodology facilitate isolating the variables in question and providing essential information to help establish causality; particularly when used in combination with laboratory bioassays and field measurements of factors affecting growth.

There is an increasing trend toward measuring sublethal responses like growth in laboratory and field studies; instead of, or in addition to measuring mortality. Reduced mussel growth has been associated with a variety of contaminants in both laboratory and field bioassays (Stromgren 1982, 1986; Stephenson et al. 1986; Salazar and Salazar 1987, 1988, 1991, 1994; Stromgren and Bongard 1987; Widdows et al. 1990; Valkirs et al. 1991; Widdows and Donkin 1992). Juvenile mussel growth was the most sensitive sublethal indicator of TBT measured in San Diego Bay microcosm experiments (Salazar and Salazar 1987; Salazar et al. 1987).

Advantages of Synoptic Measurements

The *in-situ* mussel bioassay assesses environmental quality with direct, synoptic measurements of tissue accumulation and mussel growth in field exposures. It is important to make the distinction between the use of biological indicators as detectors of environmental contamination by monitoring tissue accumulation versus their use as indicators of environmental effects by measuring biological responses like growth. Bioaccumulation is an exposure measurement and is used to determine the relative bioavailability of contaminants; growth is a bioeffect measurement and is used to identify adverse effects associated with that exposure. Both must be used to address the question of whether elevated concentrations of contaminants in the environment (water, sediment, or tissues) and reduced growth constitute an environmental problem. The *in-situ* mussel bioassay facilitates the synoptic measurements of bioaccumulation and growth to help answer that question. It should also be recognized that bioaccumulation can be regarded as both a chemical and a biological process and that bioaccumulation in itself, should not be considered an adverse biological effect. Synoptic measurements have other applications in the risk assessment process. Widdows has pioneered using synoptic measurements of bioaccumulation and physiological responses (scope for growth) to predict tissue concentrations where adverse effects are expected in mussels (Widdows and Donkin 1992). This approach is gaining importance because of the applications to ecological risk assessment and several investigators have advocated moving toward criteria based on tissue burdens; in addition to, or instead of the concentration of contaminants in water or sediment (McKim and Schmieder 1991; Calabrese and Baldwin 1993). Although these predictions can be based on chemical models, the best method is to establish the dose-response curves from direct, synoptic measurements of the dose and the associated response.

METHOD SUMMARY AND RECOMMENDED PROTOCOLS

Most of the protocols presented here were developed between 1987 and 1990 as part of a long-term research program during which nine transplant studies with juvenile mussels were conducted in San Diego Bay, California, (Salazar and Salazar 1994). In summary, the exposure period was 12 weeks, 18 caged juvenile mussels were deployed at each site, and growth was measured with whole-animal wet-weights weekly or on alternate weeks. These were water column studies to evaluate the distribution and effects of TBT antifouling coatings associated with vessels. At most sites, mussels were transplanted 1 meter below the surface, but some sites like the Shelter Island Yacht Basin (the most contaminated) also had mussels 1 meter above the bottom. The emphasis was on juvenile mussels 10 to 12 mm in length. A single 12-week study was conducted in Elliott Bay, Washington, during the winter of 1990 to

1991 (Salazar et al. 1994). In summary, the exposure period was 12 weeks, and 18 to 54 juvenile mussels and 200 to 300 adult mussels were caged at each site. Juvenile mussel growth measurements were made at the beginning and end of the test. Individual adult mussels were only measured at the end of the test; adults were sized (not measured individually) for the 50 to 60 mm range at the beginning of the test. Juvenile mussels from 24 to 30 mm were also used. Bioaccumulation was measured at the end of the tests in San Diego Bay and Elliott Bay.

Based on the San Diego Bay and Elliott Bay results, and subsequent studies, we recommend that mussel transplant studies be conducted with both juvenile (≤30 mm) and adult (≥50 mm) animals. The size range should be minimized in each group and synoptic measurements should include bioaccumulation and growth. It is also important to make multiple measurements on individual mussels to estimate growth. This increases the statistical power of the test and maximizes the ability to discriminate differences among sites. We recommend measuring whole-animal wet-weights and lengths as a non-destructive method to achieve those multiple measurements on individuals. We also recommend cages or bags with individual cells to facilitate the measurements. The collection site is largely dependent on the question to be answered and the availability of test animals. Regardless, animals should be collected with extreme care to avoid injury and other experimentally-induced stressors that might affect bioaccumulation and growth. An exposure period of approximately 90 days is recommended. It should be remembered that the purpose of the *in-situ* mussel bioassay is to estimate both exposure and bioeffects in a minimum period of time and to use those data to detect differences among sites in assessing relative environmental quality.

Experimental Design

Size--We recommend minimizing the size range for juvenile and adult mussels to reduce variability in bioaccumulation and growth attributable to size. A target range of 2 to 5 mm is suggested. Since size and reproduction are two of the most important factors influencing mussel bioindicator results (Bayne et al. 1981; Lobel et al. 1990, 1991, 1992; Widdows and Donkin 1992; Phillips and Rainbow 1993), these effects can be minimized by restricting the size range as we have done. The absolute size is less important than the range of test animals. We have successfully completed juvenile mussel transplant studies using animals 10 to 12 mm, 24 to 30 mm, and 26 to 30 mm. The majority of our work was conducted with juvenile mussels in the smallest size group (10 to 12 mm). We used small juveniles almost exclusively in the San Diego Bay studies for the following reasons: (1) to avoid the effects of gametogenesis on bioaccumulation and growth; (2) to maximize growth potential throughout the test and allow for greater separation among sites; and (3) to utilize what we believed was greater sensitivity to contaminants in juvenile mussels. We used adult mussels less frequently, but animals 50 to 60 mm and 59 to 65 mm were successfully used in San Diego Bay and Elliott Bay. Although the target size range of 2 mm is realistic, it is not always practicable and adjustments should be made to minimize the range to the extent possible. It is more difficult to use a broad size range to estimate bioaccumulation or growth because of the differences in bioaccumulation and growth attributable to size and associated physiological differences.

By limiting the overall size range (2 to 5 mm in length), the discriminating power of the bioassay improves dramatically. Statistically significant differences among sites based on growth can be identified even if growth rates differ by only 25 mg/wk (≈ 25%) (Salazar et al. this volume). Similarly, sites can be differentiated by the concentration of contaminants in tissues even if tissue residues differ by a factor of two or less (≤100%). We believe these statistical

differences are also environmentally significant and could not be as easily identified with a wider size range of animals. The Mussel Watch Program initially set a goal of identifying "hot spots" of contamination with differences exceeding a factor of 10, or greater than 1,000% (Goldberg et al. 1978), but the recommended size ranges in adult mussels vary from 10 to 30 mm (Stephenson et al. 1980; Weber 1988; National Oceanic and Atmospheric Administration 1989).

Based on results from our studies, we recommend using juvenile mussels between 10 and 30 mm with a minimum range. The smallest recommended size is 10 mm, for several reasons. First, it is the minimum practicable size to collect, cage, and measure. Secondly, more somatic tissue is added in animals between 10 and 40 mm than in any other size range. To take advantage of the potential for rapid tissue gain, it is better to start with the smallest practicable animals. The maximum recommended size for the juvenile component of the *in-situ* mussel bioassay at the beginning of the test is 30 mm. At 30 mm, animals are still growing rapidly and they will usually not exceed 50 mm during the test. At a length of approximately 50 mm, gamete production begins to exceed somatic production (Rodhouse et al. 1986). Most monitoring programs use mussels >50 mm but the recommended range is 10 to 30 mm (Stephenson et al. 1980; Weber, 1988; National Oceanic and Atmospheric Administration 1989).

Juveniles may be more representative of short-term changes than adults because of their more rapid growth rates and corresponding addition of tissue and contaminants (Fischer 1983, 1988). In the San Diego Bay study, animals that began the test in the range 10 to 12 mm provided sufficient tissue for chemical analysis at the end of the test since the contaminants of concern were limited to TBT, selected metals, and a few organic compounds. In the Elliott Bay study, we used larger juveniles (24 to 30 mm) to increase the amount of tissue available for chemical analysis. Even so, it was not enough for the complete priority pollutant list of analytes using regional EPA protocols. Low winter temperatures and high concentrations of contaminants reduced growth rates and the amount of tissue available for chemical analysis. Therefore, juvenile mussel tissues were only analyzed for metals and TBT. Since analytical laboratories differ in the amount of tissue required to achieve a particular level of detection, this criterion should be used to determine the amount of tissue (and the number of mussels) required for chemical analysis. Survival and growth rates both affect available tissue and should be taken into account.

For adults, we recommend using animals ≥50 mm within the 2 to 5 mm range. Adult mussels provide substantially more biomass for chemical analysis and they provide important information on exposure and bioeffects that is significantly different from that provided by measuring bioaccumulation and growth in juveniles alone. We have previously used growth in juveniles to assess effects and bioaccumulation in adults to assess exposure, but results based on this partial characterization could be misleading. Although their growth rates are much slower than juvenile mussels, adults may be more sensitive to contaminants during gametogenesis, as well as during temperature and nutritive stress (Bayne et al. 1985). It is generally believed that juveniles, and larvae in particular, are the most sensitive life stage. However, Widdows and Donkin (1992) suggest that adult mussels are more sensitive to contaminants than both juvenile and larval mussels. This elevated sensitivity may be attributable to a reduction in the efficiency of the immune response system of the older, larger individuals (Hole et al. 1992). Adult mussels could also be more sensitive to particular contaminants. Based on our most recent San Diego Bay transplant study (1993), adult growth rates, as estimated by whole-animal wet-weights, may be more sensitive to PAHs than juvenile growth rates.

Number of Test Animals/Frequency of Measurement--For both juvenile and adult mussels, we recommend using 50 to 100 test animals per site to achieve the resolution that is necessary to detect statistical differences in growth, to provide sufficient tissue for chemical analysis, and to provide a reasonable estimate of population exposure and response. Based on our previous studies with juveniles, 100 data points are usually necessary to detect statistically significant differences. In San Diego Bay, this was accomplished by transplanting 18 animals/site and measuring them weekly or biweekly. Using a 12-week exposure period, this provided 234 and 126 data points per site, respectively (including measurements at the beginning of the test). In Elliott Bay, a similar level of replication was achieved by transplanting 54 animals per site and making growth measurements only at the beginning and end of the test (54 x 2 = 108), and we found statistically significant differences. No statistically significant differences in adult growth were found among any of the Elliott Bay sites by measuring individuals only at the end of the test (100 measurements). This was probably attributable to the 10 mm size range and associated variability in growth, and the uncertainty regarding differences in size among cages at the beginning of the test.

We recommend a minimum of 50 juveniles per site to provide enough tissue for chemical analysis. More animals might be required depending on analytical requirements, growth rates, and mortality. For example, 50 juveniles (24 to 30 mm at the start of the test) did not provide enough tissue for analyzing all the priority pollutants in the Elliott Bay studies, whereas tissues from 100 adult animals did. In the San Diego Bay experiments, 18 juvenile mussels provided sufficient tissue for chemical analysis of TBT and some metals. The use of more animals than the minimum for tissue analysis better represents the population and reduces the overall variability in the chemical measurements attributable to differences among individuals. Gordon et al. (1980) recommend a sample size of 16 to 30 individuals in order to detect a statistically significant difference in tissue concentrations when the means differ by 20 to 40%. However, analyses were conducted on individual mussels and only for selected contaminants.

Collection Site-- The source of test animals is largely dependent on three factors: (1) the question to be answered; (2) the availability of test animals; (3) logistics involved in collection, initial measurements and sorting, and transport to the test site. Animals should be collected from a site where environmental conditions have been documented, contaminant concentrations are low, and the animals are in good health. Options include collection from a natural site or purchase from commercial culturing facilities that utilize field or laboratory grow-out. For repetitive studies, population effects can be eliminated by always collecting animals from the same population as we did in the 3-year San Diego Bay study. A similar approach has been used by the State of California Mussel Watch program for over 17 years (Martin and Severeid 1984). Population effects can also be eliminated by using hatchery-raised animals. The transplant methodology facilitates studying population effects and site effects by reciprocal transplants of animals from different populations (Dickie et al. 1984; Roesijadi et al. 1984; Mallet et al. 1987).

We have used animals from a variety of sources and obtained useful information with each. In the San Diego Bay studies, mussels (*Mytilus galloprovincialis*) were collected from a site near the mouth of the bay where contaminant concentrations were lower than those further inside the bay. These intertidal animals were alternately submerged and exposed to air during normal tidal cycles. In the Elliott Bay study, mussels (*Mytilus trossulus*) were collected from a mussel farm situated in relatively pristine waters near Whidbey Island. These animals were attached to floating platforms and were continuously submerged. (The

effects of submergence could also be studied with reciprocal transplants.) We have also used oysters (*Crassostrea virginica*) from a laboratory culture facility for field transplant studies in Tampa Bay, Florida, and microcosm tests in San Diego, California. These three different approaches demonstrate the versatility of stock choice for optimizing transplant methodologies.

Exposure Period-- We recommend an exposure period of approximately 90 days if the major priority pollutants are being assessed. This should provide sufficient time for mussel tissues to reach equilibrium with contaminants in the environment, maximize the period where growth is most rapid, and still avoid most of the effects of gametogenesis on bioaccumulation and growth. We used a 12-week exposure period in all the San Diego Bay and Puget Sound studies to accomplish this. Preliminary San Diego Bay experiments on the rate of TBT uptake in transplanted adult mussels and in microcosm exposures suggested that approximately 60 days was required to reach equilibrium (Fig. 2). A subsequent reciprocal transplant with juveniles and adults suggested that TBT equilibrium was reached in 21 days or less (Fig. 3) but the apparent exposure concentration was significantly lower (72 vs 500 ng TBT/L). Depuration was also quite rapid in this experiment. With all other conditions in the microcosm experiment relatively equal, there was a good correlation between seawater concentration and tissue concentration. Another bioaccumulation experiment in southern California with transplanted mussels has shown that DDT equilibrium was reached in approximately 90 days (Young et al. 1976). The California State Mussel Watch routinely uses 4 to 6 months exposure for bioaccumulation studies of all contaminants (Martin and Severeid 1984; Martin 1985) but has used 3 to 4 month deployment periods for growth studies (Stephenson et al. 1986). Alternatively, for some petroleum hydrocarbons, equilibrium is approached with mussel tissues within days or weeks (Widdows and Donkin 1992). Widdows et al. (1990) transplanted tropical mussels for only 11 to 12 days and achieved elevated concentrations of PAHs, TBT, PCBs, and lead. Although we have found statistically significant differences in growth after only one week when comparing the most contaminated site (Shelter Island Yacht Basin - surface) with other sites, this exposure period may not be long enough to detect more subtle differences and chronic effects.

Cages

We recommend cages that maximize water flow, confine yet maintain adequate space among individuals, and facilitate monitoring growth of individual mussels. Measuring individual mussels for growth improves the statistical power of the test because there are multiple measurements of the same individuals, even if measurements are only made at the beginning and end of the test. This is facilitated by using compartmentalized cages (i.e., trays or mesh bags with one mussel per cell). Compartmentalized cages are preferred because each animal is provided similar holding conditions and animals are not permitted to clump. Clumping could limit exposure to water and contaminants for animals in the center of the clump. With clumped mussels, the bags themselves can also restrict valve opening that, in turn, could affect bioaccumulation and growth. In all our work, plastic mesh cages with individual compartments were used for the juvenile mussels and mesh bags were used for the adult mussels. We have never used marking methods for the following reasons: (1) marks can be obscured by fouling or rubbed off; (2) marking increases the time out of water; (3) some methods could affect the results, and (4) marking increases preparation time at a time-critical portion of the study.

Rigid plastic cutlery trays (Hutzler Manufacturing, Canaan, Connecticut) were subdivided with semi-rigid plastic mesh to create 18 individual cells per cage. Overall dimensions of the cage were 30 x 16

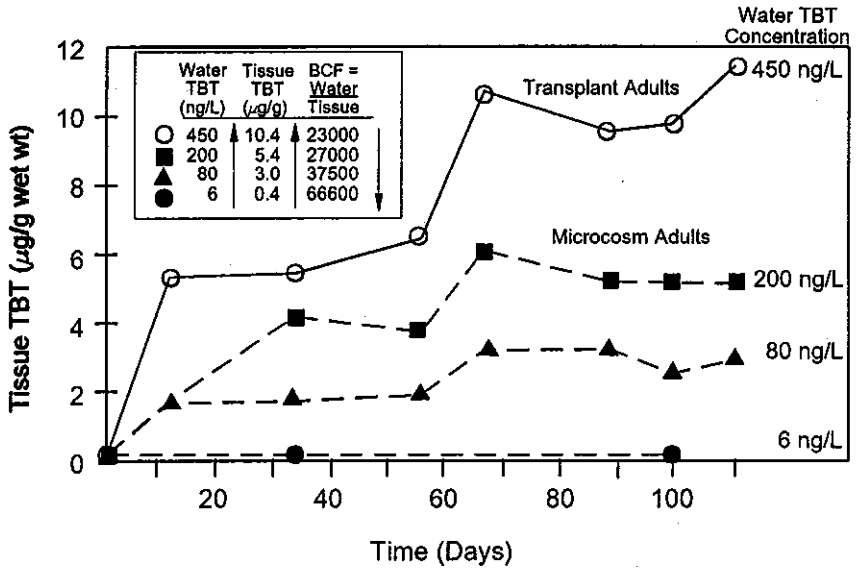

FIG. 2--Tissue concentrations of TBT in adult mussels over time in a field transplant (Shelter Island Yacht Basin) and a flow-through microcosm tank experiment. Results suggest equilibrium is reached in approximately 60 days. The inverse relationship between BCF and TBT concentration is also shown with arrows.

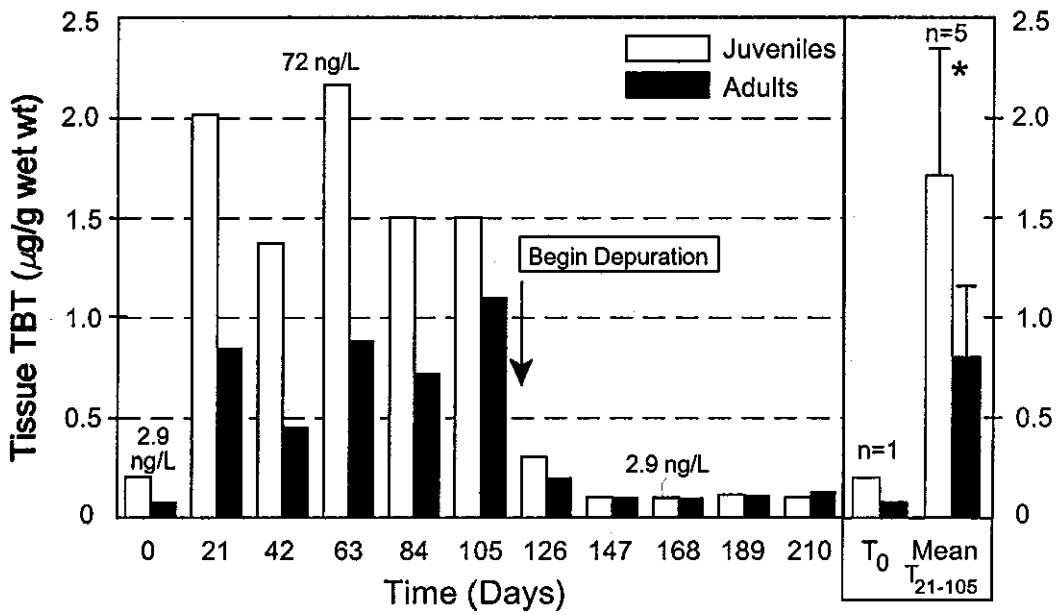

FIG. 3--Tissue concentrations of TBT in juvenile and adult mussels over time in a reciprocal transplant experiment (Shelter Island and a reference site). Results suggest TBT equilibrium is reached in approximately 21 days and concentrations are higher in juveniles than adults. Means from days 21 - 105 (± 2 Standard Errors) are also shown. * = statistically significant difference (= 0.05).

x 3.5 cm with a mesh size of approximately 3 mm. Individual cells were approximately 5 x 5 x 1.6 cm. Flexible plastic mesh (3 to 6 mm mesh size) was used as a top. Increasing mesh size maximized water flow through the cages with larger mesh tops used as the animals grew. The cages and protocols used for the adult mussels in the Elliott Bay study were similar to those developed by the California State Mussel Watch (Stephenson et al. 1980). Oyster culch netting, a plastic mesh tube with a mesh size of approximately 15 mm (Norplex Enterprises, Auburn, Washington), was used to hold the adults. Previous work has shown that clumps of 10 or more mussels can reduce bioaccumulation and growth (Stephenson et al. 1980; Okamura 1986) so we adopted the protocol of 10 adult mussels per clump. Each clump was separated by plastic cable ties. Each deployment consisted of 10 clumps (i.e., 100 mussels).

We now believe that bioaccumulation and growth should be measured in juvenile and adult mussels and that flexible mesh bags are more versatile than rigid trays. The mesh bag approach is being modified to include monitoring individual juvenile and adult mussels. Bags will be compartmentalized to include one animal per clump. A commercially available netting (Norplex Industries, Auburn, Washington) will reduce set-up as well as cleanup time and allow unlimited flexibility in the number of animals deployed at each site. There are no limits to bag length, number of bags that can be prepared for deployment, and the number of individuals per bag. The bags are also disposable. Rigid trays may still be preferable for field tests with a large number of repetitive measurements due to time savings in placing the animals back in the trays. Using beginning and end-of-test measurements only, the mesh bag approach seems best.

Collection and Sorting

We recommend that all animals be carefully collected and sorted by hand. The primary concerns during collection and sorting are animal health and chemical contamination. The collection and processing procedures for chemical monitoring have been carefully developed over many years to avoid cross contamination. Bioresponse protocols are poorly developed because mussels have been traditionally used to estimate only exposure, and animal health has largely been ignored. As is the case with laboratory bioassays, care must be taken in all stages of a field bioassay to prevent stress attributable to experimental protocols. Improper handling techniques can have marked adverse effects on mussel survival and growth (Salazar 1992). Even when we thought our protocols were conservative in terms of experimentally-induced stress, we discovered that weekly measurements had an adverse effect on juvenile mussel growth. During collection, sorting, and measurement, byssal threads should be cut with scissors or gently broken at the point of attachment to avoid injury. Mechanical sorting or ripping mussels apart can remove or tear the byssal gland and lead to death. Mussels can be exposed to air during collection and sorting, but they should be kept cool and moist. Mussel clumps with attached organisms and debris should not be kept in seawater during sorting because the water quickly becomes fouled.

To achieve the minimum recommended size range (2 to 5 mm in length), animals are first "rough sorted" into the approximate size range desired and then "fine sorted" into 0.1 mm groupings for distribution. For both the juvenile and adult mussels, a "rough sort" is done by eye or coarse measuring device to verify that there are enough animals in the appropriate size range to begin the test. The "rough sorted" mussels should be placed in containers filled with clean seawater. They are held in seawater from this point on because initial weights are significantly influenced by air in the mantle cavity. Submergence provides mussels an opportunity to open their valves and release captured air bubbles (burping). For the "fine sort," length

measurements to the nearest 0.1 mm are made with plastic vernier calipers and animals are divided into groups of 0.1 mm increments. Prior to placement in the cage, whole-animal wet weights are measured with an electronic balance (nearest 0.01 g) and the length remeasured. Animals that are floating on the surface of the water prior to measurement are generally not used to begin the test because floating indicates the presence of air within the mantle cavity. If animals float prior to weighing at the end of the test, they are given additional time to purge the trapped air and reduce measurement error.

After the fine sort, the size range for both juvenile and adult mussels should be selected based on the minimum range with the most animals. To assure an even distribution of test animals among sites, all animals in a particular 0.1 mm grouping are distributed among the cages. This process is repeated for the remaining size groups until the cages are filled; each cage then has approximately the same number of individuals from each size group. To ensure statistical similarity among cages, an Analysis of Variance (ANOVA) is run on both length and whole-animal wet-weight data. If the means are statistically different, test animals are redistributed to bring the means closer together. Using this method, we have never found a statistically significant difference among cages in weights or lengths after the fine sort. Even if the distributions are not statistically different, any differences in mean weights or lengths can be minimized by replacing or switching individual mussels. If a size range of 5 mm cannot be achieved, the range should still be minimized and animals evenly distributed (not randomly) to the extent practicable. Using size ranges above 5 mm may necessitate larger groupings for the "fine sort;" eg., 1 mm intervals.

Measurements

The *in-situ* mussel bioassay is based on measurements of growth and bioaccumulation, but survival is recorded during the process and could add significant information to the study. In combination with growth, survival can be used to determine if the test was successful. Poor survival at the control/reference site (<50%) is a good indication that the test animals have been mishandled and that test results should be rejected (Salazar 1992). The number of surviving individuals should be recorded at each measurement interval. Since the methods for chemical analysis are well developed, they will not be addressed here. We have already discussed the importance of measuring individual mussels at the beginning of the test to minimize variability. The primary growth metrics are whole-animal wet-weight and shell length. Length measurements should be made to the nearest 0.1 mm and weight to the nearest 0.01 gram. Lengths can also be used to estimate weights and compare test results with other studies since length is a commonly measured parameter in mussels and is also used in the mariculture industry.

Weight measurements are more accurate than length measurements because of variability in shell shape. Weight is also closer to a constantly increasing function than length, which levels off as the maximum length is approached. Whole-animal wet-weights can also provide a good estimate of wet and dry tissue weights (Dauble et al. 1985) although dry weights are more accurate than wet weights due to variability in water (Widdows and Donkin 1992). Shell weights and tissue weights can also increase at different rates (Widdows and Donkin 1992). Nevertheless, measuring whole-animal wet-weights using the transplant methodology provides the opportunity for multiple, non-destructive measurements on individuals. Although we have never made dry weight measurements because of the time and effort involved, research should be conducted to confirm the differences in the discriminating power of the two methods. We have always measured tissue and shell weights at the end of each test because we feel they provide

useful data and the measurements are easily made as the tissues are being removed for chemical analysis. We recommend making these measurements in both juvenile and adult mussels.

Due to the economy of scale, it is probably most efficient to measure growth at the beginning and end of the test. To understand physiological and toxicological processes, however, it would be better to measure animals more frequently, but not more than once every two weeks. Although we attempted to maximize replication with weekly measurements, this frequency reduced mussel growth rates and caused additional stress to the test animals (Salazar and Salazar 1994). The effects of handling were minimized by measuring animals on alternate weeks in subsequent tests. Similar growth rates were obtained when mussels were measured on alternate weeks or only at the beginning and end of tests.

Data Analysis and Interpretation

The bioaccumulation and growth data can be statistically analyzed to determine if there are differences among sites. The recommended statistical procedures are an ANOVA followed by Duncan's New Multiple Range Test. For analysis of the growth data, the individual mussels in each cage are treated as replicates to increase the statistical power of the test. Only data for surviving individuals are used in the final analyses to reduce data biases due to mortalities (Dauble et al. 1985). To evaluate the data from two sites in the Shelter Island Yacht Basin, we compared the weighted mean growth slopes for each site by test and across tests with a t-test (Zar, 1974). Although the discriminating power in determining the short-term changes and variability in bioaccumulation and growth is reduced with only beginning and end of test measurements, the statistical power is enhanced by using more animals at the start of the test (i.e., 50 to 100). A similar procedure was followed for the seawater data. In order to conduct statistical analyses on bioaccumulation data, it is necessary to have replicated deployments. We pooled the bioaccumulation data by site across tests and made a single comparison using a t-test.

Short- and long-term trends can be analyzed to determine if there is a significant regression over time. Then the regressions for different sites are compared for statistically significant differences. Temporal and spatial variability can be assessed by measuring that variability over time and then comparing the variability among sites and across tests. Bioaccumulation can be calibrated for specific contaminants by comparing bioaccumulation versus growth to estimate these correlations and comparing them statistically. Dose-response can be evaluated by calculating the regression for bioeffects vs exposure (growth vs bioaccumulation) to determine if the regression is significant. The next procedure is to determine the inflection point and estimate where the critical concentrations occur. This information can be used in an ecological risk assessment to predict the concentrations where effects are expected, where they are not expected, and where effects are uncertain.

SAN DIEGO BAY CASE STUDY

We successfully used the mussel transplant bioassay to identify site-specific differences in bioaccumulation and growth at sites in San Diego Bay (Salazar and Salazar 1991) and Elliott Bay (Salazar et al. this volume). We also used seawater TBT concentrations in San Diego Bay and sediment contaminant concentrations in Elliott Bay to distinguish differences among sites and correlated contamination in environmental media with bioaccumulation and growth in mussels. The discriminating power of the mussel transplant approach will be illustrated primarily

with data obtained for two sites in the Shelter Island Yacht Basin: a surface and bottom site separated by only 3 meters vertical distance. The surface site had the highest concentrations of contaminants and the lowest growth rates of all San Diego Bay sites. Since these two sites were so close together, they were expected to be more similar than most other sites. If the *in-situ* bioassay method can detect differences between these sites, it seems reasonable to assume that it could also detect differences among other sites. Fig. 4 shows seawater TBT, tissue TBT, and juvenile mussel growth rates for the surface and bottom sites: by test and by means for all tests. Since bioaccumulation was only measured at the end of the test there was only one measurement per test and there are no error bars on a per-test basis. This figure and those that follow show the utility of the mussel field bioassay in conjunction with chemical monitoring to identify short-term and long-term trends, temporal and spatial variability, site-specific differences, source identification, and dose-response. Since the Elliott Bay study was a single experiment at one point in time, the data do not address trends, variability or dose-response. Serial transplants at the test sites could address those issues, as we have shown with the San Diego Bay data. However, the Elliott Bay data were used to identify site-specific differences and potential contamination sources (Salazar et al. this volume).

Short-term and Long-term Trends

Short-term trends can be estimated by comparing measurements made during one test period with those made during another. Long-term trends can be estimated by serial transplants over time and comparing several tests over a longer time period. The short- and long-term trends in seawater TBT concentration, tissue TBT concentration, and growth for the Shelter Island sites are illustrated in Fig. 4. The seawater concentration of TBT decreased significantly at both sites between 1987-1990, although the decrease at the surface site was much more dramatic. The concentration of TBT in mussel tissues also decreased concomitantly over the first four tests at the surface site, but then showed a dramatic increase before declining again. Since we have shown that weekly measurements reduced mussel growth rates, and a correlation between growth rate and bioaccumulation, we believe that this increase in tissue TBT may be attributable to higher growth rates associated with the switch to measurements on alternate weeks in Test 5. Growth rates increased steadily at both sites through Test 7 and we attribute the decrease in Test 8 to the extremely low winter temperatures. We did not have enough replication over seasons, or enough synoptic measurements of temperature and chlorophyll-*a* to extract seasonal effects, or the effects of other natural factors. Other comparisons could be made between seawater TBT and natural factors to assess covariance. By making multiple measurements of seawater TBT during each test, it is possible to estimate variability and calculate a regression for the rate of change and determine if the change is statistically significant.

Temporal and Spatial Variability

Compared to seawater TBT concentrations and growth rates, it appears that tissue TBT concentrations for these two Shelter Island sites were the most variable over space and time (Fig. 4). The relationship between seawater TBT and growth is better than the relationship between tissue TBT and growth (Salazar and Salazar 1994). By comparing scales however, the tissue concentrations only varied by a factor of two or three. Seawater TBT concentrations and individual growth measurements varied by more than a factor of six (twice as much) on a weekly basis during the same tests (Salazar and Salazar 1988; Salazar and Chadwick 1991). This variability is one of the reasons for measuring tissue concentrations; to normalize exposure concentrations. The variability in tissue measurements may be attributable, at least in

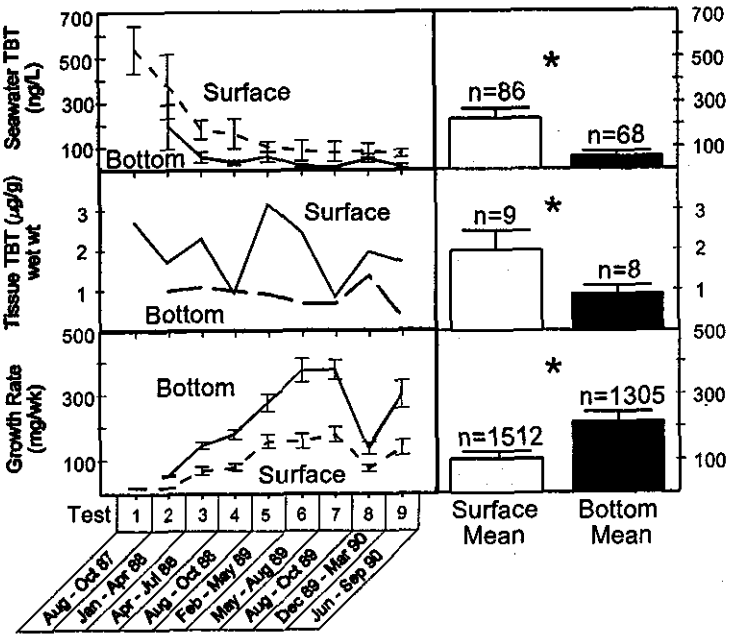

FIG. 4--Temporal and spatial variability and differences in seawater TBT concentrations, tissue TBT concentrations and mussel growth rates at two mussel transplant sites (Shelter Island). Data points for seawater and growth represent 12 week means (±2 Standard Errors). Tissue TBT data points represent end-of-test measurements only. * = statistically significant difference (= 0.05).

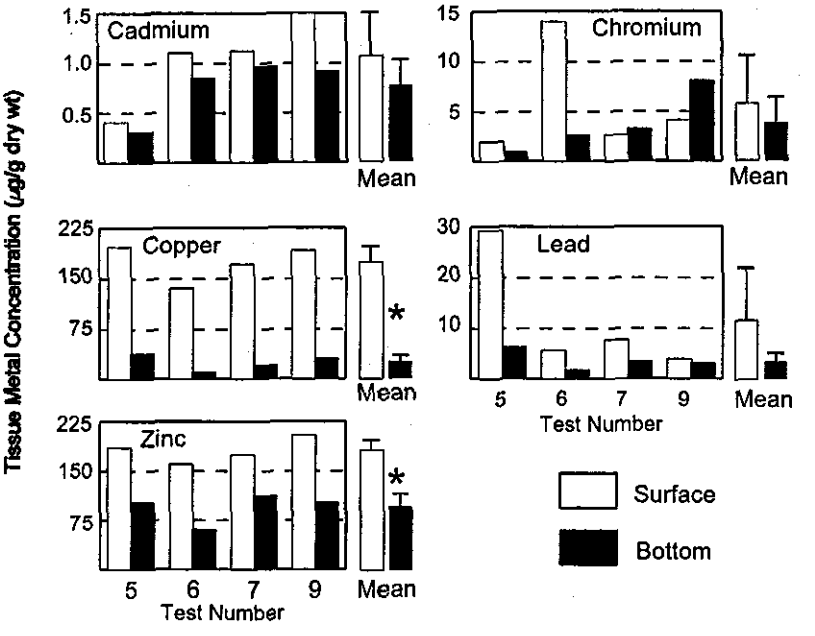

FIG. 5--Concentrations of five metals in juvenile mussel tissues from the surface and bottom site shown in Fig. 3, during the same tests (5, 6, 7, 9) and mussels from the same cages. Means across tests ± 2 Standard Errors are also shown. * = statistically significant difference (=0.05).

part, to the lack of replication in tissue measurements. Although the variability in mussel growth appears low, variability in growth rate among individual mussels and between seasons is high. We were able to normalize some of this variability by making many individual growth measurements for each individual mussel. Similar replication is accomplished by increasing the number of test animals and only measuring at the beginning and end of the test as we did in Elliott Bay. It should also be remembered that variability in seawater TBT concentrations decreased at both sites over time as the absolute concentrations decreased. Variability in mussel growth rates increased at both sites over time as the absolute growth rates increased. Although the long-term trends shown here are very similar to those shown for TBT in a monitoring program for water, sediment, and tissues of resident adult mussel populations in San Diego Bay (Valkirs et al. 1991), the fine structure of temporal and spatial variability that we have shown would not have been detected using only resident mussels and measuring less frequently. Mussels are not found at the bottom site in the Shelter Island Yacht Basin, quarterly monitoring did not capture the weekly variability we observed, and bioeffects were not measured in the resident mussel monitoring program.

Site-specific Differences

Even with the variability associated in all three measurements, we found statistically significant differences between the two Shelter Island sites in seawater TBT, tissue TBT, and mussel growth rates across tests (= 0.05). Even though these sites were separated by only 3 meters, they were very different. Growth was significantly different in every test; seawater TBT concentrations were significantly different in all tests but two. Fig. 5 shows the differences in cadmium, chromium, copper, lead, and zinc for the Shelter Island surface and bottom sites. In almost every comparison, the tissue concentrations are higher at the surface site. For copper and zinc, these differences are statistically significant (= 0.05). We also found significant differences in temperature and chlorophyll-*a* between the sites (Salazar and Salazar, 1994). Copper, zinc, and temperature were higher at the surface and chlorophyll-*a* was higher at the bottom. Because the differences in temperature and chlorophyll-*a* were small, we do not believe they were the primary cause of differences in mussel growth between the Shelter Island sites.

Source Identification

Since the concentrations of TBT were significantly higher in both seawater and mussel tissues from the surface site (Fig. 4), it appears that the primary source of TBT contamination is associated with the surface water and not the bottom sediment. This is reasonable since the primary source of TBT is the antifouling paint on the ship hulls moored in the Shelter Island Yacht Basin. With the ban on the use of TBT in 1988, the concentration of TBT in surface water declined dramatically and mussel growth rates increased significantly. This would seem to confirm that the source was at the surface. It was speculated that high concentrations of TBT in bottom sediment would be bioavailable, but we did not find high concentrations of TBT in the tissues of mussels suspended 1 meter off the bottom. Similarly, mussels caged at the surface site had consistently higher concentrations of five different metals (i.e., cadmium, chromium, copper, lead, and zinc) when compared to mussels from the bottom site (Fig. 5). The differences were statistically significant for copper and zinc (= 0.05). Similar differences were found for other metals as well. Collectively, the TBT and metals data all suggest that the source of this contamination was in the surface water and not the bottom sediment.

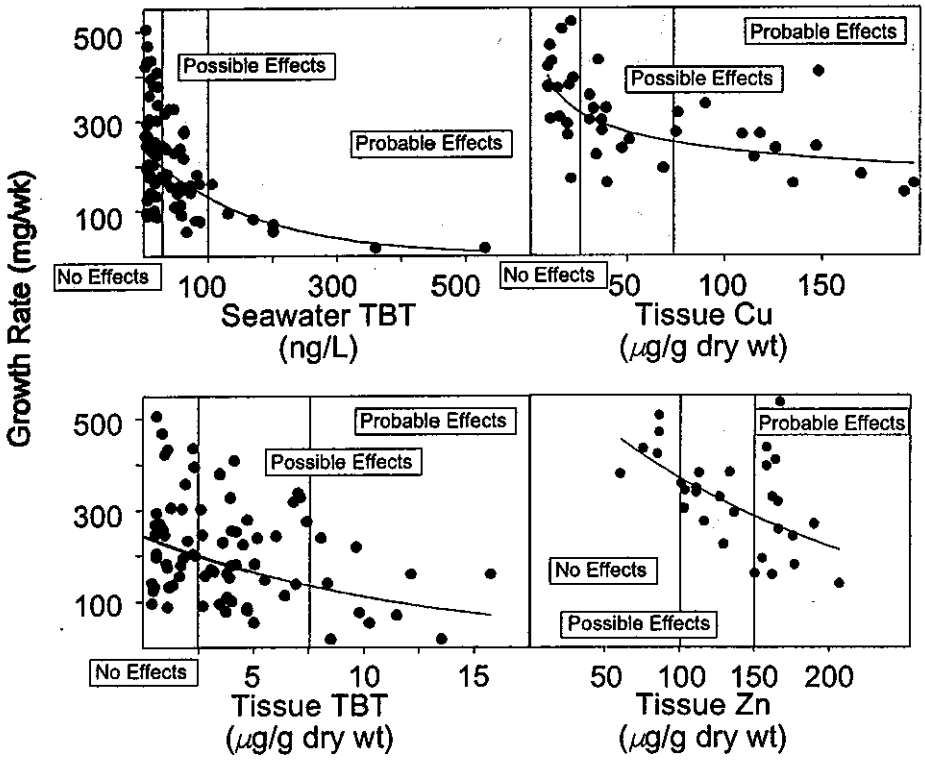

FIG. 6--Statistically significant relationships between juvenile mussel growth rates and seawater concentrations of TBT, and tissue concentrations of three different contaminants: TBT, copper, and zinc. Also shown are first order approximations of effects zones.

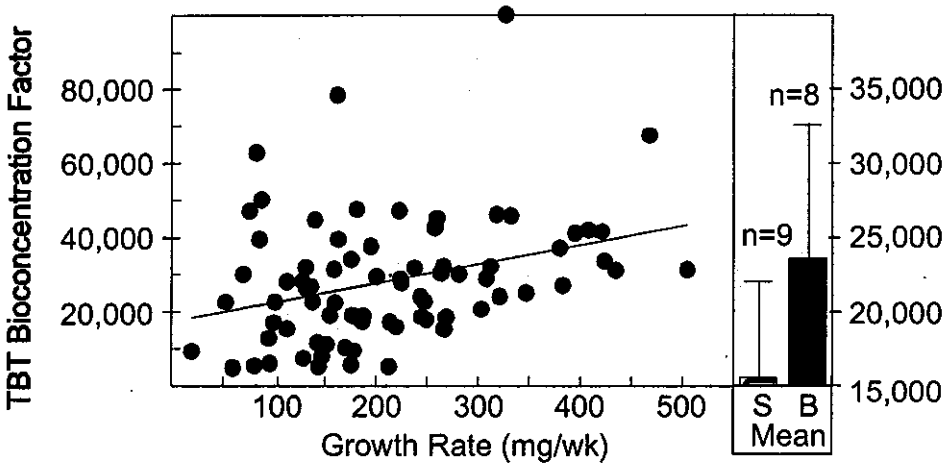

FIG. 7---The relationship between growth rate and apparent bioconcentration factor for TBT is statistically significant (= 0.05). The mean BCFs from the bottom (B) site in Fig. 3 are much higher than the surface (S) site, but the difference is not statistically significant. Error bars represent ± 2 Standard Errors.

Dose-response

By using serial transplants with repetitive measurements of seawater TBT, tissue TBT, and mussel growth rates, we constructed dose-response curves using field data. Although these field bioassays are more variable than laboratory bioassays, the results are more environmentally realistic and it is possible to achieve first-order approximations for water concentration and tissue concentration effects. Fig. 6 shows dose-response curves for seawater TBT concentrations and concentrations of tissue TBT, copper, and zinc. Each curve is statistically significant (= 0.05). The details of the regressions are provided elsewhere (Salazar and Chadwick 1991; Salazar and Salazar 1994). By using the inflection points in the curves, it is possible to predict first-order approximations of apparent-effects-thresholds and no-effect levels. There is a better relationship between seawater TBT concentrations and growth rate than tissue TBT concentrations and growth rate because the bioaccumulation process is more affected by natural factors than TBT in seawater. Interestingly, our predicted threshold tissue concentrations for effects are almost identical to those predicted for TBT and copper in mussel tissues from scope-for-growth measurements (Widdows et al. 1990; Widdows and Donkin 1992).

Although there was tremendous variability in the apparent bioconcentration factors (BCF) associated with surface and bottom sites, BCFs were much higher at the bottom site than the surface site (Fig. 7), but the difference was not statistically significant (= 0.05). Similar differences are often attributed to the kinetics of TBT, but we attribute them to differences in mussel growth rates. There is a statistically significant relationship between BCF and mussel growth rate (= 0.05). This is another reason for measuring mussel growth rate – to calibrate bioaccumulation. Just as there are criteria for animal survival in bioassays to confirm animal health, mussel growth could be used to confirm animal health in the field bioassay. Clearly, if animals are close to death, they will accumulate lower concentrations of contaminants. If growth rates are extremely low, it could be a signal to evaluate the results of bioaccumulation with more caution than is customary. Just as laboratory bioassays have a standardized survival requirement for acceptance, the *in-situ* bioassay could have a similar survival requirement. Additionally, since survival is not a very sensitive measure of animal health, growth could be used to provide a finer assessment of animal health and associated bioaccumulation.

Using data from synoptic measurements of seawater TBT, temperature, and growth rates, we constructed three-dimensional graphs to evaluate the interaction between TBT and temperature on mussel growth (Fig. 8). Interestingly, the optimum temperature for growth predicted from these field data is identical to that predicted from laboratory studies (Almada-Villela et al., 1982). This is a field validation for temperature effects. We were able to detect these correlations with temperature and differences among sites by using an *in-situ* meter (Ryan Instruments, Redmond, Washington) that recorded temperatures at 30-minute intervals during the 12-week exposure period. Since chlorophyll-*a* was highly variable and was only measured weekly or biweekly when the animals were measured, we did not have sufficient replication for appropriately sensitive statistical analysis. We recommend measuring temperature and chlorophyll-*a* at a frequency that is commensurate with variability in the environment to assist in the field validation using the *in-situ* mussel bioassay. The following approach could be used in a field validation process: quantifying exposure-response in the field; quantifying exposure-response in the lab; quantifying the effects of other variables. Laboratory experiments could be used to isolate particular variables and field manipulations could be used to achieve various combinations of test conditions with more *in-situ* measurements of natural factors affecting growth.

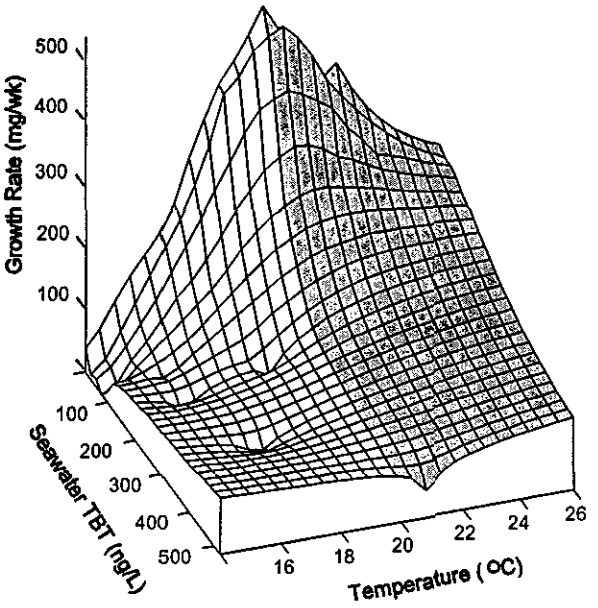

FIG 8.--Effects of seawater TBT concentrations and temperature on growth predicted from 3-dimensional surface plots using weighted means. Shaded area represents growth rate reductions at temperatures above 20°C. Reduced growth at low temperatures and high concentrations of TBT in seawater (>100 ng/L) are also apparent.

SUMMARY AND CONCLUSIONS

The most unique feature of our approach is the combined use of the following: (1) synoptic measurements of bioaccumulation and growth using both juvenile and adult mussels; (2) mussel growth as an indicator of bioeffects; (3) individual measurements to minimize the initial size range and maximize statistical power by multiple measurements of individuals; and (4) compartmentalized cages to facilitate individual, repetitive measurements.

Advantages

The mussel transplant approach combines the advantages of experimental control and environmental realism. Control is gained by manipulating the exposure time and the exposure site. These parameters cannot be controlled if natural populations of mussels are used to estimate exposure and bioeffects. Environmental realism, which is often lacking in laboratory tests, is achieved because the animals are deployed *in-situ*. Other advantages of this approach include the ability to make repetitive, synoptic measurements on individual animals, the ability of test animals to integrate bioavailable contaminants, and the effects of natural factors that provide a meaningful dose and a natural response. The technique is relatively simple, does not require specific expertise or sophisticated equipment, and is relatively inexpensive. There is also a vast mussel literature that can be used to help provide mechanistic explanations for observed responses as well as help direct the coordination of laboratory and field studies that can be used to

establish the dose-response relationships used in ecological risk assessments. Almost all clinical measurements are possible, and the *in-situ* mussel bioassay, in essence, could be viewed as simply an exposure system.

Disadvantages

The *in-situ* mussel bioassay also has a number of disadvantages and potential pitfalls. Bioaccumulation and growth are both affected by natural factors that cannot be controlled, such as external abiotic factors and internal biotic factors. It should also be mentioned that growth effects are not contaminant-specific. Mussels are not equally sensitive to all contaminants, nor do they accumulate them equally. Mussels can actually close to avoid some contaminants, and since multiple contaminant exposures are common in the field, this makes discrimination difficult. Extensive chemical measurements are required to document apparent exposures (in water or sediment), and a true field control site is virtually impossible.

Prospectus

Even with these shortcomings, *in-situ* bioassays are powerful assessment tools. Many have suggested using laboratory bioassays in conjunction with monitoring contamination and community assemblages, and this approach has been used with reasonable effectiveness in many monitoring programs. We suggest that field bioassays using more direct measurements of bioaccumulation and growth to estimate chemical exposure and bioeffects should be included as part of a comprehensive program for sediment or water quality monitoring. An integration of laboratory and field bioassays could provide very useful information. Although we used mussels in refining this *in-situ* bioassay, the generic approach is applicable to many bivalve species indigenous to freshwater, estuarine, or marine environments. We have outlined the utility of mussel transplants as a field bioassay, demonstrated the need to standardize protocols for this approach, and presented basic protocols that could be used as a framework for standardization.

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