MUSSELS AS BIOINDICATORS: EFFECTS OF TBT ON SURVIVAL, BIOACCUMULATION, AND GROWTH UNDER NATURAL CONDITIONS

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ABSTRACT

During nine field-transplant experiments (1987-1990), juvenile mussels were exposed to mean tributyltin (TBT) concentrations from 2 to 530 ng L^{-1} for 12 weeks under natural conditions in San Diego Bay. Mussels were used as biological indicators and monitored for survival, bioaccumulation, and growth. Mussel growth was the primary biological response used to quantify TBT effects. Chemical analyses were used to estimate TBT contamination in water and tissues.

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Integrating intensive measurements of chemical fate and biological effects increased the environmental significance of the data. Multiple growth measurements on individuals increased the statistical power. Size effects were minimized by restricting test animals to 10-12 mm in length, and methods were developed to minimize handling effects. This monitoring approach also permitted documenting temporal and spatial variability in TBT and its effects that have not been previously reported. Survival, bioac-cumulation, and growth were generally higher than predicted from laboratory studies. Survival was not directly affected by seawater or tissue TBT concentrations. Growth was significantly related to both seawater and tissue TBT, with the bioconcentration factor inversely proportional to seawater TBT concentration. Threshold concentrations always causing significant reductions in juvenile mussel growth are estimated at 100 ng L^{-1} TBT for seawater and 1.5 μg g^{-1} TBT for tissue, but growth could be affected by much lower concentrations of TBT under the most adverse conditions. Temperatures above 20°C were also found to reduce juvenile mussel growth rates.

15.1. INTRODUCTION

Biological indicators provide integrated information about environmental contamination and effects that cannot be defined with chemical analysis of water samples. Nevertheless, chemical analyses quantify contamination in a way that is essential in explaining biological effects. Measuring survival, bioaccumulation, and growth quantifies both contamination and effects. It is important to make the distinction between the use of biological indicators as detectors of environ-mental contamination by monitoring tissue accumulation with chemical analyses versus their use as indicators of environmental effects by measuring other biological responses (Waldock *et al.*, Chapter 11). Mussels have been used as biological indicators in many field monitoring programs because of their cosmopolitan distribution and ability to concentrate many different contaminants. Their demonstrated utility in transplant experiments and monitoring is another significant advantage. Mussels have been used most extensively to monitor contamination by measuring tissue accumulation with chemical analyses (Phillips, 1980). They have also been used to monitor the biological effects of contamination by measuring biological responses related to growth, physiology, and reproduction (Bayne *et al.*, 1985). It should be recognized that bioaccumulation can be regarded as both a chemical and a biological process; however, biological effects are not necessarily related to tissue accumulation. We suggest that caged mussels should be regarded as a bioindicator system. This indicator system consists of the entire suite of biological responses.

Bioaccumulation is the process through which organisms integrate exposure to environmental concentrations of bioavailable contaminants. The results of these integrated chemical and biological processes can be quantified with chemical analyses. Laboratory and field studies have shown that tributyltin (TBT) is highly toxic to molluscs and that filter-feeding bivalves readily accumulate TBT (Hall and Bushong, Chapter 9; Laughlin *et al.*, Chapter 10; Waldock *et al.*, Chapter 11; Gibbs and Bryan, Chapter 13; Henderson and Salazar, Chapter 14; Laughlin, Chapter 16). Natural mussel populations have been used as indicators of TBT contamination by measuring tissue concentrations of TBT (Wade *et al.*, 1988; Short and Sharp, 1989; Uhler *et al.*, 1989; Roberts *et al.*, Chapter 17). Chemical measurements of TBT concentrations in seawater and tissues of mussels from natural populations (Grovhoug *et al.*, Chapter 25) or transplants (Zuolian and Jensen, 1989) are more informative than measuring seawater or tissue levels alone. The combination of

seawater and tissue measurements of TBT provides a quantitative relationship between the chemistry of the environment and the chemistry of the organism. However, the relative influence of environmental and biological factors on this relationship is highly variable and difficult to predict (Cain and Luoma, 1990).

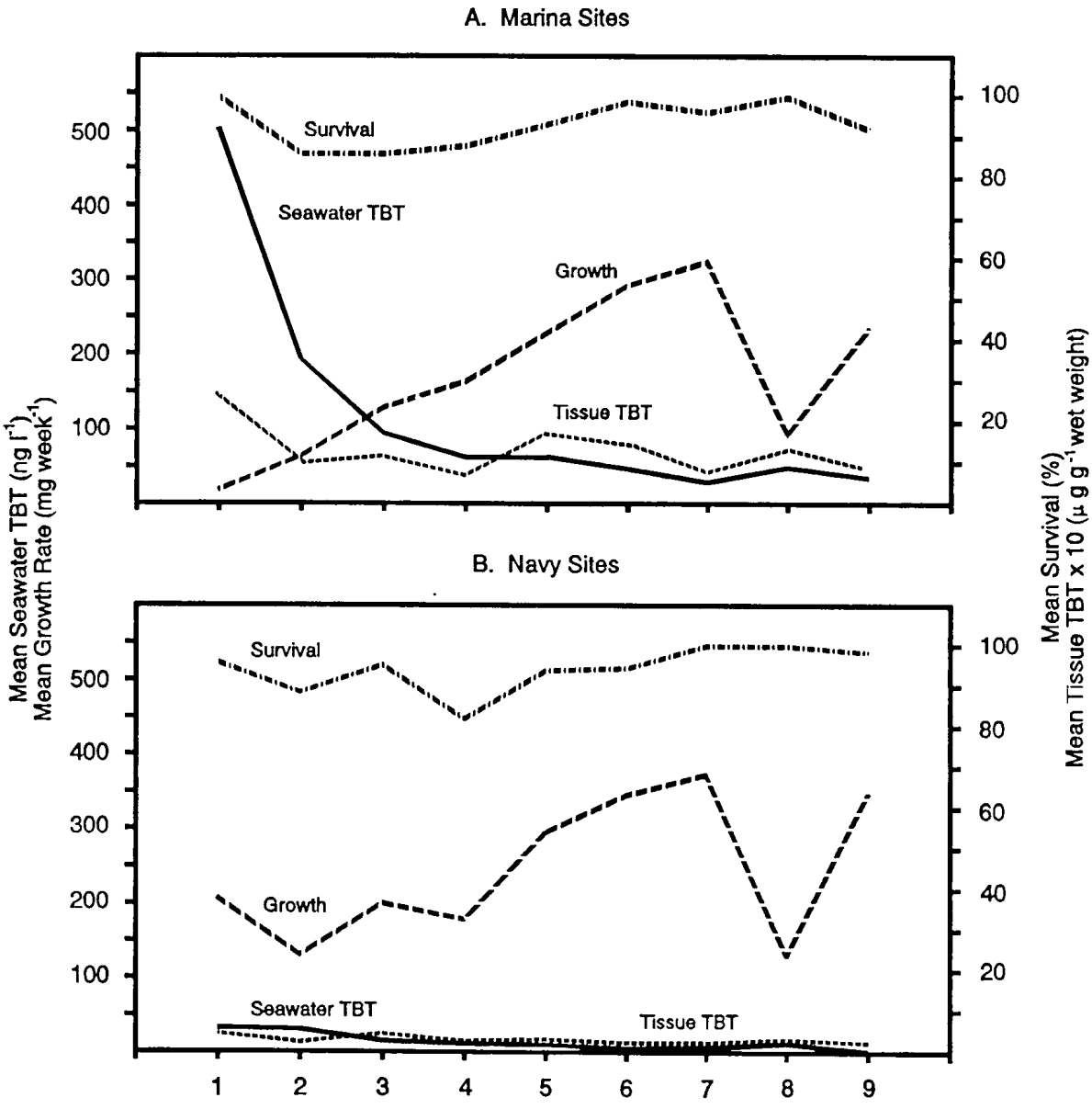
Survival and growth are biological responses that also integrate exposure to environmental concentrations of bioavailable contaminants. These responses are more directly related to animal health and do not depend on chemical analysis. Survival is the least sensitive to environmental effects because it is an all-or-nothing response. Therefore, survival data are not always informative. Growth is more sensitive because there is a graded response to environmental conditions that can be quantified through repetitive, non-destructive measurements. Reduced growth represents adverse environmental effects and possible effects on the population. Both natural and pollution-related stresses have been shown to reduce mussel growth rates (Bayne *et al.*, 1985). Reduced mussel growth has been associated with TBT in laboratory and field studies (Thain and Waldock, 1985; Stephenson *et al.*, 1986; Salazar and Salazar, 1987; Stromgren and Bongard, 1987; Valkirs *et al.*, 1987; Salazar and Salazar, 1988). Juvenile mussel growth was the most sensitive indicator of TBT measured in San Diego Bay microcosm experiments (Salazar and Salazar, 1987; Salazar *et al.*, 1987; Henderson and Salazar, Chapter *14*). Juvenile mussels have advantages over adults as bioindicators: (1) they grow faster and provide a greater range of response; (2) the growth process is not affected by gametogenesis (Rodhouse *et al.*, 1986); (3) bioaccumulation in short-term tests with fast-growing juveniles more accurately reflects recent environmental changes (Fischer, 1983, 1988); and (4) they may be more sensitive to TBT (Hall and Bushong, Chapter 9).

Survival, bioaccumulation, and growth in caged mussels can be routinely measured in field-transplant experiments. Field-transplant experiments provide an opportunity to corroborate and validate mussel performance under laboratory conditions. It is important to measure performance under natural conditions because that is where the biological effects, if any, will occur. The main advantage in using trans-planted animals over monitoring naturally settled populations is the experimental control. Experimental control is achieved by using mussels of similar genetic and environmental stocks at all test sites, pre-selecting test animal size or age group, and monitoring individual animals during the test. Animals can also be transplanted to areas where they might not normally be found. Serial transplants and monitoring facilitate the examination of both short- and long-term trends in contaminant distribution and related effects.

The work reported here had three primary goals: (1) determine the effects of TBT on survival, bioaccumulation, and growth in juvenile mussels under natural conditions; (2) identify long-term trends in the distribution of TBT and its effects; and (3) refine the use of the juvenile mussel bioindicator for environmental assessment.

15.2. METHODS

Nine field-transplant tests were conducted in San Diego Bay between 1987 and 1990 (Salazar and Salazar, 1991c). Juvenile mussels *(Mytilus* sp.) were transplanted from the collection site to test sites and monitored during 12-week exposure periods. Test dates are shown in Fig. 15.1. Serial mussel measurements were made in the field, and water samples collected weekly during tests 1-4 and on alternate weeks (biweekly) during tests 5-9. Growth measurements included whole animal wet weights and lengths. Caged mussels were removed from the water for ≈20 min during these measurements, and byssal threads were carefully cut with scissors. Water was measured for chlorophyll-*a*



Test Dates	Aug-Oct 1987	Jan-Apr 1988	Apr-Jul 1988	Aug-Oct 1988	Feb-May 1989	May-Aug 1989	Aug-Oct 1989	Dec 89 - Mar 90	Jun-Sep 1990
Temp Range (°C)	20 - 24	16 - 18	17 - 23	18 - 23	16 - 18	18 - 22	19 - 24	14 - 15	20 - 26
SW TBT (ng l^{-1})	8 - 530	9 - 360	7 - 200	5 - 169	5 - 105	5 - 86	6 - 82	6 - 67	2 - 72

Figure 15.1 Regional differences and temporal changes in seawater TBT, tissue TBT, survival, and growth for marina sites (A) and Navy sites (B). Test dates are also given with temperature and seawater TBT ranges (rounded mean values).

and TBT concentrations (Venrick and Hayward, 1984; Stallard *et al.*, 1988). Mean chlorophyll-*a* and seawater TBT concentrations for each test were determined from weekly or biweekly measurements. Temperature was measured at half-hour intervals with in-situ monitors. Whole animal wet weights, lengths, shell weights, and tissue wet weights were measured at the end of each study. Tissues for a given site were pooled for TBT analysis on a wet-weight basis (Stallard *et al.*, 1988). Average weekly growth rates based on weight (mg $week^{-1}$) and length (mm $week^{-1}$) were calculated from regression analyses for animals transplanted at each site. Only weight growth rates will be discussed because there is a greater range in response than with length growth rates (25 X compared to 10 X), and weight measurements are more accurate.

Naturally settled mussels were collected from pilings where mean TBT concentrations in seawater and mussel tissues were low, ≈5 ng L^{-1} TBT and ≈0.15 μg g^{-1} TBT wet wt, respectively. The collection site, a Navy site, is located near the mouth of San Diego Bay and characterized by high current speeds, nearshore ocean temperatures, few vessels, and low levels of contaminants in mussel tissues. Mussel growth rates in this area are among the highest in San Diego Bay. The collection site was used as a test site for comparative purposes. Animals were sorted by length for convenience. Test mussels were 10-12 mm in length ($\bar{x}$ ≈11.0 mm) and initial mean weights ranged from 100 to 250 mg ($\bar{x}$ ≈175 mg). Eighteen mussels were caged and transplanted to each site. There were no statistically significant differences in weights or lengths among these mussel groups at the start of any test. Animals were continuously submerged either 1 m below the surface or 1 m above the bottom. The 18 monitoring sites included 11 Navy sites with low seawater TBT concentrations and seven marina sites with high seawater TBT concentrations (Grovhoug *et al.*, 1986). The most significant refinements in methods include the use of (1) field transplants; (2) serial growth measurements on individuals; (3) minimizing the size range and maximum size of test animals; and (4) synoptic measurements of bioaccumulation and growth.

Bioaccumulation in transplanted and natural mussels was compared to determine if the transplanted bioindicator was responding like natural populations of undisturbed mussels. Bioaccumulation in juveniles and adults was compared to determine if there were size or age differences. During tests 4, 6, and 9, juvenile ($\bar{x}$ length <35 mm) and adult mussels ($\bar{x}$ length >50 mm) were collected from natural populations at one marina and one Navy transplant site. These sites were characterized by the highest and lowest concentrations of TBT in seawater, respectively. This Navy site was also the collection site. Tissues from these mussels, along with tissues from juvenile transplants (tests 4, 6, and 9) and adult transplants (test 9), were analyzed for TBT. In a different experiment, handling and measurement effects on growth of juvenile mussels were assessed at five sites, including the most contaminated marina site. Growth rates of animals measured weekly were compared with growth rates from those animals measured only at the beginning and end of the 12-week exposure (untouched). Some comparisons were also made with animals measured on alternate weeks (biweekly).

The data for survival, seawater TBT concentration, tissue TBT concentration, and growth were pooled by test for both marina and Navy sites to calculate regional means for comparison with other San Diego Bay monitoring studies (Grovhoug *et al.*, Chapter *25*). Regional means for seawater TBT and growth rates were compared with a one-way analysis of variance on a per-test basis as well as for data pooled across tests. Regional means for tissue TBT and individual site data were pooled across tests and compared using a one-way analysis of variance and multiple linear regression analyses. Although these regional divisions are somewhat arbitrary, they can be used to illustrate the effects of

low and high exposure to TBT in seawater and convey important general information about the physical-chemical characteristics of the sites in each region. Details regarding temporal and spatial variability, site locations, and site-specific responses are presented elsewhere (Salazar and Salazar, 1991a,b,c).

Graphical methods are used to display the general relationships among environmental levels of TBT and mussel survival, bioaccumulation, and growth. Each data point represents a 12-week exposure and the response of approximately 18 animals. The significance of each relationship was determined by linear regression analyses. The significance of the regression (*p*); the correlation coefficient, which provides a measure of association intensity between the two variables (r); and the total variation in the dependent parameter that is explained by the fitted regression (r^2) were used to compare regressions. Stronger relationships are indicated by higher *p*, r, and r^2 values. Lines and slopes can also be compared on a relative basis. The r^2 statistic estimates the predictive strength of each relationship and possible environmental significance. Statistical significance was determined at the 95% confidence level.

15.3. MUSSELS AS BIOINDICATORS

In recognition of the limitations of laboratory bioassays and chemical monitoring, there has been a shift in emphasis toward biological monitoring and field bioassays (Chapman, 1983; Chapman and Long, 1983; Phillips and Segar, 1986; Parrish *et al.*, 1988). Specific problems with the interpretation and environmental significance of TBT studies have been discussed previously (Stebbing, 1985; Salazar, 1986, 1989; Salazar and Champ, 1988). Using mussels as biological indicators of contamination and effects is a potentially powerful tool, but there are many pitfalls regarding interpretation and environmental significance (Phillips, 1980; White, 1984). Many of these pitfalls were avoided in this mussel bioindicator study by integrating chemical and biological measurements and by demonstrating the differences between using mussels as indicators of contamination versus their use as indicators of effects. Most status and trends programs in the United States have used biological indicators as detectors of environmental contamination of TBT by monitoring tissue accumulation with chemical analyses (Wade *et al.*, 1988; Short and Sharp, 1989; Uhler *et al.*, 1989). The predictive value of these studies is extremely limited. Measuring seawater TBT in addition to accumulation in natural populations (Grovhoug *et al.*, Chapter 25) or field transplants (Zuolian and Jensen, 1989) establishes a quantitative relationship between environment and organism that is much more informative. Such studies are using mussels as detectors of environmental contamination. By measuring survival and growth in addition to bioaccumulation, we used mussels as indicators of environmental contamination and effects (Waldock *et al.*, Chapter 11). The approach was further integrated by including chemical measurements of seawater. This type of integration is very important.

15.3.1. GENERAL TRENDS

Mean concentrations of TBT in seawater at the 18 sites ranged from 2 to 530 ng L^{-1} and tissue TBT concentrations from 0.1 to 3.2 μg g^{-1} wet wt. Mean 12-week temperatures ranged from 14.3 to 25.7°C and chlorophyll-*a* from 0.79 to 6.07 μg L^{-1} Mean temperatures for winter tests ranged from 14.3 to 14.8°C while mean temperatures for summer tests ranged from 20.1 to 25.7°C. Chlorophyll-*a* was lowest in the winter. End-of-test survival ranged from 50 to 100%. Mean growth rates of caged mussels ranged from 17 to 505 mg $week^{-1}$ (0.2 to 2.5 mm $week^{-1}$). The lowest growth rates were measured at the most contaminated marina site. Mussels increased

from ≈190 to 420 mg (11 to 14 mm in length). This was an increase of only 230 mg in weight (3 mm in length) after 12 weeks. The highest growth rates were measured at the Navy site nearest the mouth of the bay. Mussels increased from ≈160 to 6200 mg (11 to 40 mm in length). These mussel growth rates are among the highest reported (Kiorboe *et al.*, 1981). The minimum concentration of TBT in seawater predicted to always reduce juvenile mussel growth was estimated at 100 ng L^{-1} TBT. The minimum concentration of TBT in tissues predicted to always reduce juvenile mussel growth was estimated at 1.5 μg g^{-1} (wet wt). These concentrations were estimated from statistical analyses of mussel responses under representative conditions in San Diego Bay. Because biological responses are so site specific, site selection influenced the significance of each relationship and comparisons between regions. Therefore, the most meaningful comparisons are those between sites. Both water and tissue TBT con-centrations are similar to those predicted to reduce oyster growth (Waldock *et al.*, Chapter 11). A comparable concentration of tissue TBT was reported to adversely affect adult mussel physiology (Page and Widdows, 1990).

Regional differences and temporal changes in survival, seawater TBT, tissue TBT, and growth for the marina and Navy sites are shown in Fig. 15.1. Seawater and tissue TBT concentrations generally decreased while mussel growth rates increased over time. Although the lowest growth rates were associated with the highest concentrations of seawater TBT, extremely low growth rates were also associated with winter seawater temperatures <15°C. Seawater and tissue TBT concentrations were significantly higher and growth rates significantly lower at marina sites than Navy sites. There were no differences in survival. There was a significant decrease in seawater and tissue TBT concentrations at marina sites and Navy sites. This was associated with a general increase in growth. These changes were not consistent at all sites, however, and argue against pooling (Salazar and Salazar, 1991b). There was also a significant decrease in growth rates during test 8 that demonstrates the significance of natural factors in influencing mussel growth rates. The mean concentration of seawater TBT at marina sites Navy sites as well as the decreases in sea-water and tissue TBT concentrations were similar to those described for San Diego Bay by Grovhoug *et al.* (Chapter 25) except that we did not find significant decreases in tissue TBT concentrations at all Navy sites. Neither tissue TBT concentrations, survival, nor growth rates consistently followed sea-water TBT concentrations. However, the ratio of tissue to seawater TBT, growth rates, and survival generally increased at both marina and Navy sites after test 4.

15.3.2. SURVIVAL

Although survival is not a sensitive indicator of environmental effects, important information was gained for some sites where exceptionally high or low survival was different than expected based on other measurements and indicators of water quality. Survival is not significantly correlated with seawater or tissue TBT concentration. The four lowest survival measurements were between 50 and 65%. They were associated with mean concentrations of TBT in sea-water between 34 and 130 ng L^{-1} at one marina and one Navy site. Conversely, the five highest mean concentrations of TBT in seawater were between 169 and 530 ng L^{-1}. They were measured at a different marina site and were associated with mussel survival between 94 and 100%. There was no difference in measured survival between marina and Navy sites even though seawater and tissue TBT concentrations were significantly higher at marina sites (Fig. 15.1). Survival was higher in tests 5-9 (96.8%) than in tests 1-4 (90.2%), but the difference is not statistically significant. One hundred percent survival

was measured in 42% of the transplant in tests 1-4 and in 70% of the transplant in tests 5-9. The increase in survival after test 4 was associated with the decline in TBT concentrations in marina seawater following restrictions imposed in January 1988 (State of California, 1988) and reduced handling after test 4 when weekly growth measurements were changed to alternate weeks. Since handling was reduced at the same time seawater TBT concentrations decreased, the relative effects of each on survival are unclear.

15.3.3 BIOACCUMULATION

There is a significant positive linear relationship between TBT accumulation in juvenile mussel tissues and seawater TBT concentration (Fig. 15.2). Based on regression analysis of all data, only 38% of the variance in tissue TBT concentration can be explained by seawater TBT concentration. The regression equations calculated using all data and only data associated with seawater values >105 ng L^{-1} are very similar. Six of the seven data points for seawater TBT concentrations >105 ng L^{-1} are from the most contaminated marina and strongly influence the regression for all data. The data appear to fall into two separate groups. The slope of the regression for tissue TBT values associated with seawater TBT concentrations <105 ng L^{-1} is almost five times higher than the slope of the regression for tissue TBT values associated with seawater TBT concentrations >105 ng L^{-1}. The ratio of tissue TBT to seawater TBT

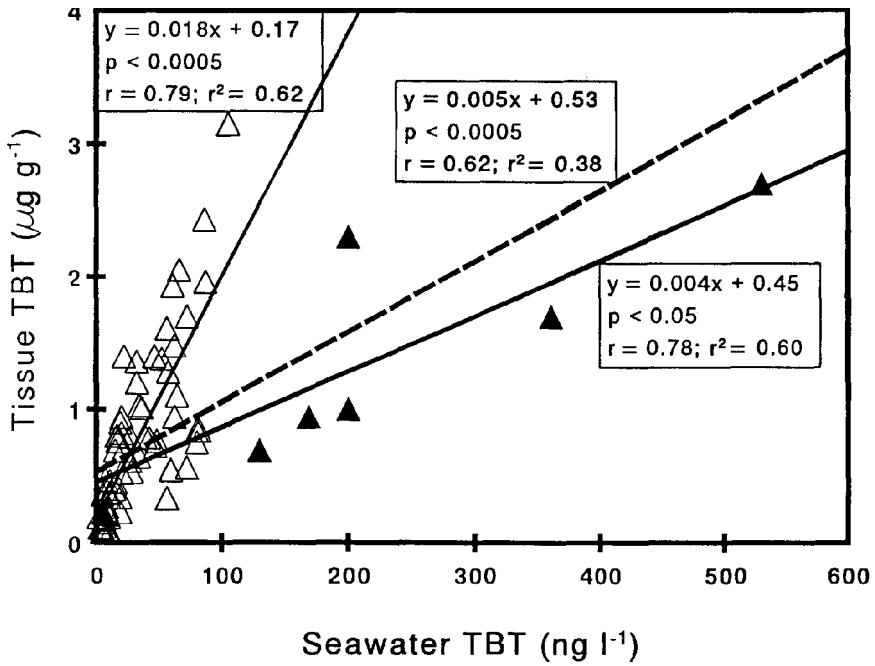


Figure 15.2 Relationship between TBT concentrations in seawater and juvenile mussel tissues. Regression lines show differences and similarities in tissue TBT accumulation between low (≤105 ng l^{-1}) and high (>105 ng l^{-1}) concentrations of TBT in seawater and all data (dashed line). The regression equations and relevant statistics are also given.

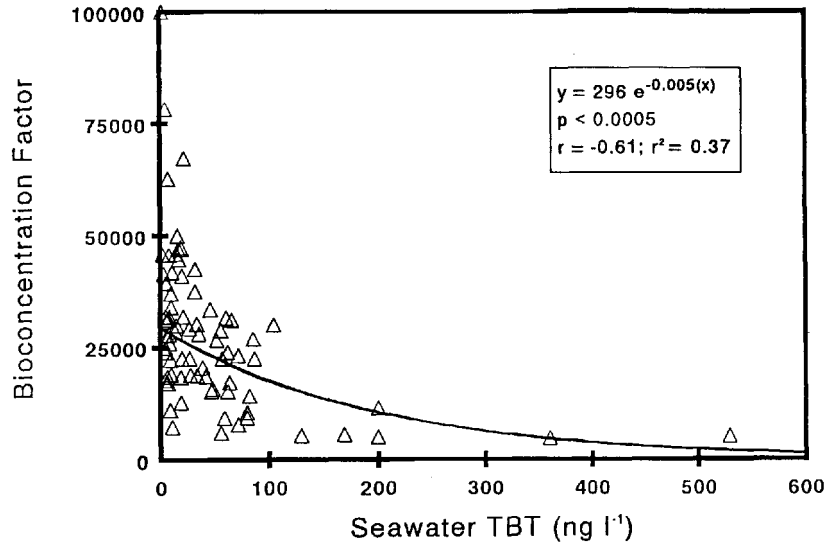


Figure 15.3 Relationship between seawater TBT concentration and bioconcentration factor. The equation of the best-fit line and relevant statistics are also given.

concentration estimates a bioconcentration factor (BCF). BCF values ranged from 4,700 to 105,600 with the majority between 20,000 and 40,000 (Fig. 15.3). An inverse exponential function best describes the relationship between BCF and seawater TBT concentration. The higher ratios of tissue TBT to seawater TBT at lower seawater TBT concentrations shown in Fig. 15.3 were suggested by the regressions in Fig. 15.2 and the shift in ratios from the trends in Fig. 15.1. Lower concentrations of TBT in seawater were associated with higher BCF values. At seawater TBT concentrations <105 ng L^{-1}, BCF values range from 5,000 to ≈100,000. Above seawater TBT concentrations of 105 ng L^{-1}, BCF values were <9,000. This inverse exponential relationship between TBT concentration in seawater and in mussel tissue was found in laboratory studies (Laughlin *et al.*, 1986; Laughlin and French, 1988), microcosm studies (Salazar *et al.*, 1987), other field-transplant studies (Waldock *et al.*, Chapter 11; Zoulian and Jensen, 1989), and natural San Diego Bay populations (Grovhoug *et al.*, Chapter 25).

Using tissue accumulation in field bioindicators to quantify environmental levels of contamination is a potentially powerful tool, but the limitations of this approach must be recognized (Phillips, 1980). Since the relationship between seawater and tissue TBT concentrations (BCF) is not constant, accurate predictions of one using the other are not possible. Even accumulating elevated levels of contaminants does not *a priori* indicate environmental effects on the bioindicator or other species (Peddicord, 1984). Initial reports on mussels as bioaccumulators emphasized the utility of identifying order-of-magnitude differences in seawater contamination and minimized potential interference from extraneous environmental factors (Goldberg *et al.*, 1978, 1983; Farrington *et al.*, 1983). Other reports have outlined potential problems in using tissue concentrations of contaminants for environmental prediction (Phillips, 1980; Luoma, 1983; White, 1984; Phillips and Segar, 1986; Cain and Luoma, 1990). Bioaccumulation is an important link between

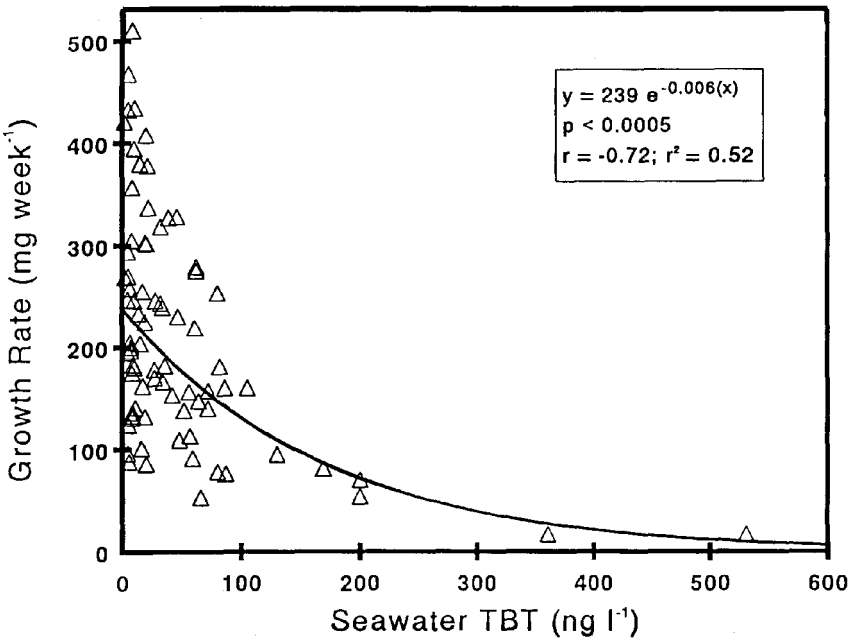


Figure 15.4 Effects of seawater TBT concentration on juvenile mussel growth rate. The equation of the best-fit line and relevant statistics are also given.

organism and its environment, but the relationship between concentrations of TBT in seawater and mussel tissue is very complex. Bioavailability of TBT may be affected by a number of natural factors, and the relationship between bio-availability and chemical analysis of TBT is unclear (Laughlin *et al.*, 1986; Salazar, 1986, Laughlin and French, 1988).

15.3.4. GROWTH

Juvenile mussel growth is significantly related to both seawater and tissue TBT concentrations. The statistical relationship is stronger for seawater TBT concentration. The relationship between juvenile mussel growth rate and seawater TBT concentration is negative and exponential (Fig. 15.4). Based on regression analysis, ≈52% of the growth variance can be explained by seawater TBT concentration. There is a high degree of variability at the lowest concentrations of seawater TBT. The seven data points for seawater TBT concentration <100 ng L^{-1} strongly influence the significance of the regression for all data. They also demonstrate the influence of the most contaminated marina where six of the seven highest measurements were made. The equations for all data and seawater TBT > 100 ng L^{-1} are quite similar. A power fit best describes the relationship for seawater TBT data >100 ng L^{-1} ($p = 0.0001$, $r = -0.97$, $r^2 = 0.94$). There is also a significant negative exponential relation-ship when the seawater TBT data <100 ng L^{-1} are analyzed separately ($p<0.0025$, $r = -0.36$, $r^2 = 0.13$), but only 13% of the growth variance can be explained by seawater TBT concentration. Some statistically significant relationships ($p = 0.0357$) were also found with seawater TBT data <70 ng L^{-1}, but the r^2 value was <0.09 and suggests little environmental significance.

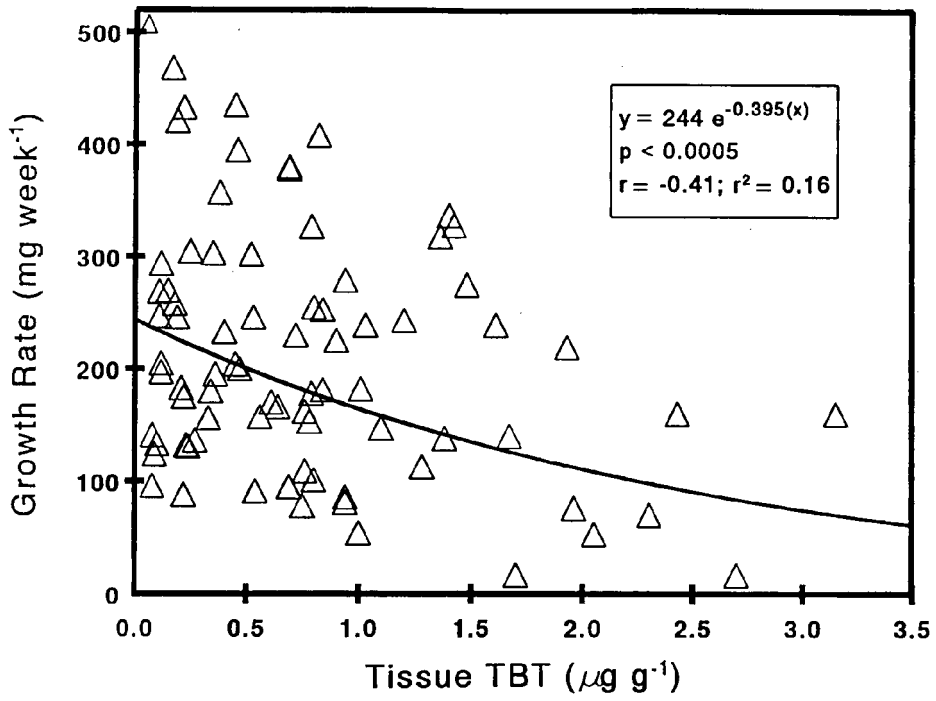


Figure 15.5 Effects of tissue TBT concentration on juvenile mussel growth rate. The equation of the best-fit line and relevant statistics are also given.

Although it has been suggested that bivalve growth is directly affected by accumulated TBT (Waldock and Thain, 1983), it is not clear from the weak relationships found in these field-transplant studies if accumulated TBT is regulating growth rate or growth rate is regulating the accumulation of TBT. There is a significant negative exponential relationship between juvenile mussel growth and tissue TBT concentration (Fig. 15.5), but variability in growth is high at all tissue TBT concentrations. Based on regression statistics, TBT in seawater has a more direct effect on mussel growth than TBT ac-cumulated in mussel tissues. Only 16% of the variance in growth can be explained by tissue TBT concentration. There are no significant regressions when the analyses are limited to data <1.5 μg g^{-1} TBT in tissue (wet wt). Many high growth rates are associated with elevated concentrations of TBT in tissue even at low concentrations of TBT in seawater.

Growth has a significant effect on the bioaccumulation process as shown by the significant linear relationship between growth rate and BCF. The highest growth rates are associated with the highest BCF values (Fig. 15.6). The highest growth rates are also associated with the lowest concentrations of TBT in seawater and reduced stress from decreased handling. These data suggest that growth rate affects bioaccumulation. At similar seawater TBT concentrations, faster growing mussels may accumulate more TBT. If mussels do not grow, they will not accumulate much TBT. Growth rates were very low in tests 2 and 8 due to winter conditions, but accumulation was similar even though TBT concentrations were significantly lower in test 8.

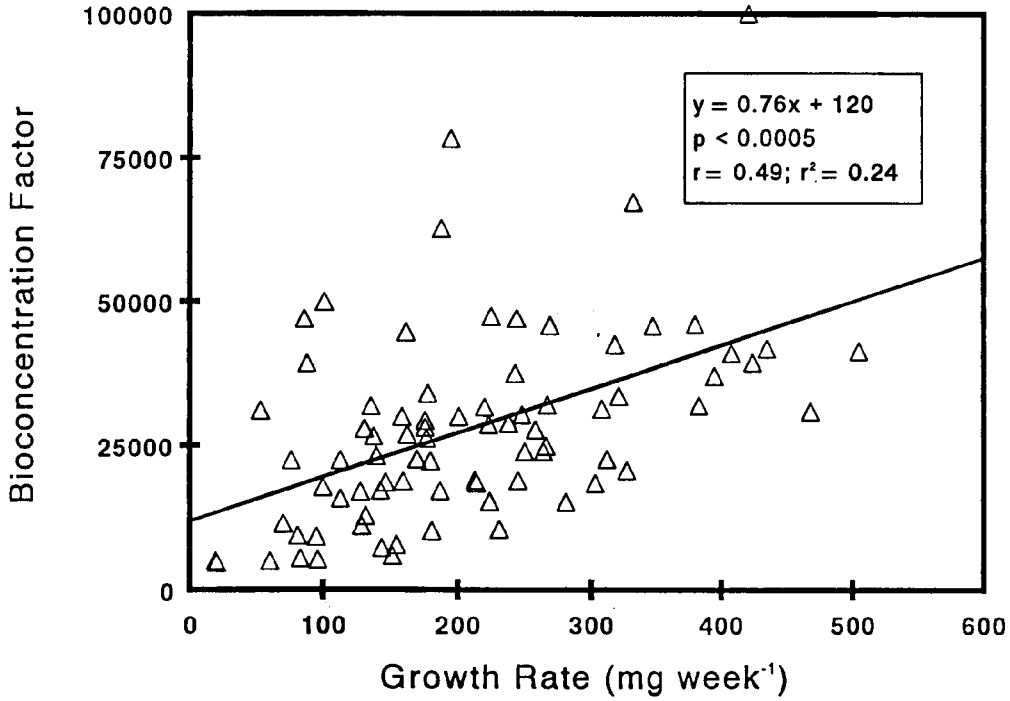


Figure 15.6 The effects of growth rate on bioconcentration factor. The equation of the best-fit line and relevant statistics are also given.

Using biological responses such as growth in field bioindicators to quantify environmental effects is also a potentially powerful tool, but, as with bioaccumulation, the limitations must be acknowledged (White and Champ, 1983; Cairns and Buikema, 1984; Malins *et al.*, 1984; Moller, 1987; Cairns, 1988). Based on statistical analyses, the data show that TBT accumulated in mussel tissue has less of an effect on mussel growth than TBT in seawater. These relationships demonstrate that other factors have a significant effect on growth rate. Nevertheless, growth rates can provide other information and perhaps be used to calibrate bioaccumulation (Fischer, 1983, 1988). Variable growth rates could explain some of the apparent anomalies in tissue accumulation shown here. For example, mussels severely stressed by TBT or other factors will not grow and will not accumulate much TBT. Without supporting measurements of growth and seawater TBT concentrations, only analyzing tissues could be very misleading. Survival was the least sensitive indicator measured here, but it helped explain the results. Clearly, natural factors can affect survival, bioaccumu-lation, and growth in juvenile mussels.

15.4. LABORATORY VS FIELD

There is a tremendous gap between correlations and causality when using biological indicators in the field. Biological responses under natural conditions are often very different from biological responses measured under controlled laboratory conditions, and they are affected by many different factors (White and Champ, 1983; Mallet *et al.*, 1987; Salazar, 1989). For example, conditions in laboratory and field tests appear to be as responsible for observed biological responses as the toxicant being tested. Temperature and nutritive stress have been shown to cause adverse physiological changes in mussels under

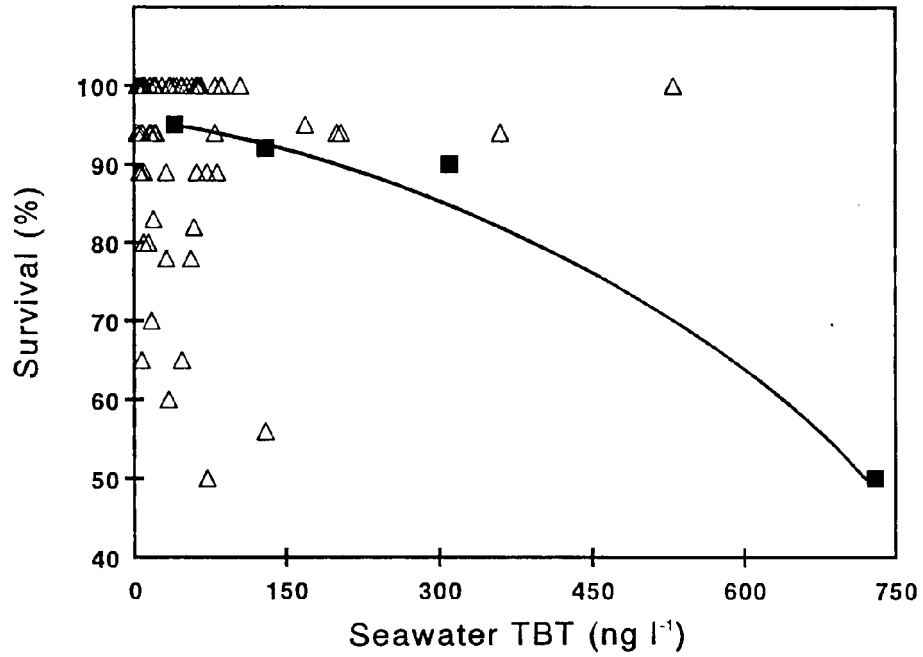


Figure 15.7 Comparison of survival in juvenile mussel field transplants (triangle) with survival in adults from laboratory studies (square) exposed to similar concentrations of TBT in seawater. A best-fit line is estimated.

laboratory conditions (Bayne and Thompson, 1970; Bayne, 1973). Mussel survival, bio-accumulation, and growth were generally much higher in these field studies than in comparable laboratory studies. Laboratory-induced stress may have enhanced the effects of TBT and account for the reduced performance of laboratory animals.

Survival of juvenile mussels in the field-transplant experiments was higher and did not demonstrate the dose dependency shown for adult mussels in laboratory toxicity tests (Thain, 1983; Valkirs *et al.*, 1987; Salazar and Salazar, 1989). These differences are best demonstrated by comparing results from a laboratory experiment with conditions most similar to field exposures (Fig. 15.7). In this laboratory experiment adult mussels were exposed to TBT for 66 d in a flow-through system (Valkirs *et al.*, 1987). Predicted decreases in survival with increasing seawater TBT concentrations found in the laboratory were not observed in the field-transplanted mussels. This apparent dose dependency in the laboratory may be due to a limited number of test concentrations and the use of extremely high seawater TBT concentrations (>500 ng L^{-1}) that strongly influence statistical analyses and yet are environmentally unrealistic. Dose dependency may not be manifested in field studies due to natural factors that modify mussel performance. At similar TBT exposure concentrations, survival was higher in field-exposed animals, even though the exposure period was longer and the test animals were also exposed to high concentrations of other contaminants.

It is extremely difficult to compare tissue TBT concentrations in mussels from these studies with tissue concentrations in mussels from laboratory studies. Analytical methods and units for reporting results are highly variable (Salazar, 1986; Page and Widdows, 1990). Another potential problem is that total contaminant content per individual may be more biologically significant than

measured tissue concentrations (Fischer, 1983, 1988; Cain and Luoma, 1990). One apparently common unit for comparison is BCF. This convention is commonly used in the TBT literature. The majority of BCF values calculated for mussels in this and other field-transplant studies, microcosm experiments, and natural populations are ≈30,000 (Salazar *et al*., 1987; Zuolian and Jensen, 1989; Grovhoug *et al*., Chapter 25). Only one laboratory experiment has provided comparable BCF values, and it was conducted under flow-through conditions with apparently healthy animals (Waldock *et al.*, Chapter 11). In most other laboratory experiments, BCF values were much lower with a reported maximum of ≈7000 (Laughlin *et al.*, 1986; Laughlin and French, 1988). The combined stresses of static-renewal conditions, overcrowding, and poor nutrition probably affected animal health and limited the ability to accumulate TBT. These obser-vations support measuring growth to quantify animal health and calibrate bio-accumulation.

Comparing BCF values from the laboratory and field can be misleading because they do not represent the same conditions. Since mussels may be exposed to TBT through various routes, such as seawater and food, field exposures include TBT from all sources. Technically, the BCF only accounts for the TBT concentration in seawater, which is generally the primary source in laboratory studies. The bio-accumulation factor (BAF) includes TBT from seawater and food. Higher BCF values from these field-transplant studies could be attributable to measuring only the TBT dissolved in seawater. These values could decrease substantially after ac-counting for TBT associated with the food, if food is a major source of accumulated TBT. Quantifying the food and water components of the BAF is important for understanding the bioaccumulation pro-cess and explaining effects attributable to TBT.

Laboratory-held mussels generally grow more slowly than mussels main-tained under natural field conditions (Kiorboe *et al.*, 1981). Temperature and nutritive stresses often associated with la-boratory experiments may cause physio-logical changes (Bayne and Thompson, 1970; Bayne, 1973) that preclude optimum growth rates and influence the relative effects of toxicants. In Fig. 15.8 juvenile mussel growth in our field-transplant studies is compared with juvenile mussel growth under laboratory (Thain, 1986) and flow-through microcosm conditions (Salazar and Salazar, 1987; Salazar *et al.*, 1987). The rate of juvenile mussel growth in field-transplant experiments is much higher than in laboratory or microcosm animals. Growth rates under laboratory and microcosm conditions were very similar and suggest a linear relationship between seawater TBT concentration and mussel growth. The field data show an exponential relationship. Growth rates of transplanted mussels approach those of juvenile mussels under laboratory and microcosm conditions only when exposed to seawater TBT concentrations >100 ng L^{-1}. At seawater TBT concentrations <100 ng L^{-1} growth rates for transplanted mussels are as much as an order of magnitude greater. Microcosm studies suggest significant re-ductions in juvenile mussel growth at sea-water TBT concentrations between 70 and 80 ng L^{-1} (Salazar and Salazar, 1987; Salazar *et al*., 1987). However, these mussels were stressed by overcrowding, high temperatures, and inadequate food associated with the test system and experimental procedures. These stressful conditions in laboratory and microcosm tanks may overestimate the effects of, TBT in most San Diego Bay environments. They may accurately represent the most stressful San Diego Bay environments.

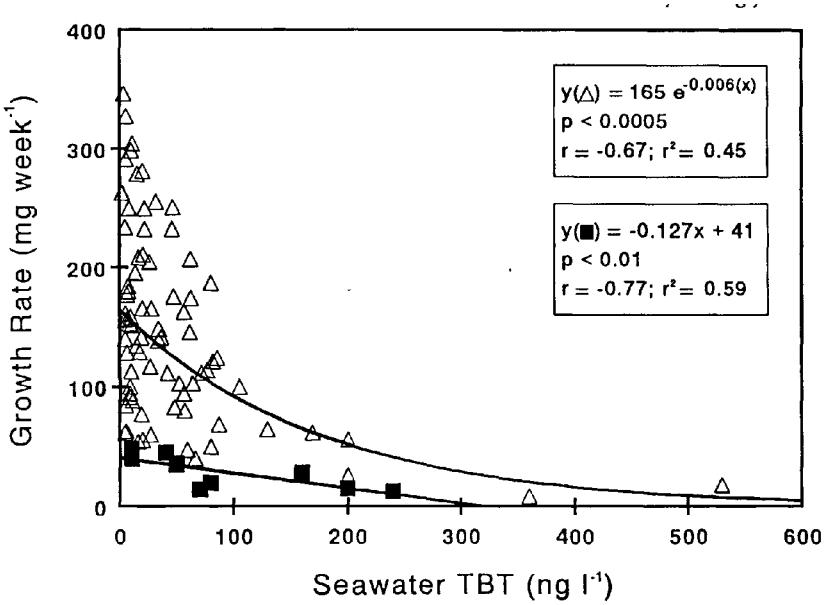


Figure 15.8 Comparison of 49-d juvenile mussel growth rates at various TBT concentrations from this and other studies. The relationship in the field (triangle) is exponential while the relationship in the laboratory and microcosm (square) is linear. The equations for the best-fit lines and relevant statistics are also given.

15.5. INFLUENCING FACTORS

15.5.1. HANDLING EFFECTS

Experimental procedures in the field can also induce stress that affects test results. Transplanting mussels to different environments and removing them from the water for growth measurements both modified performance. This was demonstrated by comparing growth rates of mussels measured weekly to those measured only at the end of the test (untouched) and by comparing bioaccumulation in transplanted and natural populations of mussels. Untouched mussels had significantly higher growth rates than animals measured weekly (Fig. 15.9). The results from five different sites confirm that frequency of measurement affects mussel growth rates. The largest differences were measured at the three highest concentrations of TBT in seawater where growth rates were approximately double those measured weekly. This suggests that stress attributable to handling enhanced the effects of TBT on juvenile mussel growth rates. Rates from biweekly measurements are similar to end-of-test measured rates and suggest they may be a reasonable indicator of growth in the natural population.

Measurements were changed from weekly to biweekly after test 4 to reduce the handling stress affecting growth rates. Increases in survival, growth, and a shift in the relationship between concentrations of TBT in seawater and in tissue after test 4 suggest that handling affected survival, bioaccumulation, and growth (Fig. 15.1).

Even though concentrations of TBT in seawater were relatively constant, the largest increase in mean growth rate occurred between tests 4 and 5 when measurements were changed from weekly to biweekly. The dramatic increases in growth and survival after test 4 suggest that switching to biweekly measurements had as much or more of an effect on growth as the concentration of TBT in seawater.

Collectively, these results suggest that transplanting and frequency of measurement both affect bioaccumulation. The effects of handling on bioaccumulation are also shown in the comparison between transplanted mussels and the natural population (Fig. 15.10). At the marina site with the highest concentrations of seawater TBT, transplanted juveniles accumulated much more TBT than natural juveniles in two of the three comparisons. Differences in growth rates associated with lower concentrations of seawater TBT and reduced handling could explain higher accumulation than expected at lower seawater TBT concentrations. Although only one comparison was made, transplanted adults accumulated more TBT than natural adults. Juvenile transplants measured for growth on a biweekly basis did not accumulate significantly different amounts of TBT than transplants measured only at the end of the test. Although only one comparison was made, accumulation of TBT in juvenile mussel transplants measured bi-

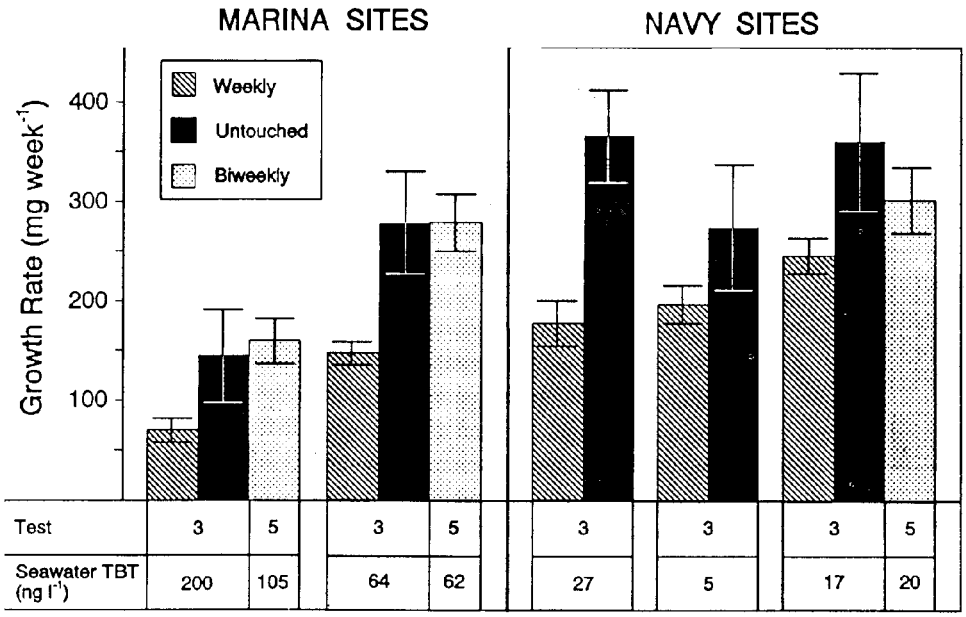


Figure 15.9 The effects of handling on juvenile mussel growth rates at two marina sites and three Navy sites on test 3. Growth rates were compared with animals measured weekly versus animals measured only at the beginning and end of the test. Growth rates for animals measured biweekly in test 5 are shown for comparative purposes. Mean concentrations of TBT in seawater and error bars (± 2 standard errors) are also given.

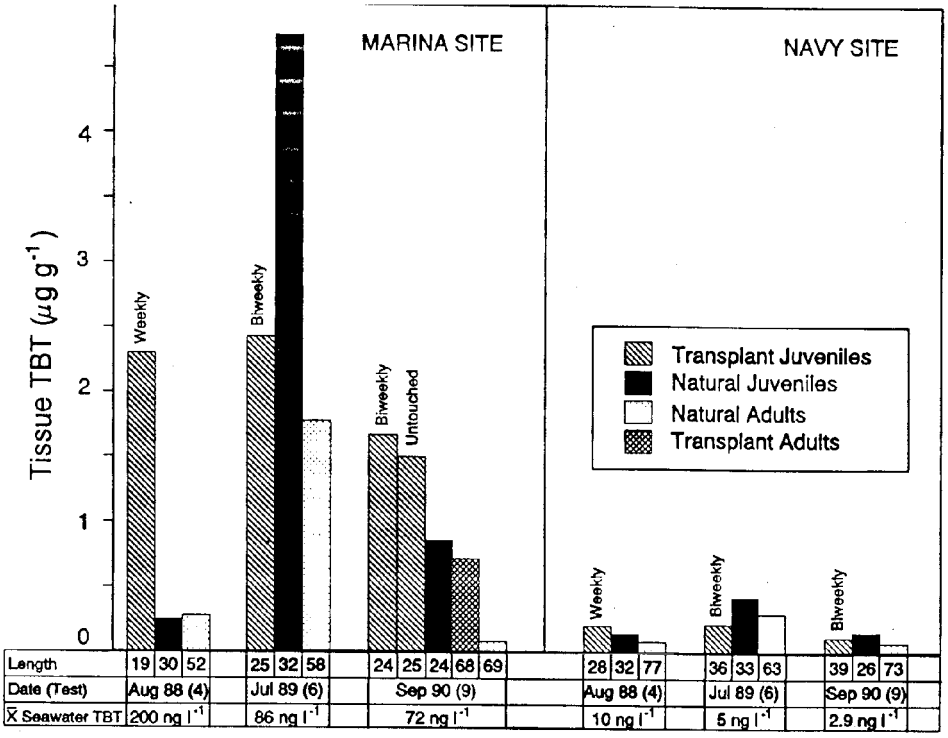


Figure 15.10 Effects of handling on bioaccumulation of TBT at the most TBT-contaminated marina site and the least TBT-contaminated Navy site. Juvenile and adult transplants as well as natural populations of juvenile and adult mussels are compared. Juvenile transplants measured biweekly for growth are also compared with those only measured at the end of the test (untouched).

weekly was slightly higher than in transplants measured only at the end of the test. At the Navy site concentrations of tissue TBT were significantly lower than at the marina site and generally too low for meaningful comparisons.

15.5.2. JUVENILES VS ADULTS

It is often assumed that juveniles of most species are more sensitive to contaminants than adults. Hall and Bushong (Chapter 9) suggest such a difference in sensitivity to TBT based on laboratory studies. In the field, rapid growth and low mortality have been associated with smaller animals while slow growth and high mortality have been associated with larger individuals (Freeman and Dickie, 1979). Juvenile mussels in our field studies exhibited higher survival when exposed to TBT than adults in a laboratory study (Valkirs *et al.*, 1987). These apparent differences between juveniles and adults could be attributable to the differences in laboratory and field experiments. However, test animals in this particular laboratory study were under severe nutritive stress, and mortalities were highest among the largest mussels. Their findings and our results are consistent with relationships established from physiological measure-ments in the laboratory and field observations. Laboratory experiments demonstrate increasing energy losses from respiration as a function of mussel size (Bayne *et al.*, 1985). These energy losses are enhanced by increasing temperature and decreasing food ration. Additional energy losses occur with gametogenesis. Therefore, under natural conditions the highest mortalities and lowest growth rates would be expected in the

largest mussels during gametogenesis when temperatures are highest and chlorophyll-*a* lowest (Freeman and Dickie, 1979; Incze *et al.*, 1980; Bayne *et al.*, 1985; Mallet *et al.*,. 1990). The introduction of TBT or any other contaminant could significantly increase the effects of these stressful conditions.

It is well established that juvenile mussels grow faster than adults (Rodhouse *et al.*, 1986), but reported effects of size and age on bioaccumulation are conflicting (Phillips, 1980). Fischer (1983, 1988) suggests that bioaccumulation in juvenile mussels represents a short-term integration while bioaccumulation in adults represents a longterm integration. Many authors have reported that smaller mussels accumulate more heavy metals than larger mussels (Lobel and Wright, 1982; Ritz *et al.*, 1982; Calabrese *et al.*, 1984; Amiard *et al.*, 1986). Juvenile oysters have been shown to accumulate more TBT than adult oysters (Ebdon *et al.*, 1989). In our study juvenile mussels generally accumulated more TBT than adults (Fig. 15.10). Our field-transplanted juveniles also accumulated more TBT than adult mussels in another San Diego Bay monitoring study of natural populations (Grovhoug *et al.*, Chapter 25). The differences in accumulated TBT may be due to the combined effects of size, handling, and natural factors.

15.5.3. NATURAL FACTORS

Even in the absence of contaminants, natural factors affect survival, bioaccumulation, and growth. They could also alter the effects of TBT. Important natural factors include temperature, food, current speed, salinity, suspended sediment, and tidal position (Seed, 1976; Newell, 1979; Kiorboe *et al.*, 1981). In our field-transplant studies there is no statistically significant relationship between chlorophyll-*a* (food) and growth rate, but chlorophyll-*a* measurements may not provide the best estimate of available food. Particulate organic carbon has been related to growth in southern California coastal waters (Page and Hubbard, 1987). Our weekly and bi-weekly chlorophyll-*a* measurements may have been too infrequent to detect a statistically significant effect. On the other hand, chlorophyll-*a* levels in San Diego Bay may not be a limiting factor. Due to a paucity of freshwater inputs, salinity is not highly variable in most areas of San Diego Bay and is probably not a significant factor in mussel growth rates. Salinity could be an important factor in other more typical estuaries. Current speed appeared to be very important, but measurements were too infrequent to quantify a significant relationship. Suspended sediment was not measured but has been very important in other TBT studies (Waldock and Thain, 1983).

Temperature had more of an effect on growth than any natural factor quantified in our studies. There is a significant linear relationship between temperature and growth rate ($p<0.0005$), but only 11% of the variance in growth can be explained by temperature. The interaction between temperature and seawater TBT on growth of juvenile mussels is shown in Fig. 15. 11. The three-dimensional surface plot predicts optimum growth near 20°C and at the lowest concentrations of TBT in seawater. Optimum growth near 20°C has been reported in several other laboratory and field studies (Incze *et al.*, 1980; Almada-Villela *et al.*, 1982; Bayne *et al.*, 1985). The lowest growth rates are predicted at the highest TBT concentrations (>100 ng L^{-1}) and temperature extremes (>22°C, <16°C). Therefore, the highest and lowest temperatures may impose natural limits on mussel growth rates and survival in San Diego Bay (Wells and Gray, 1960). It is possible that high summer temperatures alone adversely affect survival, bioaccumulation, and growth, and limit natural populations in the southern portion of San Diego Bay.

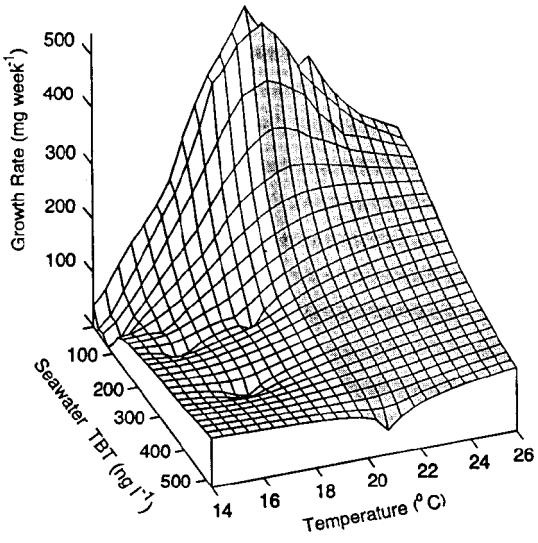


Figure 15.11 Effects of seawater TBT concentration and temperature on growth predicted from three-dimensional surface plots using weighted means. Relative growth is the *z*-axis. Shaded area represents growth rate reductions at temperatures above 20°C. Reduced growth at low temperatures (<16°C) and high concentrations of TBT in seawater (>100 ng l^{-1}) are also apparent.

Comparing the correlation coefficients of growth rate versus temperature and concentrations of TBT in seawater shows how the relationships change (Fig. 15.12). This also establishes a statistical correlation for the graph in Fig. 15.11. There is a significant linear relationship between growth and temperature in the range of 14-20°C ($p<0.0001$, $r = 0.50$, $r^2 = 0.25$). The relationship improves when the seawater TBT data <100 ng L^{-1} are analyzed separately ($p<0.0001$, $r = 0.57$, $r^2 = 0.32$). The combination of TBT masking temperature effects and too few exposures >20°C precluded detecting a statistically significant relationship at higher temperature ranges. Considering all the data, growth rates are correlated with both seawater TBT concentration ® = -0.72) and temperature (r = +0.33); although TBT concentration is more important. Using only seawater TBT data <100 ng L^{-1}, the importance of seawater TBT concentration is reduced by half. Figure 15.12 also shows the dramatic shift from a positive to a negative correlation at temperatures above 20°C that is consistent with the literature. TBT appears to modify the effects of temperature and temperature appears to modify the effects of TBT on mussel performance.

15.6. MUSSEL BIOINDICATOR MODEL

White (1984) has cautioned against the arbitrary use of mussel monitoring systems with out developing a model to be tested. The mussel bioindicator model in Fig. 15.13 emphasizes the importance of natural factors in modifying the environmental effects of TBT in San Diego Bay and depicts the inherent cycles of natural factors, TBT inputs, and mussel biology. It is suggested that natural factors act directly on TBT by altering bioavailability and directly on mussels by altering biochemistry and physiology. Other contaminants are also involved. For example, the marina with the highest seawater TBT concentrations also had the copper concentrations (≈10 μg L^{-1}) (Krett Lane, 1980; Johnston, 1989). These copper concentrations were approximately 150 times higher than seawater TBT concentrations and about three times higher than the US Environmental Protection Agency (US EPA) water quality criterion (US EPA, 1985). Similar copper concentrations reduced mussel growth rates in laboratory studies (Stromgren, 1982; Manley *et al.*, 1984). Even though they are much less toxic than TBT, accumulated petroleum hydrocarbons were shown to have more of an adverse effect on mussel physiology than accumulated TBT (Widdows *et al.*, 1990).

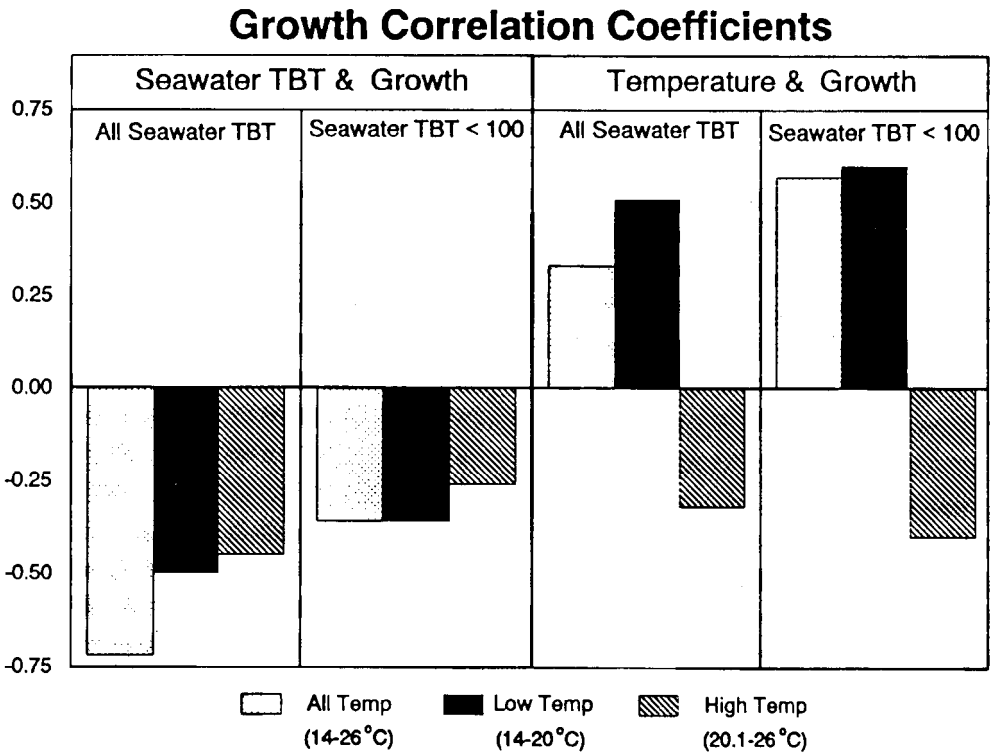


Figure 15.12 Comparison of correlation coefficients of growth rate versus temperature and concentrations of TBT in seawater to show how the relationship with growth changes at different temperatures and TBT exposures.

In addition to being modified by natural factors and TBT, mussel biology is also affected by internal biochemical and physiological cycles. These factors act in concert to modify mussel growth, bioaccumulation, and survival. The key to calibrating the mussel bioindicator is separating the effects of natural and biological factors from the effects of TBT. The clear arrows in the model (Fig. 15.13) represent the direct effects of relatively uncontaminated environments on mussel growth and survival. The dark arrows show direct effects from TBT contamination on growth, bioaccumulation, and survival.

This mussel bioindicator model has been developed based on field studies in San Diego Bay and existing knowledge of mussels and TBT. It demonstrates the difficulties in quantifying bioindicator responses to TBT by illustrating the complex and dynamic interactions among various factors that affect mussel growth, bioaccumulation, and survival. All these factors must be measured to test and verify the model and calibrate the bioindicator. The model presented here could be used for other contaminants and other bioindicators; however, given the unique and variable mussel responses to TBT exposures in San Diego Bay, extreme caution should be used in extrapolating specific results to other environments, contaminants, or bioindicators.

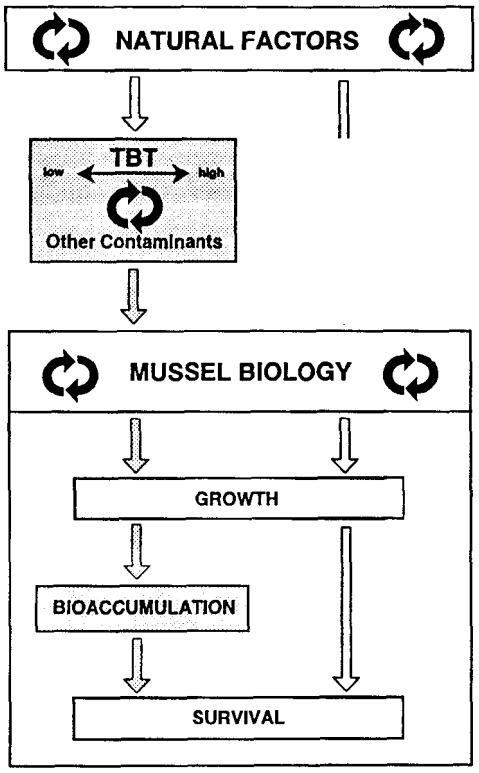


Figure 15.13 Conceptual mussel bioindicator model developed from the results of these studies. The model emphasizes the importance of natural factors in regulating mussel biology and modifying the environmental effects of TBT.

15.7. CONCLUSIONS

This TBT study in San Diego Bay demonstrates the utility of juvenile mussels as biological indicators. The most significant refinement in approach was the integration of chemical and biological measurements. Chemical measurements of TBT in seawater and mussel tissues were combined with biological response measurements of survival and growth of transplanted mussels. Mussels were used as indicators of contamination and effects. By using this approach and transplanting mussels under natural test conditions, we were able to quantify the distribution and effects of TBT, identify short- and long-term trends, and refine the use of mussels as biological indicators. The frequency of water sampling combined with multiple measurements on the same individual mussels of a very restricted size range facilitated defining statistically significant relationships. The lack of replication for tissue residue may have precluded defining a better relationship between tissue TBT concentration and juvenile mussel growth rates. It should be emphasized that a statistically significant relationship does not prove an environmentally significant relationship. Conversely, not finding a statistically significant relationship could be attributed to the influence of various factors identified here. Zar (1974) states:

> Although in many cases there is a mathematical dependence of Y on X, it cannot automatically be assumed that there is a biological cause-and-effect relationship. Causal relationships are concluded only with some insight into the natural phenomenon being investigated and may not be concluded by statistical testing alone. It must also be remembered that a regression function is mathematically nothing more than a line forced to fit between a set of data points, and may not at all describe a natural phenomenon. Although an empirically derived regression function often provides a satisfactory and satisfying description of a natural system, sometimes it does not.

Identifying some of the natural and pollution-related factors affecting mussel performance when exposed to TBT under natural conditions is only the first step in calibrating the field bioindicator and verifying the model. Crucial questions regarding environmental fate and effects of TBT remain unanswered. Factors identified in this study as affecting mussel performance need further investigation. Other contaminants and natural factors require additional study. Differences in results between the

laboratory and the field require meaningful explanations. All test conditions modifying results must be identified and their relative influence quantified.

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EPILOGUE

While it is beyond the scope of this chapter to provide all the significant findings and paradigms established since this study was conducted relative to TBT and mussels, we would like to provide an updated perspective. This new perspective will emphasize new results and new ideas concerning the three primary goals of our original work: (1) effects of TBT on juvenile mussels, (2) trends in the distribution of TBT and its effects, and (3) refinements in the mussel bioindicator system.

Most importantly, threshold concentrations predicted in this chapter to always reduce growth in juvenile mussels have not changed. Threshold concentrations for no effects remain speculative based on our data, but are probably lower than originally predicted. Although the data provided in this chapter regarding the size effects on bioaccumulation of TBT were only preliminary, additional San Diego Bay studies conducted in 1990-1991 and 1993, and a Puget Sound study, have confirmed that smaller mussels consistently accumulate higher concentrations of TBT in their tissues and consistently have higher rates of survival (Salazar and Salazar, 1995; Salazar *et al*., 1995). The interaction between size effects, concentration effects, and natural factors in determining the time required for TBT to reach equilibrium in mussel tissues remains unclear. Although the 1990-1991 study showed that small, fast growing mussels could reach equilibrium in less than 3 weeks at low TBT concentrations, we still recommend an exposure period of 60-90 d based on the time to reach equilibrium for larger mussels at high TBT concentrations (Salazar and Salazar, 1995). The trends in distribution of TBT and its effects have not changed. The 1993 San Diego Bay study shows that mussel growth rates were higher and tissue TBT concentrations lower than in 1990. This suggests that mussel growth rates have increased as a result of the decreases in TBT.

The mussel monitoring system described in this chapter is operational. A successful test could be conducted using the protocols outlined in Salazar and Salazar (1995). We continue to add improvements in logistics, handling, and interpretation from insight gained in subsequent tests. From 1990 to 1995, we have conducted transplants using six different bivalve species and over 15,000 individuals. The major conceptual refinements in experimental design that we originally introduced in this chapter remain unchanged. Since a 2 mm size range is not always achievable, 10 mm is recommended as a target size range. Absolute size is less important than minimizing the range. Other logistic refinements include using disposable mesh bags with individual compartments instead of rigid plastic cages, and interfacing digital calipers and balances with laptop computers to facilitate data entry in the field.

Although our standard protocol includes measuring whole-animal wet-weights and lengths, shell weights, and tissue weights, we only presented the whole-animal wet-weight data in this chapter. Several subequent studies have shown that under certain conditions, estimates of growth based on tissue weights are more informative than whole-animal growth. We are in the process of reanalyzing the tissue weight data from these nine transplant experiments to see if these data can provide any further clari-

ication regarding TBT effects on mussels. Our most recent statistical analysis of copper and zinc tissue data collected during tests 5, 6, 7, 9 have shown significant relationships with juvenile mussel growth rates (Salazar and Chadwick, 1991; Salazar and Salazar, 1995). The data further indicate that, in addition to TBT, smaller mussels accumuated higher concentrations of several metals than larger mussels (Salazar and Salazar, 1995; Salazar *et al.*, 1995). Some of the reationships were more informative by analying the data on a per animal basis (content) rather than on a concentration basis.

In the final analysis, bivalves in cages could be viewed as an exposure system to make any clinical measurements. This versatility is another advantage of using bivalve transplants. Further, the discriminating power of bivalve monitoring is well beyond the order-of-magnitude differences initially suggested by Goldberg *et al.* (1978) as a limit for detecting significant differences in concentrations of tissue contaminants. A number of studies, including our own, have often found statistically significant differences in both bioaccumulation and growth that we believe are environmentally significant. Some of these site-specific data differ by a factor of 2 or less. In-situ, studies with caged bivalves facilitate measurements that make this field bioassay similar to laboratory bioassays in terms of experimental control and predictive power, and retain the environmental realism of traditional field monitoring. Approaches that combine the integrating ability of filter feeding bivalves, the versatility of in-situ caging, and the relationships between bioaccumulation and bioeffects as demonstrated here, have a number of applications for environmental monitoring, risk assessment, and establishing regulatory criteria.

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