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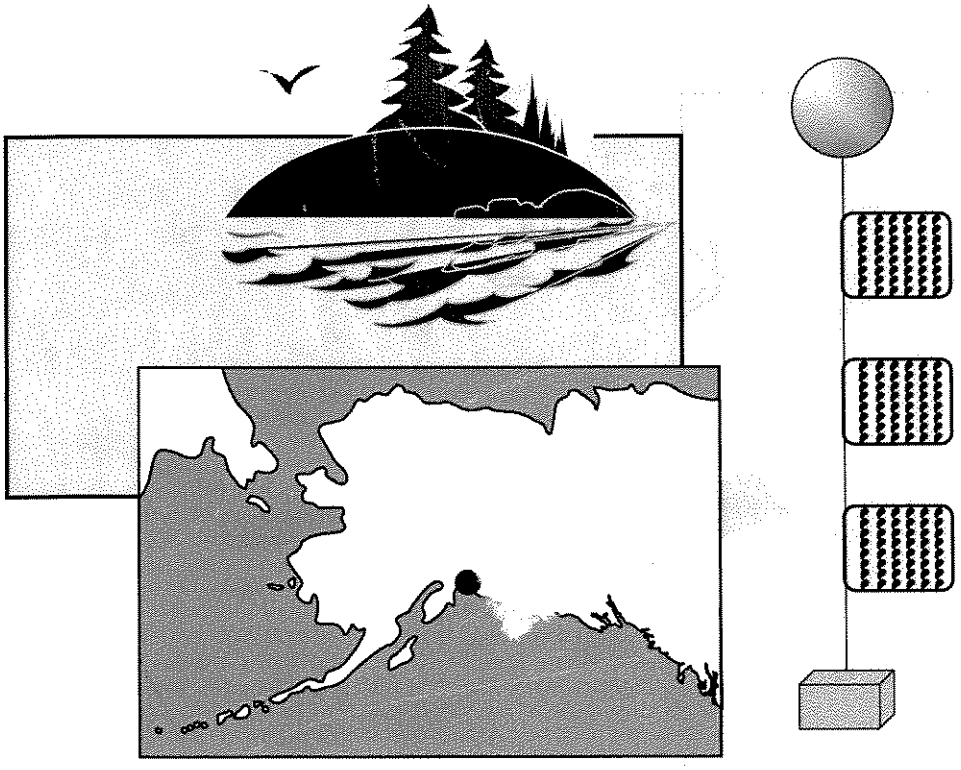
Final Report

Report Submitted to
Prince William Sound Regional Citizens' Advisory Council
Contract Number 631.1.97

Caged Mussel Pilot Study
Port Valdez
Alaska
1997



11648 - 72nd Place NE
Kirkland, WA 98034
(425) 823-3905

Applied Biomonitoring
Michael H. Salazar
msalazar@cnw.com

CAGED MUSSEL MONITORING PILOT STUDY
Ballast Water Treatment Facility

FINAL REPORT

Prepared for:

Joe Bridgman
Prince William Sound RCAC Program Manager
P.O. Box 3089
Valdez, AK 99686

Prepared by:

Applied Biomonitoring
11648 72nd Place NE
Kirkland, WA 98034

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LIST OF ACRONYMS

ADEC	Alaska Department of Environmental Conservation
ANOVA	Analysis of variance
ARI	Analytical Resources, Inc.
BOT	beginning of test
BWTF	Ballast Water Treatment Facility
CHN	carbon, hydrogen, nitrogen
CAL	Columbia Analytical Laboratories
DO	dissolved oxygen
dw	dry weight
EDR	Exposure-dose-response
EOT	end of test
EVS	EVS Environment Consultants, Inc.
MLLW	mean lower low water
NPDES	National Pollutant Discharge Elimination System
NMFS	National Marine Fisheries Service
NOAA	National Oceanic and Atmospheric Administration
PAH	polynuclear aromatic hydrocarbon
PVC	polyvinyl chloride
QAPP	quality assurance project plan
QA/QC	quality assurance/quality control
RCAC	Regional Citizens' Advisory Council
RFP	request for proposal
SE	standard error
SQT	sediment quality triad
TBT	tributyltin
TOEM	Terminal Operation Environmental Monitoring
TSS	total suspended solids
U.S. EPA	U.S. Environmental Protection Agency
WAWW	whole-animal wet-weight
WP	Work Plan
ww	wet weight

1.0 EXECUTIVE SUMMARY

A caged mussel pilot study was conducted between February and April, 1997 in Port Valdez, Alaska. The purpose of this study was to determine the feasibility of using transplanted mussels to monitor effluent from the Ballast Water Treatment Facility (BWTF) at a depth of 70 meters, where mussels are not normally found. A total of 2100 Pacific blue mussels (*Mytilus trossulus*) were transplanted from the intertidal zone in Anderson Bay to 7 stations in the vicinity of Alyeska's BWTF effluent diffuser for a period of 56 days.

The three most important questions to be answered by this study were the following:

- Will the mussels survive?
- Will the mussels grow?
- Will they accumulate chemicals associated with the BWTF effluent?

The size range of test mussels was limited to 30-36 mm to minimize the potential effects of size and related factors (e.g., tissue mass, percent lipids) on bioaccumulation and growth. Compartmentalized cages were used to provide replicate measurements on the same individual mussels at the beginning and end of the test. This improved the confidence in length and weight measurement data and the discriminating power of the test. Growth of mussel shells and tissues were used to characterize biological effects associated with exposure to the BWTF effluent. Bioaccumulation of polycyclic aromatic hydrocarbons (PAHs) was used to characterize potential chemical exposure from the BWTF effluent. To estimate changes in tissue weights and tissue concentrations of PAHs accumulated during the 56-day exposure period, tissues from an additional 300 mussels in the same size range were measured at the beginning of the test and stored for chemical analysis. These tissues were only analyzed after establishing that pre-determined survival (i.e., 50%) and growth criteria (i.e., <20% loss in tissue weight) were met.

Mussel survival was 97%. Increases in shell lengths and whole-animal wet-weights were small (< 1mm, < 1 gram respectively), but statistically significant all sites except the one closest to the diffuser. Estimated tissue weights appeared to decrease slightly at most sites (< 0.1 gram) but these changes were only statistically significant at sites further from the diffuser. It was intended that growth of mussel shells and tissues would be used to characterize associated biological effects, but there was not a clear relationship between the effluent and mussel growth. PAHs known to have been associated with the BWTF effluent accumulated in caged mussel tissues at concentrations approaching an order of magnitude higher than at the beginning of the study.

Although not explicitly stated, implicit in the objective of this pilot study were questions about the method's ability to distinguish differences in bioaccumulation and growth among stations and over space and time. Statistically significant differences were found in mussel growth among stations, and between stations inside, and outside the BWTF mixing zone, as defined in the U.S. Environmental Protection Agency's (U. S. EPA's) National Pollutant Discharge Elimination System (NPDES) permit. The chemistry data showed a gradient of decreasing concentrations of total PAHs in mussel tissues with increasing distance from the diffuser. This gradient is consistent with results from other studies, such as dye dispersion studies, plume modeling, sediment monitoring, and a previous caged mussel study conducted in the same area.

The pilot study was successful because the objectives were met. Test mussels exceeded pre-determined survival and growth criteria and accumulated target chemicals in their tissues. Results demonstrate the feasibility of using caged bivalves for monitoring the effluent discharged by the BWTF. New information was provided regarding bioavailability, pathways of exposure, and potential exposure to PAHs. Potential chemical exposure by depth and distance from the BWTF effluent diffuser was quantified with mussel tissue chemistry. These chemical measurements showed differences in bioaccumulation among stations 200 meters apart and between mussel arrays as close as two vertical meters. Simultaneously, tissue and shell growth were quantified with various metrics over space and time to assess potential effects.

Results of the pilot study will be used to evaluate the potential use of caged mussels as an environmental monitoring tool in Port Valdez. The technique has been standardized to allow temporal comparisons, and is flexible enough to help answer questions on a site-specific basis. Although the pilot study was not designed to reach conclusions about the mixing zone and impacts to the benthos, the results indicate that this method could be used to answer longstanding questions about the BWTF's potential impact on Port Valdez in the vicinity of the diffuser. Using caged mussels as sentinels of potential exposure and effects is consistent with the Prince William Sound RCAC objective of developing a monitoring strategy that will permit early detection of environmental impacts associated with BWTF operations.

2.0 INTRODUCTION

Monitoring the effects of the Valdez Marine Terminal operations on the Port Valdez environment has been a top priority of the Prince William Sound Regional Citizens' Advisory Council (RCAC). The portion of the marine terminal which is widely regarded as having the most potential for impact on the marine environment is the Ballast Water Treatment Facility (BWTF). This facility operates under a federal wastewater permit and is allowed to discharge up to 30 million gallons of water per day into Port Valdez. This water comes primarily from unladen oil tankers which arrive in Port Valdez carrying ballast water in their cargo holds for stability at sea. Treatment is required because the ballast water usually contains residual amounts of crude oil and other potentially toxic chemicals. A three-stage treatment process is used before the ballast water is discharged in Port Valdez at a depth of approximately 70 meters. Due to difficulties in sampling the effluent and associated effects at this depth, the biochemical and ecological interactions between chemicals from the diffuser and the resident biota are poorly documented and understood.

Although the effluent contains chemicals at concentrations that are within limits specified by the U.S. Environmental Protection Agency (U.S. EPA) in their National Pollution Discharge Elimination System (NPDES) permit, local citizens have expressed concerns that chronic release of chemicals, even at low concentrations, may be adversely affecting the ecology of Port Valdez. Environmental monitoring studies conducted over the last two decades do not indicate that the ecosystem is being adversely affected by this discharge, but concentrations of polynuclear aromatic hydrocarbons (PAHs) above background concentrations, and changes in the benthic community have been observed in sediments near the diffuser (Feder and Shaw, 1996). There are concerns that PAHs and other chemicals associated with the BWTF may be causing effects that cannot be identified with traditional monitoring methodologies. Chemical analysis of discrete water samples, laboratory bioassays, and studies of benthic community structure may not be sensitive enough to detect environmentally significant changes in the ecology of Port Valdez, particularly in the water column. Increased concern is based on the number of uses of this waterway that rely on its environmental health: subsistence harvesting, commercial fishing, sport fishing, salmon hatchery operation, recreational boating, commercial cruising, and others.

Many of the concerns that have been expressed are based on the following limitations of standard approaches: 1) discrete water samples only represent an instant in time and do not provide reasonable estimates of biologically available chemicals over extended periods of time; 2) laboratory bioassays provide a great deal of experimental control but are generally conducted on a small number of representative species under environmentally

unrealistic test conditions; and 3) benthic community analyses are the most environmentally realistic of traditional approaches but results are often difficult to interpret due to the effects of uncontrolled natural factors, and they do not adequately represent potential effects in the water column. Further, benthic community structure may not adequately reflect variability in, and changes resulting from, water column exposure.

Monitoring with caged mussels has been suggested on a number of occasions by the U.S. EPA, RCAC consultants, and others as a way to address these citizens' concerns. Federal scientists suggested it as a means to determine the source of PAHs found in the sediments. The ability of bivalves to bioaccumulate chemicals from their surrounding environment makes them good candidates for increasing understanding and characterizing ecological processes in Port Valdez. However, before a full-scale caged mussel monitoring program could be implemented, a pilot study (the focus of the present report) was needed to determine whether these transplanted organisms would survive, grow, and accumulate target chemicals in the vicinity of the diffuser near a depth of 70 meters.

The common blue mussel (*Mytilus* sp.) is one of the most widespread marine molluscs in the world and they form an important element in the ecology of coastal waters. Because they are sessile filter feeders and have been shown to integrate concentrations of trace toxic substances, mussels have been widely used as biomonitoring organisms for coastal water quality. They have also been used extensively as model organisms in many scientific studies and a vast body of literature exists from basic physiological, biochemical, genetic, and toxicological investigations (Gosling, 1992).

2.1 Background

Prior to installing the BWTF diffuser, a generic framework was developed for long-term monitoring and comprehensive environmental studies in Port Valdez. The effluent diffuser system was the first major oil and gas industry discharge permitted for coastal Alaska (Redburn, 1988). Regulatory authority for the NPDES permit requirements is shared by the U.S. EPA and the state of Alaska Department of Environmental Conservation (ADEC). Baseline data were obtained from monitoring studies conducted in Port Valdez between 1971 and 1972 (Hood et al., 1973) and juvenile fisheries studies conducted by the National Marine Fisheries Service (NMFS). The first NPDES permit granted in 1975 used laboratory toxicity tests to establish the limits of 0.05 mg/L oil and grease at the top and bottom of the mixing zone (Redburn, 1988).

The long-term monitoring plan developed to meet NPDES requirements needed to address several different issues. A primary consideration was the fate and effects of introduced

hydrocarbons associated with the effluent discharged into the waters of Port Valdez (Redburn, 1988). The hypothesis developed to address this concern was that hydrocarbon concentrations would not become elevated in the water column or in the tissues of aquatic organisms outside of the mixing zone. Another issue was the adequacy of the mixing zone established in 1975. It was necessary to deviate from strictly traditional monitoring approaches because oil was not acutely toxic to many organisms and any effects would probably be gradual, long-term changes in response to chronic, low-level concentrations of petroleum hydrocarbons. In 1985, Alyeska contracted with the University of Alaska to reinitiate intertidal and benthic field studies (Feder and Shaw, 1986).

The use of caged bivalves as a monitoring tool in Port Valdez was recommended by the U.S. EPA's contractors reviewing the NPDES monitoring program in early 1992 (Tetra Tech, 1992, 1993, 1994). This approach was also recommended at the Scientific Meeting on Environmental Monitoring of Port Valdez on January 17-18, 1995, and was included in U.S. EPA's (1995) Very Preliminary Environmental Monitoring Requirements as a possible key component of the new NPDES permit.

The Prince William Sound RCAC and its Terminal Operations and Environmental Monitoring (TOEM) committee agreed that this approach could be a valuable monitoring tool. A number of planning meetings and conference calls were held between 1995 and 1996 to define the program objectives. A request for proposal (RFP) was sent out in June, 1996. Applied Biomonitoring's original proposal suggested conducting a 90-day study in the fall of 1996 to maximize growth potential. However, due to a number of unforseen difficulties, the study could not be initiated until February 1997, and mussels were deployed for 56 days.

A progress report submitted on April 28, 1997, presented the preliminary findings of the survival and growth components of the study (Tier 1). These results demonstrated that the criteria established for Tier I had been met, indicating a successful test, and it was appropriate to proceed to Tier II, chemical analysis of mussel tissues. Analytes for chemical analysis were selected after consultation with RCAC, Alaska Department of Environmental Conservation (ADEC), U.S. EPA, and Alyeska. Chemical analysis was initiated in September, 1997, according to methods identified in the Quality Assurance Project Plan (QAPP; EVS Consultants, 1997a; available from RCAC); the electronic version of the tissue chemistry data became available on January 2, 1998.

The first Draft Final Report was submitted on February 1, 1998 and a second Draft Final Report was submitted on March 10, 1998. During this time, both drafts received intense review from the following: 1) RCAC; 2) RCAC Scientific Advisory Committee, 3) four

independent reviewers retained by RCAC; 4) Alyeska; 5) EPA; 6) two reviewers on the Applied Biomonitoring team; and 7) four independent reviewers who supplied a courtesy review for Applied Biomonitoring. It was during this review process that several reviewers noticed many of the alkylated homologs were conspicuously absent. Agreement was reached to have the samples re-analyzed in April, 1998 at no additional cost through the cooperation of the analytical lab; the missing alkylated homologs were identified and quantified during the re-analysis. An overview presentation describing the results of this pilot study was made to the Prince William Sound RCAC board of directors in May, 1998 where additional comments were provided. The Final Report, submitted on July 30, 1998, incorporates the most substantive comments and suggestions.

2.2 Physical Setting

The Port Valdez BWTF effluent is discharged into the head of Port Valdez, a water body that connects with Prince William Sound (Figure 1). Port Valdez is a 22 km by 6.0 km glacial fjord. In general, steep rocky shores in the western half of Port Valdez are subsequently replaced by boulder-cobble beaches to the east. In the eastern half, extensive mudflats are found where major glacier streams enter the fjord. The marine terminus of the Trans-Alaska Oil Pipeline is situated on the south shore of the port.

The waters of Port Valdez reflect seasonal fluctuations in glacial runoff and precipitation. In general, surface waters exhibit reduced salinity from late spring through fall due to glacial melt-water and precipitation. Salinities as low as 0.29 ppt have been reported in some areas. Surface water temperature shows a gradual warming trend through late summer reaching temperatures of 12°C. Minimum salinities coincide with maximum water temperatures. The freshwater influx becomes minimal in Port Valdez at the onset of freezing air temperatures, with surface salinities approaching open ocean values of 31.55 ppt from January through March. In the late winter, surface water temperatures decline to a yearly minimum of about 0.2°C. Seasonal variations in salinity and water temperature are most pronounced at the head and eastern half of Port Valdez where most of the major glacial streams discharge. The suspended sediment load also exhibits a marked seasonality. Most sediments entering the port are discharged by the Lowe River, Valdez Glacier Stream, and Mineral Creek, which discharge to the eastern half of Port Valdez (Feder and Keiser, 1980).

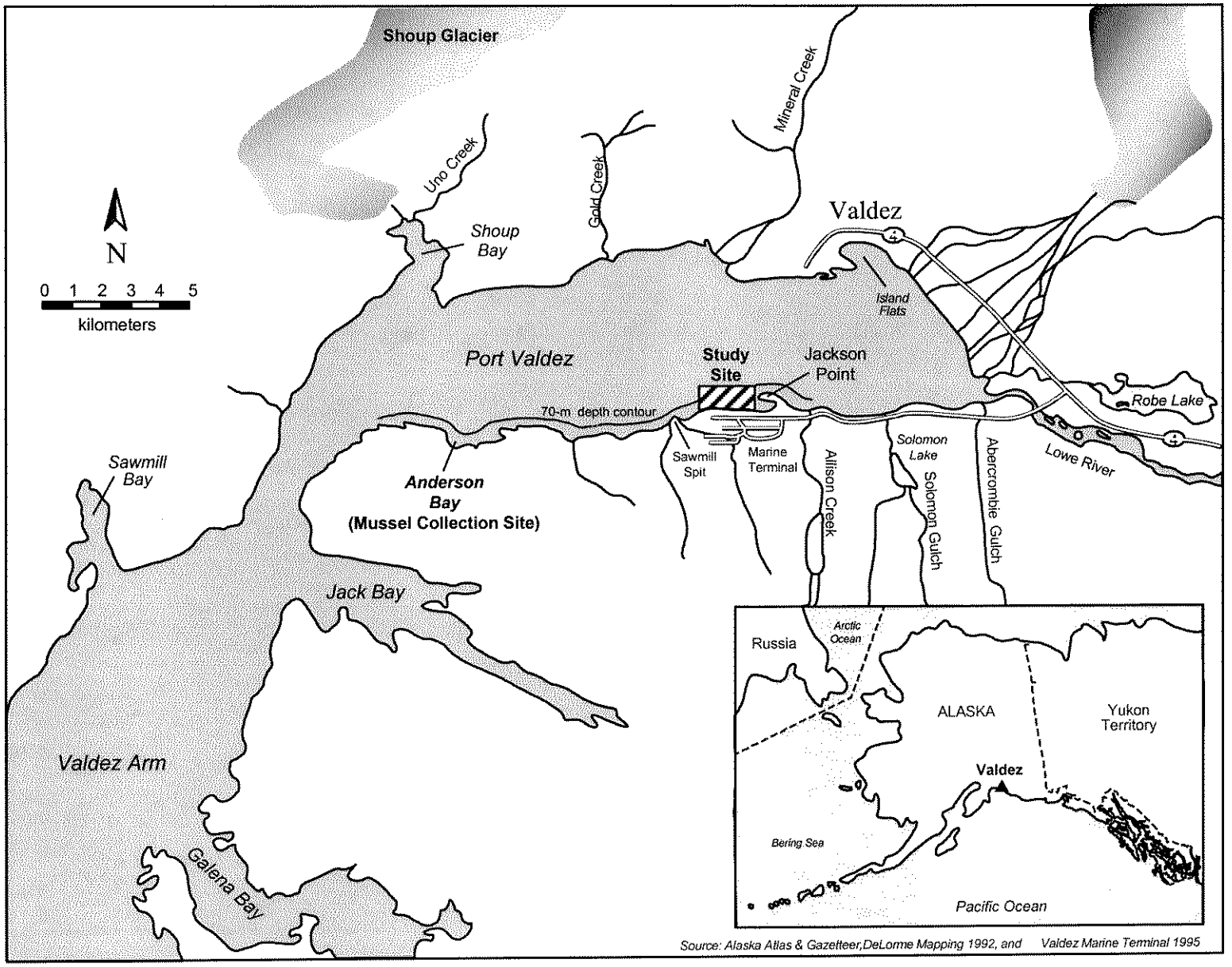


Figure 1. Overview map of Port Valdez, Alaska showing study site and mussel collection site.

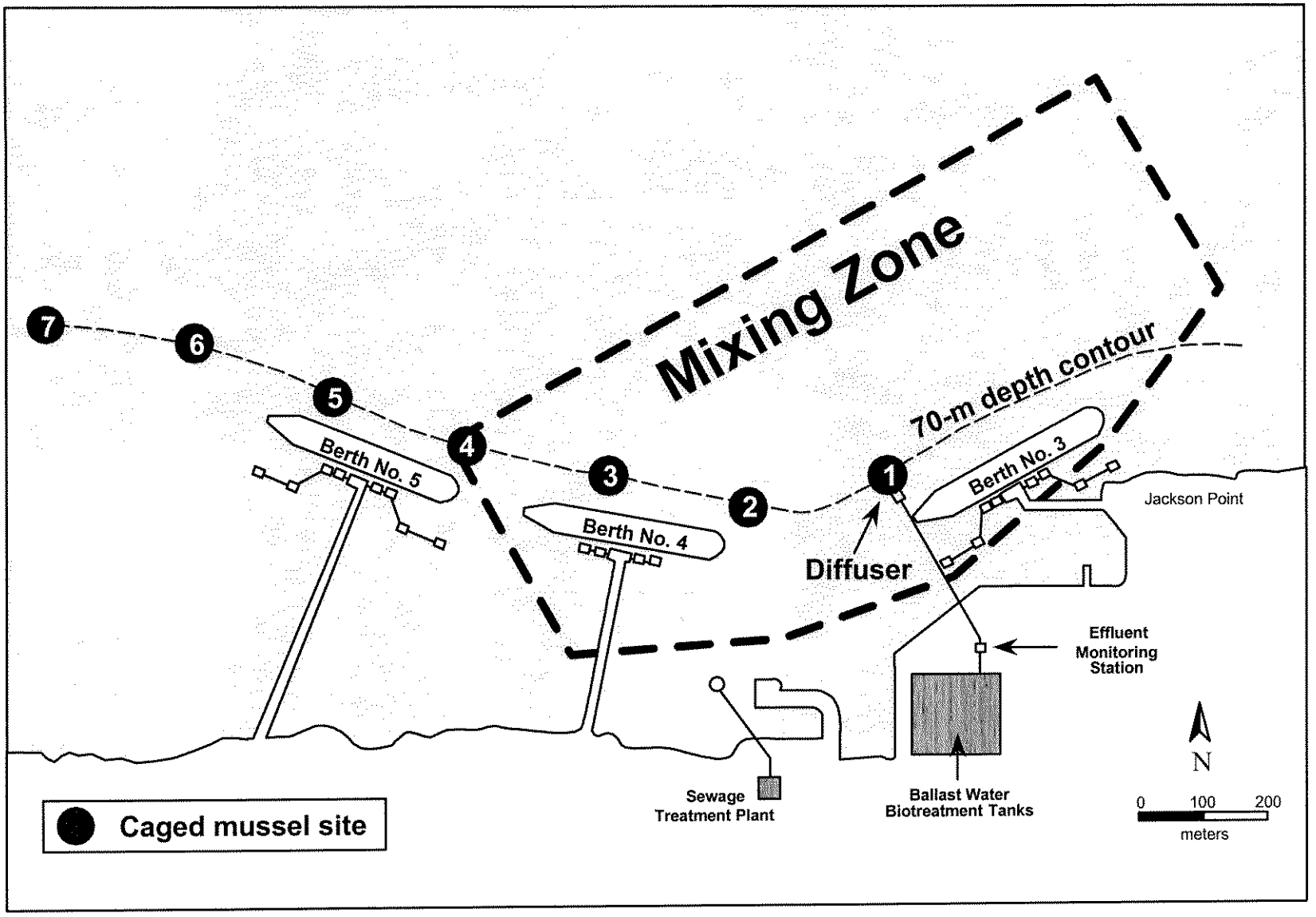


Figure 2. Detailed study site map showing marine terminal, mixing zone, and mussel monitoring sites.

Effluent from the Port Valdez BWTP is discharged through a diffuser system consisting of a 63-m long, 122 cm diameter pipe which extends bayward from the treatment system (Figure 2). The depth of the diffuser pipe ranges from 62 to 82 m. Effluent is discharged through ten 10-cm diameter ports and then ten 13-cm diameter ports spaced at 3-m intervals on alternating sides of the pipe. The ballast water treated by the facility originates from a variety of locations ranging from brackish coastal waters to open ocean water. As a result, the salinity and resulting density of the discharged effluent can range from very low to greater than the salinity of the receiving waters, which has a significant effect on the mixing provided by the diffuser (Valdez Marine Terminal, 1995). Although the ballast water comes from different locations, the oil associated with that water is primarily Alaskan North Slope crude. Therefore, chemical characteristics of that oil can be used as a tracer or "fingerprint" for identifying potential sources.

2.3 Study Objectives

The overall objective of the *in situ* field study with caged mussels was to evaluate the feasibility and scientific value of using caged mussels as a monitoring tool for evaluating chemical exposure and biological effects associated with the BWTF effluent being discharged into Port Valdez. Mussels were used as surrogate test animals because they are easy to collect, cage, and measure for exposure and effects assessments, and they can provide information to help understand and characterize ecological processes. The experimental design addressed three basic questions: 1) will the mussels survive at 70 m?; 2) will they grow at 70 m?; and 3) will they accumulate chemicals known to have been associated with the BWTF effluent? The following approach was formulated to meet the objectives and answer these questions:

- Transplant and retrieve caged mussels in Port Valdez at a depth of 70 meters
- Evaluate mussel survival and growth
- Collect sufficient tissues for chemical analysis
- Determine whether mussels accumulated chemicals known to be associated with the BWTF effluent

Based on discussions with representatives of the RCAC, Alyeska, U.S. EPA, and other interested parties, a tiered approach was used for the pilot study. Specific objectives were outlined for each tier. Section 2.3.1 describes decision criteria and rationale that were used to progress from Tier 1 to Tier 2. The different objectives of the two tiers are described below relative to mussel deployments at depths near 70 m after a period of 56 days.

Tier 1 Objectives for Mussels	Tier 2 Objectives for Mussel Tissues
• Measure survival	• Measure the concentrations of selected chemicals (PAHs , metals)
• Measure growth	• Measure the percent lipids and percent water in mussel tissues

2.3.1 Decision-making Criteria and Rationale

The following are the minimum acceptable survival and growth criteria that were used to evaluate Tier 1 data before proceeding to Tier 2, chemical analysis of the tissues. These criteria were established to help answer the questions regarding adequate survival and growth to provide a baseline for useful tissue chemistry data; i.e., significant losses in tissue weight could bias the chemistry results. These criteria were applied on a station-by-station basis; only tissues from stations that met the criteria were analyzed. Mean mussel survival and growth for the three cages at each station were used during the evaluation process.

Mussel tissues would be analyzed for selected chemicals if 1 AND 2 were met:

1. Mean survival is $\geq$ 50%. If mean survival is less than 50 percent, only tissues from those stations where survival is $\geq$ 50% will be analyzed.

2. There is no significant loss in either end-of-test tissue weights or whole-animal wet-weights when compared to measurements made at the beginning of the test (BOT). "Significant loss" is defined as being: a) more than 20% lower than BOT, AND b) statistically different than BOT ($\alpha = 0.05$).

These decision criteria based on survival and growth, with the caveats provided below, were established to ensure meaningful, relevant tissue chemistry data. Although bioaccumulation data proved to be more useful for monitoring the effluent from the BWTF than either survival or growth, mussel tissues should not be analyzed if the test animals are highly stressed and near death. Chemical analyses of highly stressed mussels in poor condition could provide bioaccumulation data that would be misleading. Highly stressed mussels can 1) underestimate chemical bioavailability when they "shut down" physiologically and decrease filtration rates, or 2) overestimate chemical bioavailability when chemicals are concentrated in their tissues due to tissue shrinkage. The amount of chemical contamination discharged from the BWTF was not expected to elicit acute, short-term effects such as high mortality or rapid reduction in growth rate which are indicative of severely stressed mussels. Rather, the concern is that continuous discharge of low

concentrations of chemicals may be causing chronic, long-term effects on the environment indicated by reductions in mussel growth. One of the reasons for the extremely conservative decision criteria (i.e., 50% survival, statistically significant differences, and 20% decreases in weight) is the potential for adverse effects due to deploying caged mussels in suboptimal conditions at 70 meters depth. Possible stressors, which may also affect survival and growth, include collection, handling, and transfer to a depth of 70 meters. Constant exposure to low temperatures and low food supplies may further stress the mussels. Alternatively, the benefits of constant immersion at depth, compared to alternating immersion and exposure to air in the intertidal zone, may offset the impacts of suboptimal conditions at depth.

Emphasis was placed on using several different growth metrics previously demonstrated to be appropriate indicators of mussel health. In addition to using changes in tissue weight as a health indicator, tissue weight metrics were used as a way to explain the chemical concentrations measured in the tissues of the exposed mussels. The vast majority of mussel studies conducted previously throughout the world have only used bivalves as indicators of exposure by measuring accumulation of chemicals in their tissues. Synoptic estimates of exposure and effects can be obtained by measuring bioaccumulation and growth. This approach, similar to the paradigm for ecological risk assessments that includes characterizing exposure and effects, provides a greater degree of environmental significance than measuring only one endpoint. The growth data serve as effects endpoints and help explain the tissue chemistry data. It is necessary to know if tissue mass increased or decreased (i.e., tissues have been metabolized) during the study (based on changes compared to baseline BOT tissue measurements. To properly interpret the tissue chemistry data so that apparent "increases" or "decreases" in tissue burdens are appropriately attributed to bioavailable chemicals and not changes in tissue mass. A thorough evaluation of the in situ mussel approach as a monitoring tool for future environmental monitoring of the BWTF effluent was possible because multiple exposure and effects endpoints were included in the experimental design.

The objective of ***Tier 1*** was to demonstrate whether caged bivalves would survive and grow in the deep waters (70 m) of Port Valdez adjacent to the BWTF diffuser. Survival and growth, however, are only the first step in answering the question of whether or not bivalves can be used as an effective monitoring tool at these depths. For a complete evaluation of the caged bivalve methodology as an environmental monitoring tool for chemicals discharged with the treated ballast water, the soft tissues of exposed mussels need to be chemically analyzed to determine the amount of PAHs and other chemicals accumulated. Such analyses provide information regarding the bioavailability of chemicals and whether there is cause to suspect that the observed effects are related to

accumulation/exposure of these chemicals, or perhaps some other factor. Chemical analysis of mussel tissues is the major element of the ***Tier 2*** assessment. However, the decision-making criteria identified above had to be met during ***Tier 1***. It should be emphasized that the ***Tier 2*** tissue chemistry data could be just as important, if not more important than the ***Tier 1*** survival and growth data for monitoring Port Valdez water quality. In fact, it is possible that the bioaccumulation endpoint may be more useful for monitoring the BWTF effluent than survival or growth endpoints because of its ability to quantify exposure to PAHs. Nevertheless, previous studies have shown that it is necessary to measure growth to help explain the environmental significance of accumulated chemicals on a site-specific basis. Similarly, chemical tissue burdens help explain observed growth.

2.4 Scope of Work

The Prince William Sound RCAC included the following scope of work in its contract with Applied Biomonitoring. This scope of work was developed as a framework to evaluate the use of caged bivalves as a monitoring tool for the BWTF effluent. The scope of work provides the detailed approach to meeting the program objectives:

- Review literature on caged bivalve studies conducted under similar circumstances (i.e., low temperatures and 70 m depth)
- Select and justify the species of bivalve to be used in the test:
  1. Research the species selected (including reproductive phases, periods of growth, bioaccumulation habits)
  2. Consult with experts on the Port's marine ecosystem to determine the best time of year to conduct the test
  3. Describe and justify the procedures for procuring the animals
- Quantify and justify minimum acceptable survival and growth for the test animals
- Consult with Alyeska's facility operator to determine the logistics of deploying caged bivalves near the BWTF diffuser and the tanker loading berths
- Determine the experimental design of the pilot test
  1. Select suitable test and reference site(s) and depths; provide justification
  2. Determine the number of replicate(s) per site, as well as the number of animals per replicate
  3. Determine and justify the placement period, and schedule for placing and recovering the animals at each site
  4. Identify the parameters to be used in measuring the success of test animals and in assessing the condition of the test animals
  5. Identify and document the procedures to be used in making these measurements

6. Describe the accuracy and precision of these procedures
7. Determine what ancillary data should be collected; describe how often and where these data will be collected
8. Determine the feasibility and utility of co-locating sediment traps at some or all of the sites; explain how such analytical data would be used

- Implement the test and document results
- Archive tissues from the test animals for possible chemical analysis; in the event of analysis, explain how such data would be used
- Communicate with the Terminal Operations and Environmental Monitoring (TOEM) Committee program coordinator and experts on the Port's marine ecosystem
- Make presentations to the TOEM Committee and/or the Council
- Submit a concise, clearly-written final report with a narrative summary understandable to the layman, including
  1. Detailed description of procedures used and data collected
  2. Investigator's views based on the test results regarding the utility and feasibility of expanded caged bivalve monitoring
  3. Design characteristics that should be included in expanded bivalve monitoring if it is undertaken

2.5 Literature Review

The RFP required a review of the literature on using caged bivalves as opposed to natural populations for monitoring, and caged bivalve studies conducted under "similar circumstances." For purposes of this report, "similar circumstances" was interpreted as studies that could be grouped into the following categories: 1) high latitudes and low temperatures; 2) depths greater than 10 meters; 3) shore-based industrial effluents; and 4) PAHs. A multi-phased approach was used to conduct this review. First, Applied Biomonitoring reviewed in-house information on the use of caged bivalves as environmental assessment tools, as well as information obtained in Aquatic Fisheries and Sciences Abstracts. Monthly updates were searched at the National Marine Fisheries Service Library in Seattle, Washington, and weekly updates on selected journals were provided by another private search company. Active researchers and research organizations working with caged bivalves were also contacted by email and telephone to provide the most up-to-date literature.

Caged mussels have been proposed for use in establishing pathways of exposure and assessing the environmental effects of oil spills (Salazar, 1993). They have been used successfully in many parts of the world (but not yet in Port Valdez for bioaccumulation and

growth) at a variety of depths and temperatures, and using a variety of species (Young et al., 1976; Widdows et al. 1980-81, 1987, 1990, 1995a,b; Wolfe et al., 1981; Salazar and Salazar, 1991, 1995, 1997a; Salazar et al., 1995, 1996). Young et al. (1976) successfully used caged bivalves to monitor chlorinated hydrocarbons at depths of up to 35 m and Karinen (1980) used caged bivalves to monitor petroleum hydrocarbons in Port Valdez near the BWTF diffuser at approximately 80 m; however, this pilot study was the first in which growth was been measured at these depths.

Caged bivalve monitoring has also been required by U.S. EPA at the Ketchikan Pulp Company in southeast Alaska as part of their NPDES permit (U.S. EPA, 1994), at the Harbor Island Superfund site in Puget Sound (Salazar et al., 1995), and at the Nyanza Superfund site in Massachusetts (Salazar et al., 1996). Recently, a caged mussel pilot study was conducted in Neroutsos Inlet on Vancouver Island in British Columbia, Canada (Applied Biomonitoring, 1997). The experimental design was nearly identical to the present study: three depths, six stations, and approximately 100 animals per replicate cage.

Early caged mussel tests were conducted in the laboratory and involved assessing the rate of byssal thread production (Salazar, 1974; Salazar and Kenis, 1973). Initial field studies using caged mussels were conducted in San Diego Bay, California (Salazar, unpublished) and at the Trident Submarine Base in Hood Canal, Washington (Peeling et al., 1976; Goforth et al., 1979). Although methods are continuously being refined, the most rapid development occurred between 1987 and 1990 when caged mussels were used to evaluate exposure and effects of tributyltin (TBT) antifouling coatings in San Diego Bay (Salazar and Salazar, 1991, 1995, 1996a). These specific methods were used to evaluate effluents and contaminated sediments in Tampa Bay, Florida (Salazar, unpublished); the controlled release of oil in the intertidal zone of Delaware Bay, Delaware (Salazar and Salazar, 1997a); and contaminated sediments in San Diego Bay, California (Salazar and Salazar, 1993), Sinclair Inlet in Puget Sound, Washington (URS, 1994), and the Hylebos Waterway in Puget Sound, Washington (Salazar and Salazar, 1996b).

Meador et al (1995) have recently completed a comprehensive review of bioaccumulation of PAHs by marine organisms. Interestingly, over 20% of the citations were on marine bivalves and less than 18% were on fish. This shows the importance of bivalve work and its common use as a surrogate species even though fish are often considered a more important resource. Many studies are conducted on bivalves because they are easier to maintain and study in the laboratory or in the field. This is also the reason why there are mussel watch monitoring programs in the State of California, the U.S. and also an international mussel watch. Over 27% of the citations in the Meador paper were of a general nature that included either conceptual papers or monitoring papers on various

animal groups. This shows the importance of understanding the general principles of bioaccumulation in developing a monitoring program. The fact that over 15% of the citations referred to sediments demonstrates the relative importance of understanding the relationship between sediment-sorbed chemicals and the bioaccumulation process.

2.6 Report Focus and Organization

This report emphasizes how the results of the caged mussel pilot study satisfied the original objectives. It summarizes the methods used to conduct the pilot study, the results, problems encountered during the study, and the ability of the method to detect differences in bioaccumulation and growth among stations and zones. The report does not speculate about the environmental significance of the data or the objectives of future studies.

This report is divided into seven sections. An Executive Summary (Section 1) provides a detailed overview of the *in situ* pilot study with caged mussels. Section 2, this Introduction, describes the monitoring priorities of the RCAC, summarizes pertinent background information, identifies project objectives, outlines the decision-making criteria, reviews the scope of work established for this project, and presents the results of the literature review. Section 3 describes the study methods, including the conceptual framework and the various types of applicable models, the experimental design, and specific methods used in the field and laboratory. All results are provided in Section 4. Section 5 is the discussion of test results and includes problems encountered during the study. Lessons learned from this pilot study that are important for successfully completing future studies are reviewed in the discussion. Acknowledgments are given in Section 6; References in Section 7.

Additional information beyond the scope of work are in the following appendices. The results of statistical comparisons for stations and zones are found in Appendix A. Appendix B contains results for the various PAH homologs and other chemicals identified in mussel tissues. The actual measurements made on each of the mussels at the beginning and end of test are provided in Appendix C. The tissue chemistry data are presented in Appendix D; ancillary seawater measurement data are presented in Appendix E. Comments from Alyeska are included in Appendix F, and the comments from the US EPA as Appendix G.

3.0 SUMMARY OF STUDY METHODS

In addition to providing a detailed description of the procedures used and data collected for the parameters described in the Scope of Work, this section details the methods used to develop the project model and experimental design.

3.1 Conceptual Framework and Project Model

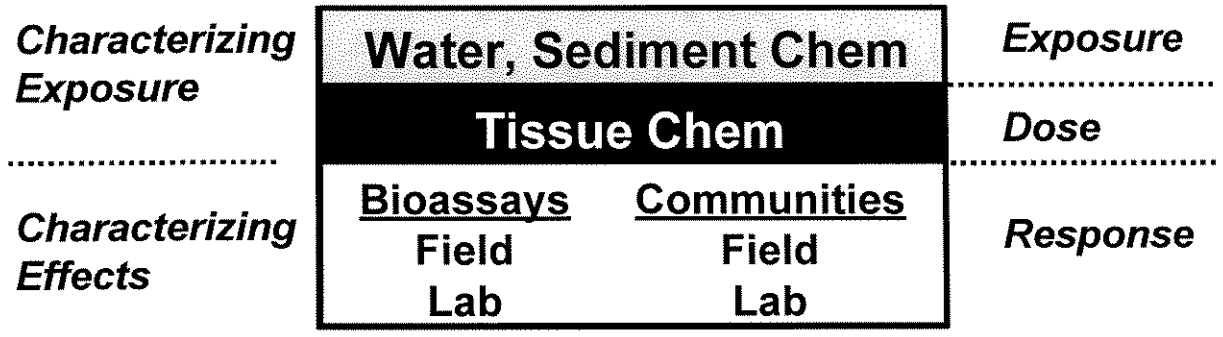
It is important to develop a conceptual framework before any routine caged bivalve monitoring is conducted (White 1984). This helps to establish a meaningful monitoring program, properly interpret the results, and use the results appropriately in a regulatory framework. The conceptual framework provides an overall plan for the most appropriate measurements to achieve the program goals. It allows development of the proper project model, identification of appropriate chemical and biological measurements, and synthesis of data to answer the hypotheses developed for the study. A conceptual framework eliminates the potential for "mindless monitoring" (Carpenter and Huggett 1984) and helps focus the work on the study objectives. It is also important to understand both the temporal and spatial characteristics of bioaccumulation and relate those to the temporal and spatial characteristics of effects. Although predictive models are often useful as first-order approximations, bioaccumulation and associated effects are often driven by site-specific factors. Collectively, these issues could be addressed with caged bivalve monitoring.

3.1.1 Exposure-Dose-Response Framework

Using the risk assessment framework paradigm as a framework, and the sediment quality triad (SQT) from Chapman and Long (1983) and Chapman (1996) as a guide, Salazar and Salazar (1995, 1996a, 1998) developed an *exposure-dose-response* (EDR) triad (Figure 3). The EDR triad emphasizes the importance of monitoring chemicals in external media, chemicals in tissues, and biological effects to support an integrated risk assessment strategy. The advantages and potential applications of this approach are also shown in Figure 3. This effects-based approach, emphasizing measurements of exposure and dose, is the type of approach that should be used for monitoring the BWTF effluent.

From an ecotoxicological perspective, the relationships between external exposure from environmental media (e.g., water and sediment), exposure at internal receptors through bioaccumulation (e.g., tissues), and any associated adverse biological effects (e.g., growth) are the keys to assessing ecological risk (Salazar and Salazar, 1998). Field bioassays bridge the gap between traditional laboratory bioassays and field monitoring of benthic

Exposure-Dose-Response Framework



Advantages

- Preponderance of evidence
- Laboratory & field
- Individuals & communities
- Bioassays & field monitoring
- Manipulative experiments

Applications

- Lab bioassay validation
- Bioaccumulation calibration
- Inputs to models
- Status & Trends monitoring
- Ecological risk assessment

Figure 3. Exposure-dose-response assessment framework showing the link between toxic chemicals in the external environment (I.e., water and sediment), in tissues, and associated adverse biological effects. The assessment "triad" also shows how toxic chemicals in tissues establish a link between environment and organism. Tissue chemistry can also be used to establish links between laboratory bioassays, field bioassays, and assessments of benthic community structure.

community assemblages by combining some elements of experimental control commonly associated with laboratory bioassays and other elements of environmental realism associated with field monitoring (Salazar and Salazar, 1997a,b).

3.1.2 Mussel Monitoring Model

A generic conceptual monitoring model, originally developed for marine bivalve monitoring of TBT antifouling coatings (Salazar and Salazar, 1996a), is potentially applicable to most biomonitoring programs, including assessment of the BWTF effluent (Figure 4). This conceptual model illustrates the interaction between natural factors and chemical exposure in affecting bioaccumulation and growth, as well as the impacts of man-made factors such as habitat alteration and destruction. It demonstrates the importance of measuring factors that affect bioaccumulation and growth. These factors act in concert to modify bivalve growth, bioaccumulation, and survival. The key to calibrating the bivalve bioindicator is separating the effects of natural and biological factors from the effects of chemicals. The measurements recommended in the mussel monitoring model facilitate interpreting the environmental significance of chemical concentrations in various environmental compartments (water, sediment, and tissues) and their potentially adverse biological effects. This preponderance-of-evidence approach in using caged bivalves takes advantage of emphasizing chemical exposure and biological effects under natural conditions in receiving waters while reducing the uncertainty associated with the effects of chemicals.

3.1.3 Characterizing Exposure and Effects over Space and Time

Caging bivalves facilitates monitoring individual organisms and sampling an almost infinite matrix of space and time. The animals can be strategically situated along physical and chemical gradients associated with both the water column and sediments (Figure 5). Most importantly, caged bivalves can be placed along known and/or suspected gradients of chemical contamination in three dimensional space and time that are in the area of concern and associated with point sources of concern. At each transplant location, the bivalves will integrate the effects of exposure. These biologically-integrated monitoring results can be easily estimated by measuring bioaccumulation for exposure, and growth for effects. This approach combines the advantages of experimental control from laboratory bioassays—placement in areas of concern where they might not normally be found, defined exposure period, and facilitation of effects measurements; and the environmental realism of traditional field monitoring—experiments are conducted *in-situ*. Even if bioaccumulation in natural populations of bivalves were measured to characterize

Mussel Monitoring Model

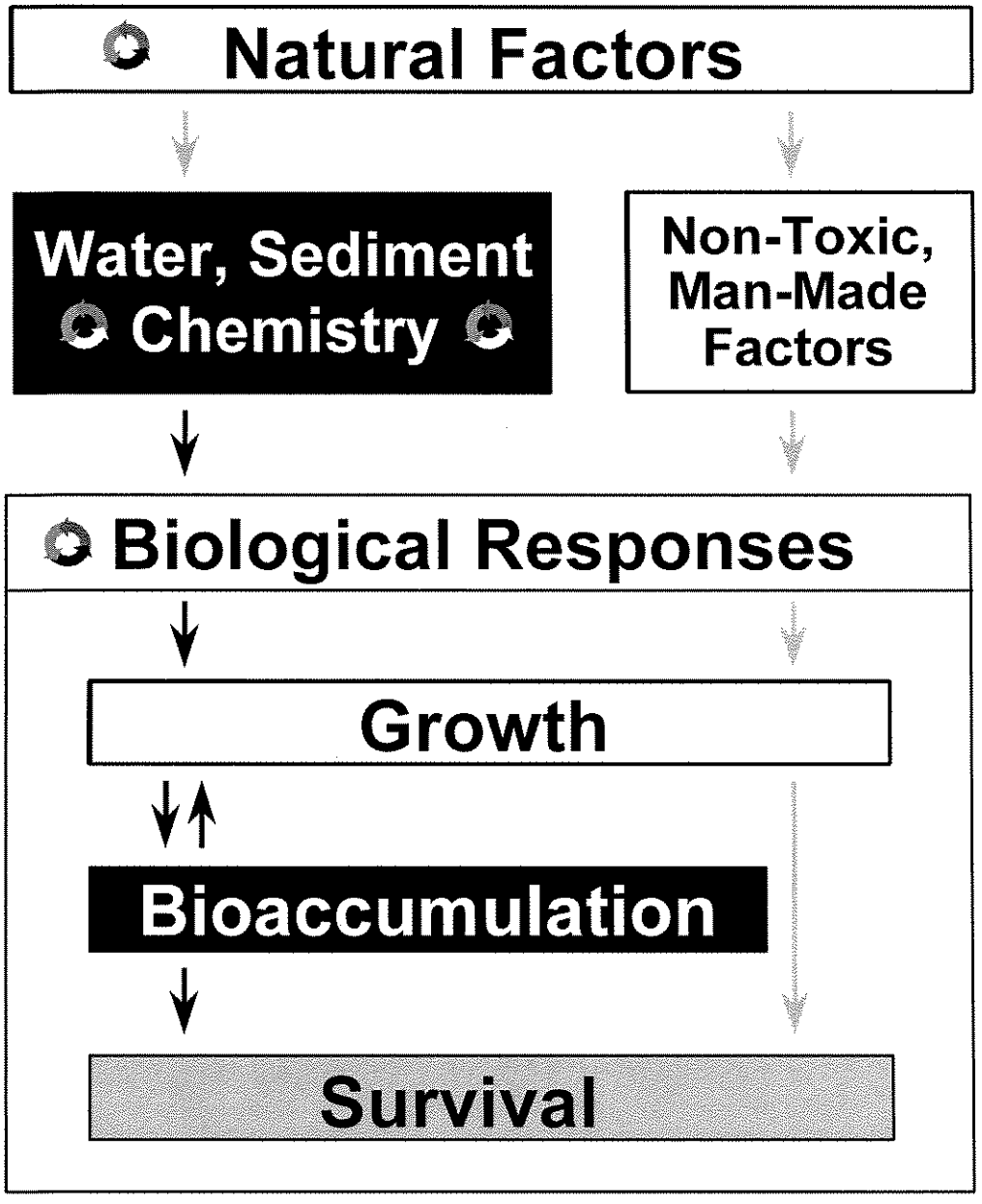
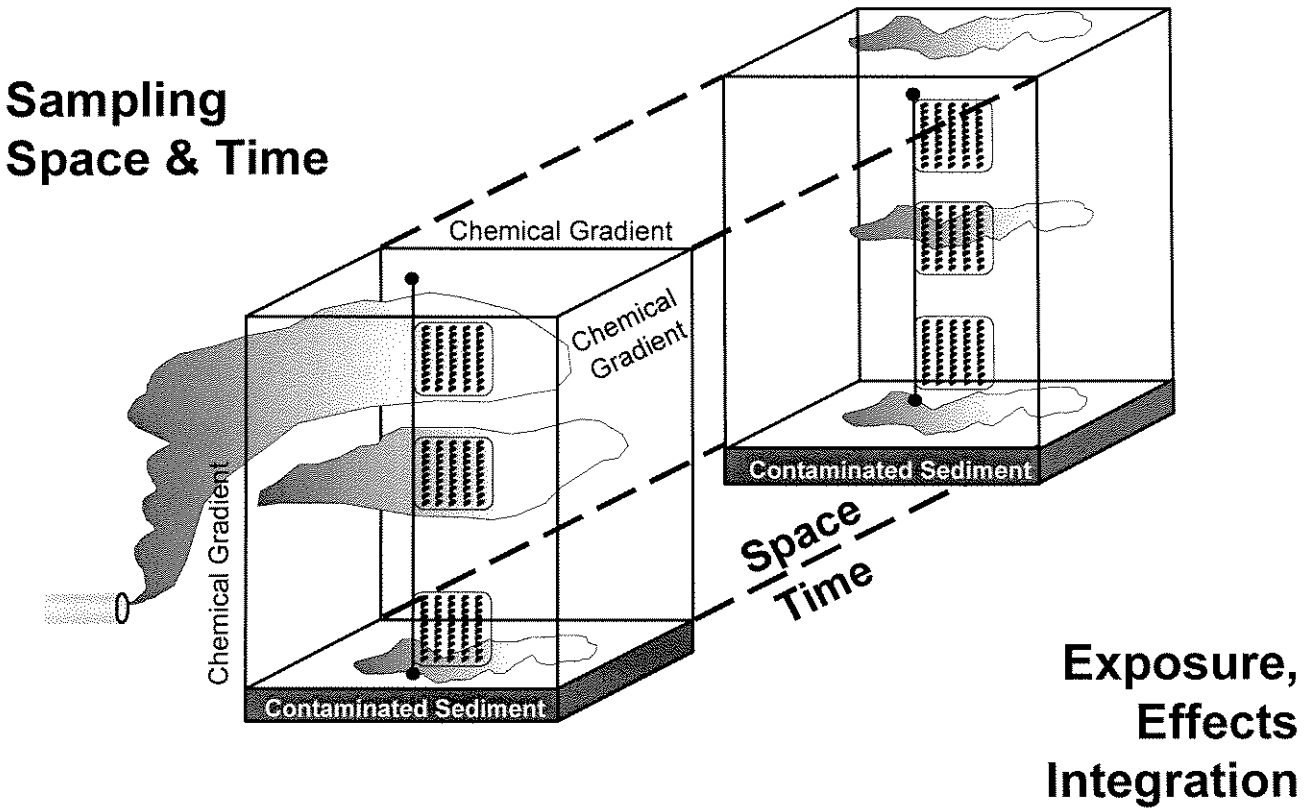


Figure 4. Mussel monitoring model showing the influence of natural factors, chemicals and non-toxic man-made factors on biological responses. Natural factors, chemical concentrations, and biological responses can also be cyclical. Double arrows are shown between bioaccumulation and growth to indicate interactions.

Characterizing Exposure & Effects over Space & Time



Advantages

- Control & realism
- Outside natural populations
- Defined exposure period
- Physical or chemical gradients
- Manipulative experiments

Applications

- Site-specific differences
- Temporal/spatial variability
- Short & long-term trends
- Source identification
- Dose-response estimates

Figure 5. Characterizing exposure and effects over space and time using caged bivalves. Figure shows bivalves transplanted along suspected gradients of chemical contamination. Two suspected sources (bottom sediment and bottom effluent diffuser), two sites, three depths, two sampling intervals, and chemical stratification are shown.

exposure, it would not be clear whether tissue chemistry represented the last day, week, month, or year. The same problems exist for effects measurements of wild bivalve populations. Since the exposure period is clearly defined in a caged bivalve monitoring program, any differences in tissue chemistry or associated effects (animal health estimated by growth) between the beginning and end of the test can be compared with bioavailable chemicals associated with BWTF effluents and possibly other sources that have been accumulated during the course of that exposure period. Strategic placement of caged bivalves along suspected contamination gradients, coupled with careful selection of chemical analytes facilitate source identification and quantification of bioavailable chemicals associated with BWTF effluent. Two comparisons that can be made with respect to tissue chemistry of BWTF-associated chemicals and associated biological effects are: 1) comparisons between beginning and end-of-test; and 2) comparisons among different sites.

3.2 Experimental Design

The experimental design addressed three basic questions: will the mussels survive?; will they grow?; and will they accumulate chemicals known to have been associated with the BWTF effluent? The experimental design involved collecting mussels from Anderson Bay, a relatively uncontaminated site, sorting mussels into size groups and assigning them to cages, and transplanting the caged mussels to selected locations along the 70-m contour near the BWTF diffuser (Figure 6). Three cages of 100 mussels were deployed at each station; cages were separated by approximately 2 m vertical distance. Stations were approximately 200 m apart. The transplanted mussels were exposed to existing conditions at these locations for a 56-day period, after which time they were retrieved and measured for changes in whole-animal weight, shell length, shell weight, and tissue weight. The survival and growth criterial presented in Section 2.3.1 were met and tissues were analyzed for chemicals accumulated during the 56-day exposure period. This design was chosen after numerous discussions with RCAC, Alyeska, and other interested parties, and was consistent with the objectives of the pilot study.

The cages were placed at three different depths to optimize exposure to the effluent plume and increase chances of success. There were concerns that placing all of the mussels too close to bottom sediments would compromise the results if there were adverse effects associated with proximity to the sediment. A gradient approach was selected to evaluate overall trends with distance from the diffuser rather than to compare treatment versus control sites since true field controls are virtually impossible to achieve. Evenly spacing the stations at 200-m intervals away from the diffuser facilitated testing the relationship

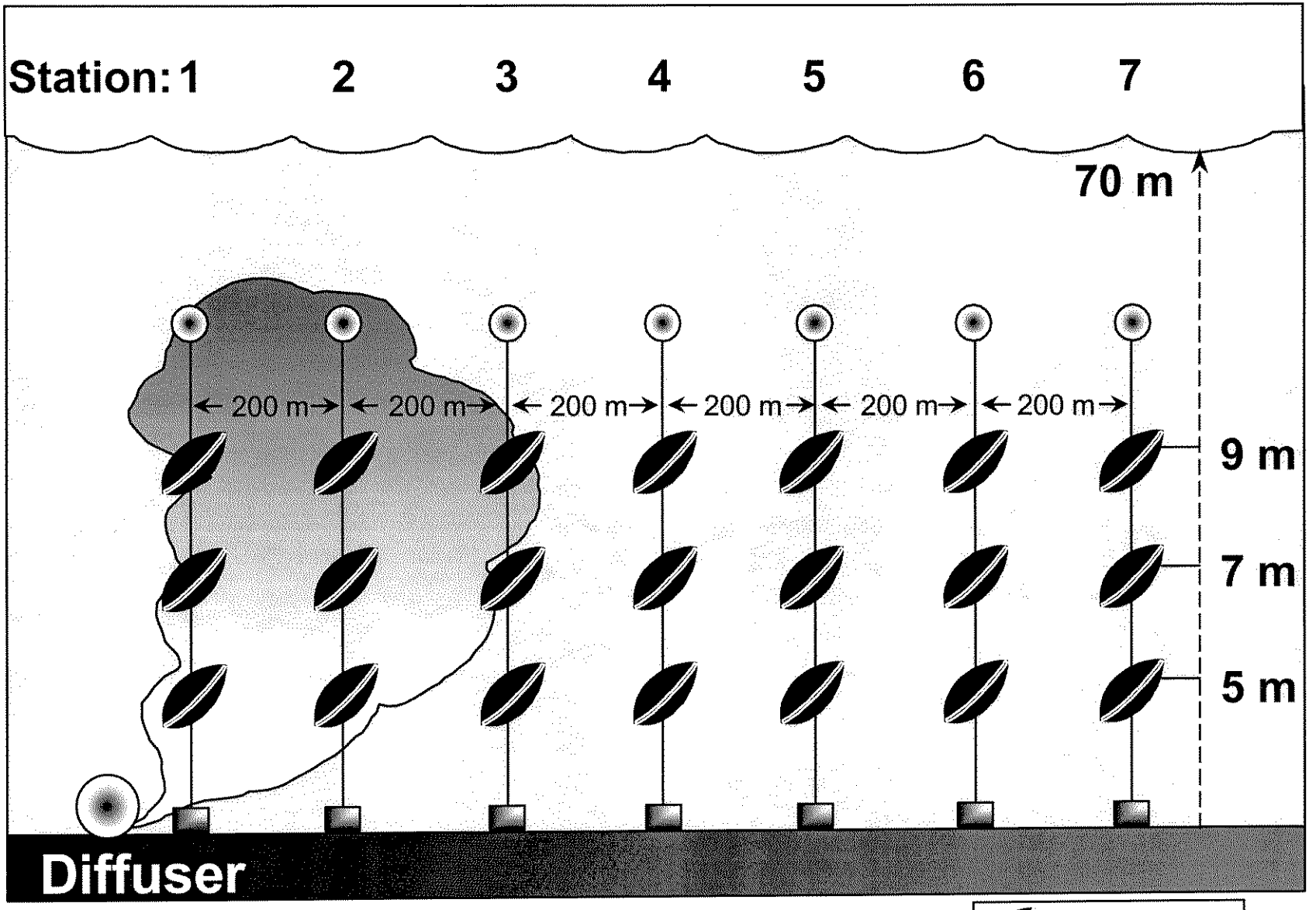


Figure 6. Overview of mussel deployment configuration.

between chemicals accumulated in mussel tissues with distance from the diffuser. In terms of feasibility, implicit in this study is the ability to distinguish differences in exposure and effects among zones, stations, and depths. Stations 2, 3, and 4 were within the mixing zone while Stations 5, 6, and 7 were outside the mixing zone, as defined by the U.S. EPA and ADEC. The statistical model used in the pilot study was a nested ANOVA, in which station and depth are nested in zone. Figure 7 shows the relationships between each level tested in this model and the level of replication.

The parameters used in assessing mussel health and mussel responses to exposure conditions were whole-animal wet-weight (WAWW), shell length, growth rates based on weight and length, tissue weight, percent lipids, and percent water. These parameters have been shown to be sensitive endpoints, are relatively easy to measure, and can be measured with a relatively high degree of accuracy and precision. Other parameters, such as byssal thread production or reproductive indices, were considered as measurement endpoints but were rejected after discussion with other experts because of the difficulty in making these measurements and interpreting the results.

For the effects characterization portion of the study, the level of replication was individual mussels. Power analyses performed on data from other similar studies conducted in Alaska (EVS, 1996, 1997b) indicate that between 100 and 300 mussels per station are sufficient to detect differences in weight on the order of 0.2 and 0.1 g, respectively. By placing 100 mussels in each cage, there was sufficient replication at each depth as well as at each station to test for such differences.

For exposure characterization portion of the study, the level of replication was composited tissue samples, created by combining the soft tissues of all living mussels from a given cage. Tissues from approximately 100 individuals were required to conduct the desired analyses. Therefore, there was only one chemistry replicate at each depth at each station. This scenario does not permit statistical comparison unless the three samples from a given station are considered replicates. For the purposes of this pilot study, it was believed that a demonstration of bioaccumulation was more important than actually identifying significant differences among stations.

The following null hypotheses were developed for characterizing effects:

- There is no significant difference in whole-animal weight or shell length among cages or among stations (3 cages pooled) at the beginning of the test.
- There is no significant mortality in mussels after the 56-day exposure period.
- There is no significant change in mussel metrics after the 56-day exposure period.
- There is no significant difference in mussel survival, whole-animal wet weight,

shell length, shell weight, growth rate, tissue weight, or condition index between zones, among stations, or among depths.

- There is no significant interaction of zone, station, and depth on mussel survival, whole-animal wet weight, shell length, shell weight, growth rate, tissue weight, or condition index.
- There is no relationship between mussel survival, whole-animal wet weight, shell length, growth rates, condition indices, or tissues weights and distance from the diffuser.

Hypotheses 1, 2 and 3 were addressed in response to the explicit objectives of this study; the remaining hypotheses were used to assess the implicit objective about the method's ability to detect differences among stations and over space and time.

The following null hypotheses were developed for characterizing exposure:

- There is no significant difference in mussel tissue total PAH concentrations between beginning and end of test.
- There is no significant difference in the concentration of total PAHs in mussel tissues within zones and among stations.
- There is no significant relationship between chemicals in mussel tissues and distance from the diffuser.

Details and discussion of station and mixing zone comparisons are provided in Appendices A and B. All null hypotheses were tested at the 95 percent confidence level ($\alpha = 0.05$).

3.3 Species Selection and Justification

The Pacific blue mussel (*Mytilus trossulus*), which, was selected as the test species. Mussels were selected over other bivalves for the following reasons: 1) their widespread distribution and ease of obtaining sufficient numbers of test species from a relatively clean source, 2) their lengthy historic and continued use as a test species in monitoring studies conducted by other researchers in other parts of the world, and 3) historical data available for mussels regarding the uptake of PAHs and effects from PAH exposure. The Pacific blue mussel *Mytilus trossulus* was selected as the specific test species because it occurs naturally in Port Valdez and was used in a previous assessments of chemical bioavailability in the vicinity of the BWTF at 70 meters (Karinen, 1980) and in the intertidal zone (Feder and Keiser, 1980). The suitability of this species for the pilot study was confirmed by discussions with local, national, and international experts in the field regarding species availability, applicability, and sensitivity.

H_0: There is no difference in mussel growth parameter among zones, stations, or depths.

The nested ANOVA was used because the design includes fixed stations and fixed depths at each station both within and outside of the mixing zone. The factors are said to be nested within zones because each of the three depths and each of three stations are found within each zone. The nested ANOVA will test for differences among zones, stations, and depths, and whether there is an interaction effect (i.e., whether the combination of station and depth significantly affect the measurement). The individual mussels are the level of replication at each depth.

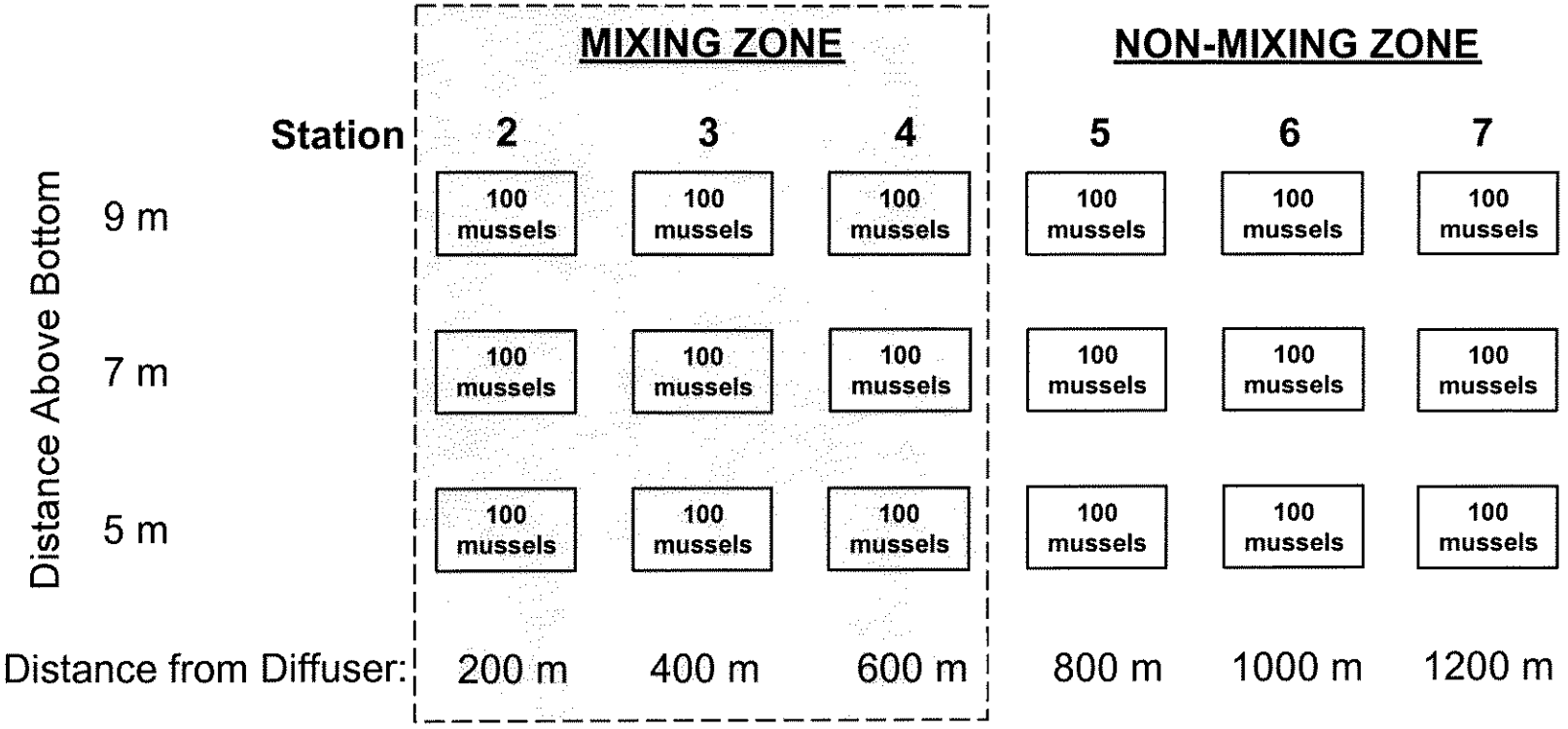


Figure 7. Model for nested ANOVA showing two zones with three stations and three depths nested within each zone. Also shown are distances above the bottom sediment and distances from the diffuser.

3.4 Test Season and Exposure Period

The literature review included information on optimum deployment seasons based on the reproductive phases, periods of growth, and bioaccumulation potential of *Mytilus trossulus* and other species of *Mytilus*. Local experts on the Port's marine ecosystem were also consulted to help determine the best time of year to conduct the test. In a study of intertidal biology, Feder and Keiser (1980) reported that mussels in Port Valdez complete spawning by mid-June with gametogenesis (i.e., the development of reproductive tissue) commencing in late October and November and gradual gametogenesis development occurring throughout the winter. The period of September through November was initially recommended as the deployment window since it provides a period when mussel tissue mass is increasing and there is minimal concern for loss of tissue due to spawning. Initial plans also included confirming the reproductive condition of the animals before beginning the test. This approach would have maximized the period of potential growth and avoided difficulties associated with winter conditions. Monitoring the reproductive state of the mussels at the proposed collection site was proposed, to ensure that the population had completed spawning and entered the growth and development phase.

However, it was not possible to conduct the test during the fall of 1996 as originally proposed due to contractual difficulties. February-April was selected as a second choice. The final decision regarding actual deployment time was made only after consultation with local experts, in particular: John Karinen (NOAA, NMFS Auke Bay), Howard Feder (University of Alaska), members of the RCAC Science Advisory Committee, Jeff Short (NOAA, NMFS Auke Bay), Chuck O'Clair (NOAA, NMFS Auke Bay), and Ken Chew (University of Washington). Deployment was initiated on February 26, 1997 and terminated on April 23, 1997.

The RFP identified a 30- to 60-day deployment period. Applied Biomonitoring originally recommended 90 days 1) to ensure tissues had reached equilibrium with respect to chemicals in the surrounding environment, and 2) to maximize growth potential. Previous studies in southeastern Alaska indicated that 60 days may not be long enough to detect differences in growth among caged mussels at different sites due to the slow growing characteristics of mussels in Alaskan waters (EVS, 1996,1997b; Feder and Keiser, 1980; Feder and Bryson-Schwafel, 1988; Feder and Shaw, 1996). Nevertheless, due to concerns about potential spawning and interference with growth, the pilot study was terminated after 56 days.

All mussels were deployed on February 26, 1997. Mussels were retrieved from Stations 2, 3, and 4 on April 22, 1997; mussels were retrieved from Stations 5, 6, and 7 on April 23,

1997. Station 1 mussels were lost and not recovered.

3.5 Mussel Collection

Once the Pacific blue mussel (*Mytilus trossulus*) was chosen as the test species, possible options for sources included: 1) collection from the cleanest possible local stocks (if animals have been exposed to chemicals, this can increase their resistance to chemical stress); or 2) collection from a more pristine location outside of Port Valdez. The decision as to source was made with the assistance of input from local experts including John Karinen of NOAA and Howard Feder of the University of Alaska. Janice Wiegers (formerly of Western Washington University) also provided significant input based on the Risk Assessment for Port Valdez. Anderson Bay was selected as the mussel collection site over Sawmill Spit, Shoup Bay, and other suggested locations. John Karinen had previously found elevated concentrations of PAHs from Sawmill Spit and Janice Wiegers had expressed concern about runoff in the vicinity of Shoup Bay. Therefore, those sites were eliminated as potential collection sites.

Anderson Bay is located approximately 10 km west of the BWTF. It is a water body with physical attributes comparable to those found near the discharge effluent (Figure 1). This area has previously been identified as a relatively uncontaminated source of mussels (Feder and Shaw, 1996) but clearly all mussels within Port Valdez are exposed to a some minimum low-level PAH concentrations. Mussels were collected on February 24, 1997, by hand during low tide. Scrapers were used to remove mussels from the rocks. Harvested mussels were stored and transported in ice chests; no water or ice was added during this time. These mussels were held overnight at the Solomon Gulch Fish Hatchery before sorting, measurement, distribution, and shucking the next day.

3.6 Mussel Sorting, Distribution, and Deployment

A summary of the methods used here are described in Salazar and Salazar (1995). Alyeska was consulted throughout the planning, deployment, and retrieval phases of the caged mussel pilot study. All mussel sorting, measurement, distribution, and shucking activities occurred at the Solomon Gulch Fish Hatchery. During the sorting phase, the mussels were held in buckets containing seawater and ice packs (i.e, plastic bags containing wet ice). The ice was used to maintain water temperatures at 6 ±3°C.

Shell length was used to select mussels for this study. Shell length (longest axis, generally from the anterior end near the beak to the leading posterior end) was determined with

vernier calipers. Initially, mussels collected from Anderson Bay were pre-sorted into 1-mm size groups. All mussels between 25 and 40 mm in shell length were retained to combine attributes of the smallest mussels with the highest growth rates and attributes of the largest mussels with the most tissue for chemical analysis. After the pre-sort, the number of mussels per size category was determined. The final size range of 31 to 36 mm was based on maximizing test animal numbers in five contiguous size groups.

Once the final size range was identified, all test mussels were measured for length (to the nearest 0.1 mm) and whole-animal wet-weight (to the nearest 0.01g) as part of the distribution process. This included 2100 mussels to be deployed in Port Valdez and 300 used to establish baseline BOT conditions in tissue chemistry, shell weight, and tissue weight. Only live animals that were fully closed, or those that closed immediately upon light physical stimulation were used. A very specific process, based on draft standard protocols (Salazar and Salazar, 1995; Salazar and Salazar, 1997c), was used to ensure an even distribution of mussels across stations based on size. First, all mussels were distributed across randomly-assigned cages (Figure 8) beginning with the smallest mussels (31 mm). When the 31-mm mussels were used up, the next size range, i.e., 32 mm, were distributed. This process was used until all the bags were filled. The 300 mussels identified for baseline BOT measurements were removed from their mesh tubes and measured. Soft tissue and empty shell wet weights measurements were made on each individual. The soft tissues for 100 mussels were composited, creating three samples, for chemical analysis. Tissues were removed and processed according to the methods given in Section 3.8.

Tubes of fine mesh plastic netting (approximately 10 cm diameter, 5 mm mesh size) were used to hold the mussels during the deployment period. Mussels were situated in the mesh netting with one individual per cell, for a total of 20 animals per tube (Figure 9). Nylon cable ties were used to separate mussels and create the individual cells. The mesh netting facilitated water circulation and exposure to environmental conditions; sufficient space was provided between cable ties to permit valve opening, growth, and movement by each animal. The "one animal per cell" approach was used to permit measuring growth effects on an individual-by-individual basis. Five tubes were prepared for each cage for a total of 100 animals per cage. After BOT processing, the mussels were held in seawater near the fish pens situated adjacent to the fish hatchery's seawater intake.

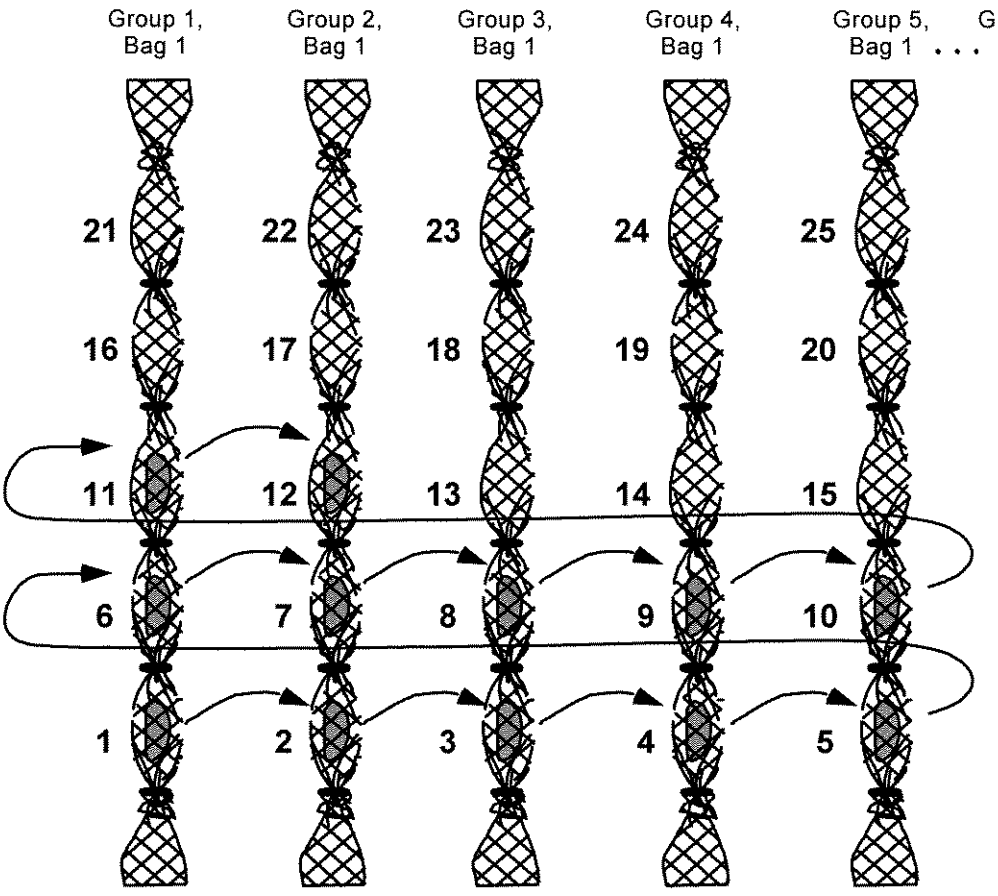


Figure 8. Distribution process for caging mussels

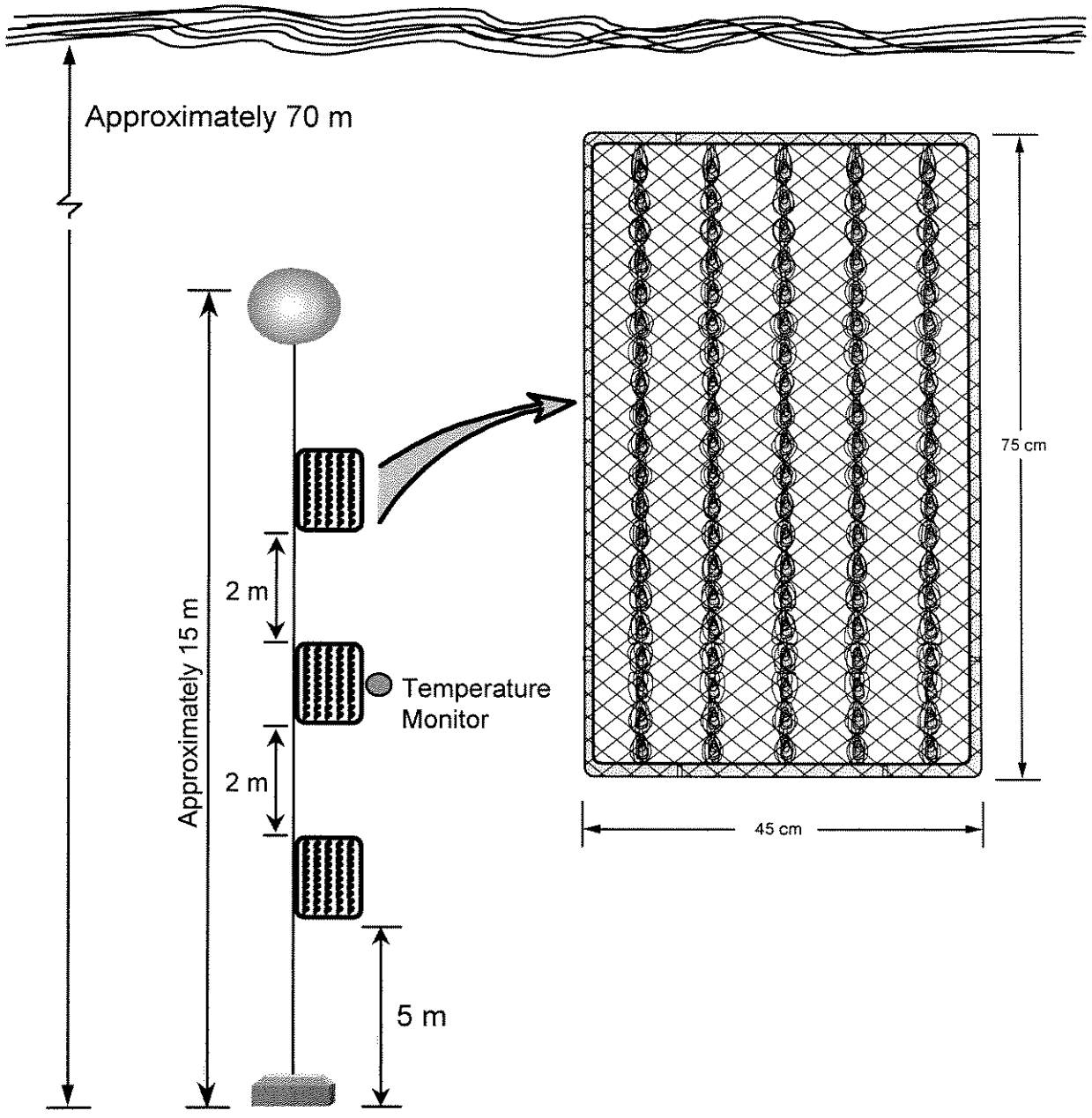


Figure 9. Detailed caged mussel deployment configuration

3.7 Deployment and Station Locations

Following overnight holding in the shallow subtidal area near the Solomon Gulch fish hatchery, the mussels were prepared for deployment. The mesh tubes containing mussels were secured to rigid polyvinyl chloride (PVC) frames with large nylon cable ties and rope. The PVC frames, or mussel cages, were wrapped with heavy-duty plastic mesh (approximately 2.5 cm mesh size) to discourage predators. The assembled cages were loaded onboard the *Tor* and transported to the stations. For each station, three cages of 100 mussels each were attached to the deployment lines with large nylon cable ties. The three cages were separated by approximately 2 m vertical distance (Figure 9). The deployment array was completed by adding an anchor and a subsurface buoy. This buoy maintained vertical position of the caged mussels in the water column after deployment (Figure 9). Anchors were used to maintain station position along the 70-m contour. A sinking tether line was attached from each array to the shore or to one of three berths to prevent the arrays from sliding down slope and to facilitate retrieval. This tether line was used to prevent drifting or movement of the deployment array into deeper water.

Mussels were deployed at seven stations along the 70-m contour of the south shore of Port Valdez between Jackson Point and Sawmill Spit (Figures 2, 6). These stations were selected because they provided a potentially decreasing PAH gradient with distance from the diffuser. It was expected that mussels would survive, meet growth criteria, and demonstrate a similar gradient of PAHs in their tissues. The first station was located immediately adjacent to the diffuser; the other six were located at 200-meter intervals west of the diffuser. Four of these stations were within the BWTF mixing zone. The remaining three stations were positioned outside of the mixing zone but in the vicinity of the marine terminal. Station positioning (Table 1) was determined using variable range radar onboard the vessel *Tor*, operated by Erling Carlson, (accuracy $\pm$10 m). All reported depths were referenced to Mean Lower Low Water (MLLW).

Although the experimental design called for Station 1 to be located immediately adjacent to the diffuser and all other stations located at 20-m intervals along the 70-m depth contour, estimates based on the positioning measurements which follow suggest that the actual distances and depths may have been somewhat different. For simplicity and because of questions regarding the accuracy of these estimates, all data analysis is based on the nominal 200-m distances. Station 1 was tethered to Berth No. 3 and was located closest to the outfall diffuser at a depth of 71 meters (Figure 2). Station 2 was located approximately 150 meters west of the diffuser at a depth of 70 meters and was the only station tethered to shore. Station 3 was tethered to Berth No. 4 and was located approximately 350 meters west of the diffuser at a depth of 70 meters. Stations 4 and 5 were tethered to Berth No. 5.

Station 4 was located approximately 650 meters west of the diffuser at a depth of 70 meters. Station 5 was located approximately 870 meters west of the diffuser at a depth of 69 meters. Stations 6 and 7 were tethered to the western-most pilling of Berth No. 5. Station 6 was located approximately 1100 meters west of the diffuser at a depth of 69 meters. Station 7 was located approximately 1290 meters west of the diffuser at a depth of 70 meters.

Table 1. Station location coordinates for the caged mussel pilot study

Station	Latitude	Longitude
1	61° 05' 25"	146° 23' 16"
2	61° 05' 23"	146° 23' 24"
3	61° 05' 22"	146° 23' 38"
4	61° 05' 26"	146° 24' 06"
5	61° 05' 27"	146° 24' 14"
6	61° 05' 30"	146° 24' 28"
7	61° 05' 33"	146° 24' 41"

3.8 End-of-Test Measurements

Mussel cages were retrieved using the vessel *Lady Sandra*, operated by Bill Crump. All mussel cages, except those at Station 1, were successfully retrieved on April 22 and 23, 1997. The tether line for Station 1 was "parted" 15 meters seaward of its attachment point to the piling of Berth No. 3. Extensive efforts to dredge ("snag") for the tether line on both sides of the berth and on both sides of the original position of the array proved unsuccessful.

The PVC cages containing the mussels were detached from the deployment lines and taken to the shallow subtidal inlet near the Solomon Gulch Fish Hatchery for overnight elimination of sediment in the gut. EOT measurements on all live mussels were made the following day, and involved WAWWs, shell lengths, tissue weights, and shell weights. The number of dead and missing animals was recorded for each station. Stations 2, 3, and 4 were processed on April 23, 1997 and Stations 4, 5, and 6 were processed on April 24, 1997.

For each cage, tissues from all live mussels (i.e., only animals that closed upon stimulation; gaping animals, with intact tissues, that did not close upon light physical stimulation were considered dead) were pooled for chemical analysis. All equipment (i.e., shucking knives and the aluminum foil covering the cutting boards) used during tissue extraction was thoroughly cleaned before processing a new batch (i.e., replicate) of mussels according to the following process: wash with Liquinox®, rinse with hot tap water, rinse with deionized/distilled water. Thin-bladed stainless steel knives were used to slice the mussels in half and remove the soft tissues. Gloves were not worn during the shucking process to reduce the potential for injury as handling and shucking wet mussels causes the rubber gloves to become slippery. Prior to processing a station, all staff thoroughly washed their hands with Liquinox®. After severing the interior muscles, the stainless steel knife was used to separate soft tissue from shell. The severed mussel was held in such a position that the excess liquid was allowed to drain. The soft tissues were kept on the shell during extraction and after complete separation. The shell was used as a "holding dish" until tissue weights were measured using weigh pans, made from decontaminated aluminum foil. The soft tissues were placed on the weigh pan using the original shucking knife.

When all the tissues from mussels attached to a given PVC frame were weighed, the tissues were transferred from the weigh pan to certified clean sample jars. The sample jar was tightly capped, affixed with a prepared label, and placed in the freezer. The aluminum foil weigh boat and cutting board cover were then discarded. All shucking equipment was decontaminated before processing the next sample.

The frozen mussel tissue samples were hand carried to Analytical Resources, Inc (ARI) of Seattle, Washington, for storage on April 28, 1997. On September 16, 1997, the samples were transported from ARI to Columbia Analytical Laboratories (CAL), Kelso, Washington, as directed by the RCAC. CAL was selected by the RCAC to perform the chemical analyses. At CAL, mussel tissues were homogenized and analyzed for selected metals, PAH compounds, TBT, percent lipids, and percent water.

Additional mussels were collected from Anderson Bay and Saw Island, which lies approximately 100 meters SSW of the face of Berth No. 5, on April 24, 1997 for tissue chemistry analyses. The mussels were gently removed from the rock substrate using plastic putty knives, minimizing stress to the organisms. Approximately 300 mussels of the same size range used in the pilot study were retained for these additional chemical analyses. Tissues from the Anderson Bay and Saw Island mussels were processed according to the methods described above, except these animals were not held at the hatchery overnight to purge the gut of sediment-associated PAHs. The main purpose for collecting these animals was to compare with other monitoring programs where gut purging

is not part of the standard protocol. These chemistry results should not be directly compared with the transplanted mussels because of differences in handling.

Samples were originally analyzed by CAL in December 1997. As part of the review process it was determined that inadequate sample cleanup with insufficient silica gel precluded an accurate quantification of both total and individual PAH compounds due to lipid interference. Remaining extracts from the first analysis were cleaned up with more silica gel according to standardized Exxon Valdez Oil Spill procedures used by the NMFS Auke Bay Lab. This re-analysis produced the expected suite of alkylated homologs which were missing from the first analysis. All samples were remeasured except Station 2 depth 9 where the extracts were lost and there was insufficient mussel tissue for another extraction.

3.9 Supplemental Measurements

3.9.1 Temperature

The effects of environmental factors on mussel growth and reproduction have been well documented. Temperature and food availability are probably the most critical factors. At each station, a continuously recording temperature monitoring device was attached to the deployment line adjacent to the middle mussel cage at a depth of 7 m above bottom sediment (Figure 7). Temperature data were collected at 20 minute intervals over the duration of the test using one *in situ* computerized data logger (HoboTemp, Onset Instruments). At the end of the study, the data were downloaded from the logging devices using the instruments' data recovery software.

3.9.2 Water Quality Measurements

Other environmental parameters (i.e., salinity, dissolved oxygen, chlorophyll-a, total organic carbon, total suspended solids) were measured at the beginning and end of the test along the 70m depth contour for each station. Water samples for these analyses were collected with a 3-L Niskin water sampler deployed from the side of the boat. The water sampler was lowered to the 9-m above bottom depth at each station. Once aboard the boat, 500-ml aliquots of water were taken from the sampler for processing. At the start of the test, three 50-ml samples were filtered for each station; one sample was used for chlorophyll-a analysis, one sample was used for total suspended solid (TSS) analysis via SM2540D, and one sample was used for carbon-hydrogen-nitrogen (CHN) analysis. At the end of the test, three 100-ml samples were collected and analyzed for the same parameters.

3.10 Data Analysis and QA on Growth Measurements

3.10.1 Data Analysis for Mussel Metrics and Bioaccumulation Data

Effects from exposure to the effluent discharged from the BWTF were assessed by evaluating survival, comparing EOT mussel metrics to BOT measurements, and comparing both survival and changes in mussel metrics across stations. Six metrics were used to assess growth and thereby animal health: shell length, WAWW, dry tissue weight, wet tissue weight, shell weight, and condition index. Only WAWW and shell length were measured for each individual at the start of the test. Therefore, growth rates based on the change (i.e., increase or decrease) over time could only be determined for these two metrics. Because of the closeness in size distribution among stations at the start of the test, it was assumed that the average tissue weight and shell weight were also similar among stations. Based on this assumption, the end-of-test tissue weights and shell weights were evaluated for statistical differences; any differences observed were assumed to have occurred during the test period.

Descriptive summary statistics (e.g., mean and standard error, standard deviation) were calculated for all survival, growth, and bioaccumulation data. These data were used to prepare graphs showing the overall mean, plus or minus two standard errors (±2SE) by station for each parameter measured. Two standard errors are presented because they approximate the 95 percentile and allow for a visual appraisal of the similarity, or difference, between two stations. The following statistical analyses were performed on EOT survival and growth data to test the hypotheses listed in Section 3.2:

Hypothesis (H_0: There is no significant...):	Statistical Process ($\alpha = 0.05$):
• Difference in whole-animal weight or shell length among cages or among stations (3 cages pooled) at the beginning of the test.	• Kolmogorov Smirnov Normality Test ANOVA
• Mortality in mussels after the 56-day exposure period.	• Compared results against Tier I criteria
• Change in mussel metrics after the 56-day exposure period (compare BOT to EOT).	• For WAWW and shell length, t-test. For tissue weight and shell weight, ANOVA & Dunnetts MRT.
• Difference in mussel survival among stations	• Contingency Table
• Difference in mussel whole-animal wet weight, shell length, shell weight, growth rate, tissue weight, or condition index between zones, among stations, or among depths.	• Nested ANOVA

Hypothesis (H_0: There is no significant...):	Statistical Process ($\alpha = 0.05$):
• Interaction of zone, station, and depth on mussel survival, whole-animal wet weight, shell length, shell weight, growth rate, tissue weight, or condition index.	• Nested ANOVA
• Relationship between mussel survival, whole-animal wet weight, shell length, growth rates, condition indices, or tissues weights and distance from the diffuser.	• Regression analysis
• Difference in mussel tissue total PAH concentrations between beginning and end of test.	• ANOVA & Dunnetts MRT
• Relationship between chemicals in mussel tissues and distance from the diffuser.	•Regression analysis

One of two computerized statistical packages were used for data analysis depending on the analysis required: GraphPad InStat (GraphPad Software, San Diego California) and/or Statistica (Statsoft, Tulsa, Oklahoma).

The first step in the analytical process was to check the data sets for normality. Normality was assessed with GraphPad InStat using the Kolmogorov Smirnov test. Parametric tests were run with all normally distributed data sets; the nonparametric equivalents were used for all data sets that failed to meet this requirement. The nested ANOVA was used to test for differences in zone, station, depth, and interaction effects. The Student-Newman-Keuls multiple range test where those differences occurred. T-tests were used to test for differences between BOT and EOT for selected parameters as well as for differences within and outside the mixing zone. All statistical analyses were run at the 95 percent confidence level ($\alpha = 0.05$).

3.10.2 Temperature

Temperature data were downloaded from the logging devices using the instruments' data recovery software. Minimum, maximum, and mean temperatures were calculated for each station. Temperature profiles were generated for each station and used to identify overall temperature trends.

Two null hypotheses were tested:

1. There is no difference in average daily temperatures among sites
2. There is no difference in weekly temperature ranges among stations.

Testing for Differences in Daily Mean Temperature

The temperatures at all stations displayed similar patterns in daily and seasonal cycles. The temperature series for all stations showed very strong autocorrelations (a measure of the dependence between observations of the same series), requiring a non-standard analysis of mean differences. To reduce variability and autocorrelation, each series was reduced to daily mean temperatures at each station. The daily mean temperature data were not normally distributed. Therefore, differences in daily average temperatures were determined using the Kruskal-Wallis non-parametric ANOVA followed by Dunn's multiple range test ($\alpha = 0.05$).

Testing for Differences in Temperature Range

To assess the effects of temperature conditions on mussel growth, temperature ranges over one-week periods were evaluated. This time interval was selected because seven days is a manageable time period, as opposed to comparisons based on an hourly or daily basis, and smaller temperature variations over longer time periods may have some biological relevance. Weekly intervals are also commonly used to measure changes in environmental conditions and growth in aquatic organisms. Although some dramatic changes in temperature were observed on a daily basis, overall temperatures decreased over time, with marked temperature declines evident over four to seven day periods. First, the minimum weekly temperature was subtracted from the maximum weekly temperature at each station, resulting in 8 observations of temperature range per station. These series were not significantly autocorrelated, and the variances were approximately equal across stations. Normality was assessed by plotting a histogram and quantile plot for residuals from an initial ANOVA fit. The data were approximately normal. The weekly ranges at each station were statistically analyzed using a one-way ANOVA and Newman-Keuls Multiple Range test. These tests identified whether the weekly ranges were similar among all stations, and if not, which stations differed.

3.10.3 QA on Growth Measurements

Accuracy and precision are fundamental to obtaining reliable, usable data. Accuracy is an expression of the degree to which a measured or computed value represents the true value, or the ability of the measuring device to provide the true value. The accuracy of measuring devices was determined according to the standard operating procedures for each measuring device. For the balance, this involved calibrating the instrument with a standard weight (200 g). After every 100 measurements made on the balance, the standard weight was applied to the balance. If the balance was off by more than 1% (2 g),

the balance was recalibrated and the previous batch of 100 individuals reweighed. The balance did not deviate from its calibrated weight by more than 0.05 g during the accuracy checks. There was no need to recalibrate the balance during the measurement process.

Precision is a measure of the reproducibility among individual measurements under similar conditions, or the ability to measure and find the same value time after time. Precision was assessed by performing multiple measurements for the parameters, using the following approach. At the beginning and the end of testing, for every 50 mussels measured, five mussel were remeasured. The remeasuring of mussels occurred throughout the measurement process as each group of 100 individuals was processed to ensure that all measurements were within the originally proposed limits of ±1.65 mm for length and ±0.35 g for weight. These limits represent an approximate ±5% and ±10% variance of the actual length and weight measurements, respectively. For length it was estimated that the average mussel shell length would be approximately 33 mm. Because the electronic calipers allow extremely accurate length measurements, only a ±5% variance in length was originally proposed. The remeasurement of weight can provide an opportunity for measurement error because of the loss of water from the outside of the mussel shells during the process. Therefore, a ±10% variance in weight was originally proposed. For weight, it was estimated that the average mussel whole-animal wet-weight would be approximately 3.5 g.

The remeasurement process used to satisfy the QA requirements indicated that field staff were consistent in the measurement technique and that the error associated with those measurements was mostly within the ±5% deviation. During the QA process, none of the length measurements exceeded the proposed variance; only one weight measurement exceeded the proposed variance (Figure 10). For the QA length portion, the average difference between the two measurements for all 100 mussels was -0.002 mm (SD = 0.145 mm); for the QA weight portion, the average difference between the two measurements for all 100 mussels was 0.014 g (SD = 0.109 g).

The differences between the QA measurements on length are probably due to

- misplacement of the calipers along the longest axis of the mussel shell
- pressing too hard on the calipers causing the mussel shell to be squished

The differences between the QA measurements on weight are probably due to

- the loss of water from the outside of the mussel shell or from the inside between the two valves
- improper taring of the balance prior to making the weight measurements

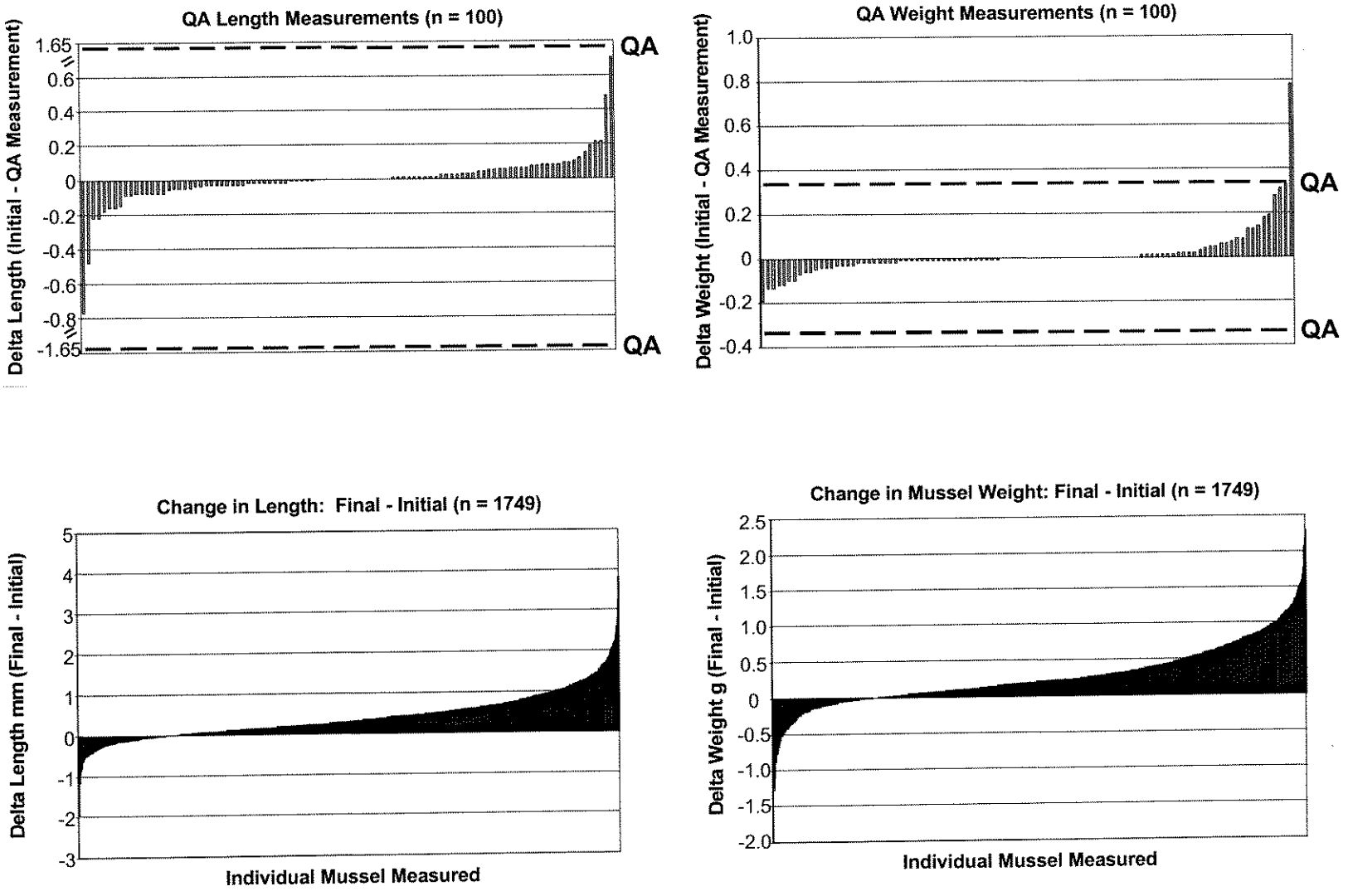


Figure 10. Results of the QA measurements on mussel length and weight and changes in mussel length and weight after the 56-day exposure period. (----- = proposed limits for QA variance)

When the EOT and BOT mussel metrics for all mussels were compared, some negative values were encountered. This is usually not expected because it is anticipated that the mussels will grow during the exposure period and increase in both shell length and tissue weight. Figure 10 shows the distribution of differences between BOT and EOT measurements for lengths and weights. For both lengths and weights, a small percentage of these measurements were negative.

4.0 RESULTS

The caged mussel pilot study was completed as proposed. All tasks as listed in the scope of work were accomplished. After a 56-day deployment period, all caged mussels were successfully retrieved, except those deployed nearest the diffuser (Station 1). Station 1 mussels were lost when the tether line split. Although Station 6 mussels were retrieved, coordinates at the retrieval site and its proximity to Berth 5 indicated that the caged mussel array had moved. This hypothesis is supported by significantly higher temperatures recorded by the *in situ* temperature meters, greater losses in tissue weight than any other station, and relatively insignificant accumulations of PAHs. These results will be discussed later.

Results demonstrated that caged mussel monitoring is feasible in the vicinity of the BWTF effluent diffuser at 70 meters. The specific questions identified by the RCAC regarding applicability of this monitoring approach were also answered:

- *Would the mussels survive?* Yes, overall survival was greater than 97%

- *Would the mussels grow?* Yes, whole-animal wet-weights and shell lengths increased significantly at every station except Station 2 closest the diffuser where increases in shell length were not statistically significant. Mussel shells and tissues appeared in good condition. Based on comparison to the baseline tissue weights, most mussels lost some tissue weight but many individuals appeared to have added reproductive tissues during the exposure period.

- *Would the mussels accumulate chemicals?* Yes, mussels accumulated chemicals known to have been associated with the BWTF effluent and some PAHs increased by almost an order of magnitude during the exposure period.. A gradient was established PAHs in mussel tissues and distance from the diffuser.

4.1 Data Quality Review

Based on external appearance, the mussels showed some growth and relatively good condition. Based on the appearance of internal tissues, most tissue masses appeared normal with the presence of reproductive tissues in some individuals. All mussel growth data are considered usable for the purpose of this report. No data were considered outliers, therefore none were excluded from the data set. Growth rates were calculated to facilitate comparisons with other studies by using the following formula:

(EOT Measurement - BOT Measurement)/8 weeks.

All tissue chemistry data are considered usable for the purposes of this report. Chemicals reported as undetected were included in statistical calculations using a value of one-half the reported detection limit. All data quality objectives outlined in the QAPP for this project were met.

4.2 Mussel Survival

Survival was calculated as initial number deployed minus number dead. For this pilot study, dead mussels were defined as those where empty shells or shells with decaying tissue were found. It is unlikely that any mussels "escaped" the mesh tubes because of the small mesh size. However, it is possible that the shells of a dead mussel could fragment and fall through the mesh. Based on the number dead, end-of-test survival was very high, ranging from 90 to 100% for individual replicates (Table 2). Average survival by station ranged from 95.7 to 99%. Survival data were analyzed for differences among stations using a contingency table. No significant differences ($\alpha = 0.05$) were detected.

Table 2. EOT percent survival for mussels

Station Number	2	3	4	5	6	7
Replicate 1	98	97	99	97	96	99
Replicate 2	98	100	93	98	99	99
Replicate 3	97	90	98	98	94	99
Mean	97.7	95.7	96.7	97.7	96.3	99
Standard Deviation	0.6	5.1	3.2	0.6	2.5	0
Total "n"	293	287	290	293	289	297

Cages from Station 1 were not retrieved and are considered lost.

4.3 Mussel Growth

Traditional growth metrics (Table 3, 4) were used to estimate mussel health and determine what effects, if any, were associated with exposure to the BWTF effluent. Results are first presented for shell length and whole-animal wet-weight, the measurements made on the individual mussels both at the beginning and end of test. These measurements provide the most accurate assessment of effects because they represent paired data for individual mussels. All other comparative metrics represent a comparison between end-of-test

measurements on individual mussels and an average determined from a surrogate number of animals measured at the beginning of the test. Table 4 also includes percent water and percent lipids data, biochemical measurements also used to assess mussel health, albeit not as accurately. Details for each of the individual metrics are presented below. Additional details of the nested ANOVA results and results of the multiple range tests for station and zone comparisons are provided in Appendix A.

Table 3. Percent change in mussel metrics

Station Number	2	3	4	5	6	7	Mean
% Survival	97.7%	95.7%	96.7%	97.7%	96.3%	99.0%	97.2%
% Change Weight	10.0%	5.5%	9.0%	7.0%	5.5%	7.1%	7.4%
% Change Length	0.7%	1.4%	1.1%	1.7%	0.9%	1.4%	1.2%
% Change Wet Tissue Weight*	-4.0%	3.8%	-3.9%	2.2%	-10.4%	-3.1%	-2.5%
% Change Dry Tissue Weight*	-0.16%	2.1%	-5.0%	-2.40%	-11.6%	-0.8%	-3.0%
% Change Condition Index*	-2.4%	-3.1%	-7.9%	-5.6%	-14.6%	-4.1%	-6.3%
% Change Shell Weight*	1.4%	4.5%	2.1%	2.4%	1.7%	2.2%	2.4%

* Estimated using baseline (BOT) measurements

Table 4. Mussel metrics summary table

	Station:	1	2	3	4	5	6	7	Mean	BOT
Initial Length (mm)	**Mean**	**33.60**	**33.53**	**33.60**	**33.54**	**33.56**	**33.60**	**33.53**	**33.56**	**33.55**
	min	31.04	31.14	31.11	31.08	31.00	31.00	31.00		31.12
	max	35.99	35.98	35.99	35.94	36.12	35.99	35.97		35.99
	stdev	1.37	1.35	1.34	1.35	1.36	1.37	1.39		1.33
	count	300	300	300	300	300	300	300		300
	2SE	0.16	0.16	0.16	0.16	0.16	0.16	0.16		0.15
EOT Length (mm)	**Mean**		**33.76**	**34.06**	**33.90**	**34.14**	**33.89**	**34.00**	**33.96**	-
	min		30.83	31.14	30.74	31.10	30.89	31.22		-
	max		38.07	36.98	36.97	38.32	36.65	37.26		-
	stdev		1.41	1.35	1.35	1.45	1.40	1.41		-
	count		293	287	290	293	289	297		-
	2SE		0.16	0.16	0.16	0.17	0.16	0.16		-
Length Growth (mm/wk)	**Mean**		**0.030**	**0.060**	**0.046**	**0.072**	**0.036**	**0.060**	**0.051**	-
	min		-0.22	-0.04	-0.22	-0.06	-0.10	-0.25		-
	max		0.32	0.27	0.25	0.40	0.32	0.48		-
	stdev		0.06	0.06	0.06	0.08	0.05	0.07		-
	count		293	287	290	293	289	297		-
	2SE		0.01	0.01	0.01	0.01	0.01	0.01		-
Initial Weight (g-ww)	**Mean**	**3.79**	**3.68**	**3.77**	**3.69**	**3.72**	**3.76**	**3.73**	**3.73**	**3.77**
	min	2.31	2.22	2.31	2.24	2.28	2.16	1.96		2.00
	max	6.69	5.71	6.10	5.36	5.95	6.53	5.95		6.05
	stdev	0.67	0.66	0.67	0.61	0.67	0.69	0.66		0.66
	count	300	300	300	300	300	300	300		300
	2SE	0.08	0.08	0.08	0.07	0.08	0.08	0.08		0.08
EOT WAWW (g-ww)	**Mean**		**4.05**	**3.97**	**4.04**	**3.98**	**3.98**	**4.01**	**4.00**	-
	min		2.43	2.64	2.90	2.60	2.52	2.86		-
	max		5.75	6.43	5.75	6.12	6.01	5.99		-
	stdev		0.62	0.62	0.57	0.64	0.64	0.61		-
	count		293	287	290	293	289	297		-
	2SE		0.07	0.07	0.07	0.07	0.08	0.07		-
WAWW Growth (mg/wk)	**Mean**		**46.2**	**25.8**	**41.5**	**32.4**	**26.0**	**33.2**	**31.2**	-
	min		-208.8	-152.5	-92.5	-110.0	-180.0	-153.8		-
	max		271.3	286.3	211.3	210.0	191.3	210.0		-
	stdev		60.7	52.2	44.6	50.6	46.8	47.3		-
	count		293	287	290	293	289	297		-
	2SE		7.09	6.16	5.24	5.91	5.50	5.49		-
EOT Wet Tissue Wt (g-ww)	**Mean**		**0.69**	**0.75**	**0.69**	**0.74**	**0.65**	**0.70**	**0.70**	**0.72**
	min		0.30	0.28	0.35	0.41	0.34	0.34		0.37
	max		1.15	1.22	1.13	1.31	1.14	1.07		1.18
	stdev		0.14	0.15	0.13	0.15	0.14	0.13		0.14
	count		291	287	290	293	289	297		300
	2SE		0.02	0.02	0.02	0.02	0.02	0.02		0.02
EOT Dry Tissue Wt (g-dw)	**Mean**		**0.116**	**0.119**	**0.111**	**0.114**	**0.103**	**0.116**	**0.113**	**0.117**
	min		0.055	0.043	0.060	0.063	0.051	0.056		0.061
	max		0.190	0.189	0.171	0.200	0.185	0.177		0.189
	stdev		0.025	0.024	0.021	0.023	0.024	0.022		0.023
	count		291	287	290	293	289	297		300
	2SE		0.0029	0.0029	0.0025	0.0027	0.0028	0.0025		0.003
EOT Shell Weight (g-ww)	**Mean**		**1.64**	**1.69**	**1.65**	**1.66**	**1.64**	**1.65**	**1.66**	**1.62**
	min		1.05	1.03	1.04	0.98	0.91	1.07		0.51
	max		2.99	3.62	2.73	2.95	2.58	2.89		2.82
	stdev		0.32	0.36	0.29	0.32	0.31	0.31		0.35
	count		292	287	289	292	289	297		300
	2SE		0.04	0.04	0.03	0.04	0.04	0.04		0.04
Percent Lipids	**Mean**		**1.07**	**1.07**	**1.07**	**0.93**	**1.03**	**1.07**	**1.04**	**1.23**
	min		1.00	1.00	1.00	0.50	1.00	1.00		1.10
	max		1.2	1.1	1.1	1.2	1.1	1.1		1.3
	stdev		0.12	0.06	0.06	0.38	0.06	0.06		0.12
	count		3	3	3	3	3	3		3
	2SE		0.13	0.07	0.07	0.44	0.07	0.07		0.13
Percent Water	**Mean**		**83.2**	**84.1**	**84.0**	**84.5**	**84.1**	**83.4**	**83.87**	**83.8**
	min		81.7	82.9	82.9	84.2	83.5	82.7		83.5
	max		84.3	84.8	84.92	84.7	84.9	84.1		84
	stdev		1.33	1.02	1.02	0.29	0.74	0.70		0.25
	count		3	3	3	3	3	3		3
	2SE		1.54	1.18	1.17	0.33	0.85	0.81		0.29
Condition Index	**Mean**		**0.072**	**0.072**	**0.068**	**0.070**	**0.063**	**0.071**	**0.069**	**0.074**
	min		0.038	0.033	0.028	0.038	0.027	0.030		0.035
	max		0.121	0.146	0.116	0.125	0.097	0.104		0.230
	stdev		0.015	0.014	0.013	0.013	0.012	0.013		0.019
	count		291	287	289	292	289	297		300
	2SE		0.0018	0.0017	0.0016	0.0016	0.0014	0.0015		0.0022

4.3.1 Shell Length

At the start of the test, individual shell lengths ranged from 31.00 to 35.99 mm, a range of 5.99 mm. Mean shell length for each of the seven stations was between 33.53 and 33.60 mm, a range of 0.07 mm. There were no statistically significant differences in mean lengths among individual cages ($p = 0.99$) or among pooled cages assigned to each station ($p = 0.99$) at the beginning of the test. Mean shell length increased at all stations during the 8-week exposure period. Many shells had an obvious and distinct leading edge of new growth that ranged between 1 and 3 mm. The percentage increase in length across stations ranged from 0.7 to 1.7% with a mean of 1.2% (Table 3). Although the increases in shell length were relatively small, results of t-tests comparing beginning- and end-of-test shell lengths showed that the increases in shell length were statistically significant at all stations, except Station 2 nearest the diffuser (Figure 11).

EOT shell length data were suitable for a parametric nested ANOVA without transformation. Results of this analysis indicated that there were no significant differences among zones, significant differences among stations ($p < 0.05$), no significant differences among depths, and no interaction effects (Table 5).

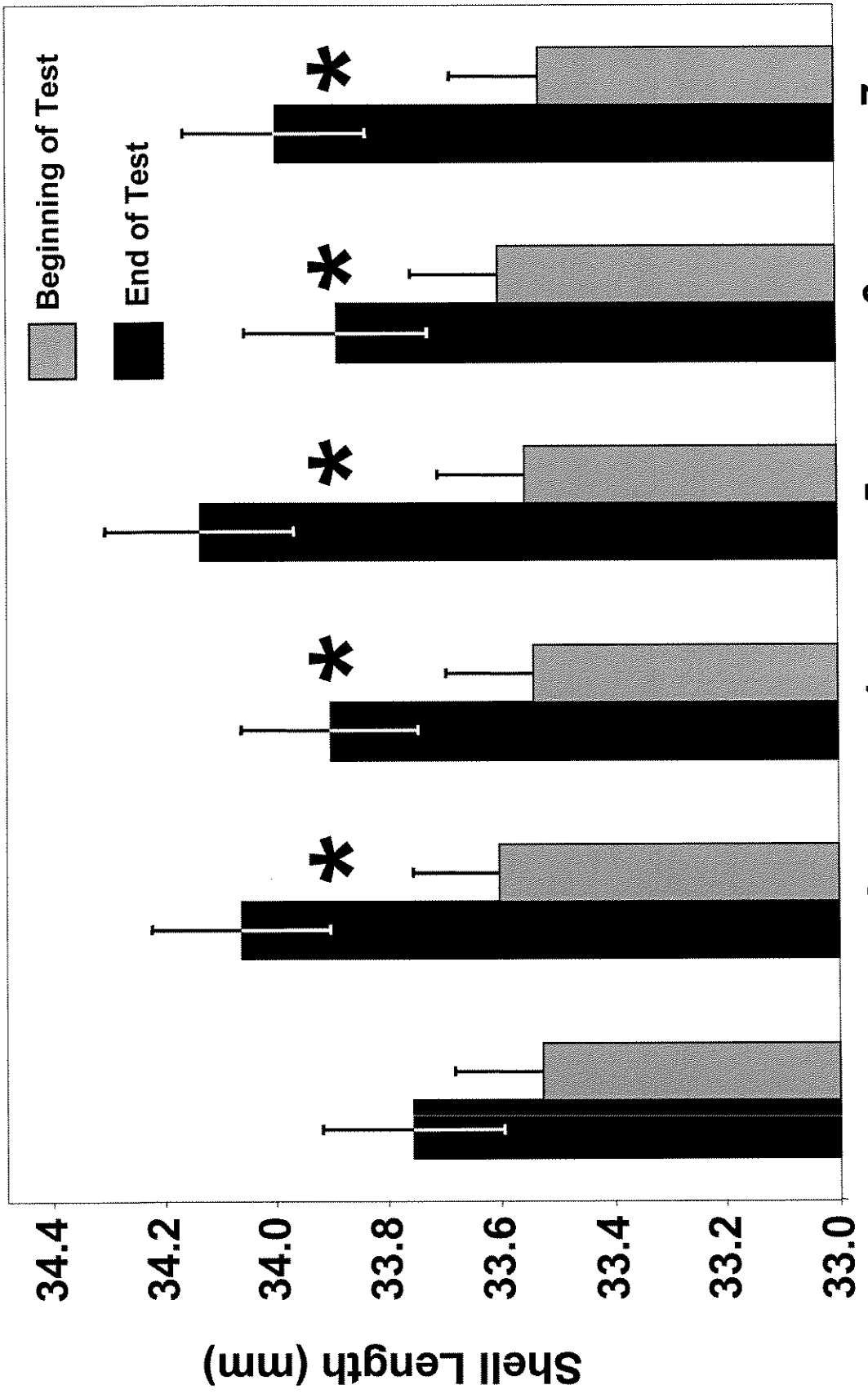


Figure 11. Comparison between BOT and EOT Shell Length (±2SE). * = statistically significant difference.

Table 5. ANOVA summary table for growth metrics[1]

	MAIN EFFECTS			INTERACTIONS	
GROWTH METRIC	Zone	Station	Depth	zone x depth	Station x depth
EOT Length	0.1200	**0.0213** *	0.390	0.612	0.407
Length Growth Rate	**0.000349** ***	**0.000000** ***	**0.000278** ***	**0.0032***	**0.0000072** ***
EOT WAWW	O.301	0.547	**0.000042**** *	0.359	0.090
Weight Growth Rate	**0.0018** **	**0.000005** ***	**0.000000**** *	0.261	**0.000014** ***
EOT Wet Tissue Weight	**0.010** **	**0.000000** ***	**0.00486** ***	0.502	**0.000007** ***
EOT Dry Tissue Weight	**0.000020** ***	**0.000000** ***	**0.000104** ***	**0.00220** ***	**0.000000** ***
Shell Weights	0.554	0.415	0.338	0.954	0.387
Condition Index	**0.000015** ***	**0.000000** ***	**0.00596** ***	**0.000042** ***	**0.000000** ***
% Lipids	0.497	0.901			
% Water	0.533	0.447			

[1] Shaded boxes indicate statistically significant results. * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

Length growth rates were calculated to facilitate comparisons with literature values that are commonly expressed in terms of length increase per unit time. Length growth rates ranged from 0.030 to 0.072 mm/wk among stations (Table 4). The highest growth rates were found for mussels at Station 5 and the lowest at Station 2, nearest the diffuser. The length growth rate data were analyzed with parametric tests without transformation. Results of the nested ANOVA indicated that there were significant differences ($p < 0.001$) among zones, stations, depths, and significant interactions (Table 5). No distinct patterns were evident in length growth rates among stations and depths when the means were compared, suggesting that depth is not affecting length growth rate the same way at each station.

4.3.2 Whole-Animal Wet-Weight (WAWW)

At the start of the test, individual WAWWs ranged from 1.96 to 6.69 g, a range of 4.73 g; mean WAWW by station ranged from 3.68 to 3.79 g, a range of 0.11 g (Table 4). There were no statistically significant differences in mean WAWWs among individual cages ($p = 0.49$) or among pooled cages assigned to each station ($p = 0.40$) at the beginning of the test. This is not surprising given the small range in mean WAWW among stations. WAWW increased at all stations during the 8-week exposure. The percentage increase in WAWW across stations ranged from 5.5 to 10% with a mean of 7.4% (Table 3). Although the increases in WAWW were relatively small, results of t-tests comparing beginning- and end-of-test shell lengths showed that the increases in WAWW were statistically significant at all stations (Figure 12). In contrast to the length measurements, the largest increase in WAWW occurred in mussels at Station 2, nearest the diffuser.

The EOT WAWW data were suitable for a parametric nested ANOVA without transformation. Results of the ANOVA indicated that there were no significant differences among zones, stations, and no significant interaction effects, but there were significant differences ($p < 0.001$) among depths (Table 5).

Weight growth rates were calculated to facilitate comparisons with literature values that are commonly expressed in terms of weight increase per unit time. The beginning- and end-of-test WAWWs were used to calculate growth rates. Weight growth rates ranged from 25.80 to 46.15 mg/wk among stations (Table 4). The highest growth rates were found for mussels at Station 2 and the lowest at Station 3. The WAWW growth rate data were suitable for a parametric nested ANOVA without transformation. Results of the ANOVA indicated that there were significant differences ($p < 0.001$) among zones, stations and depths, and there was a significant interaction ($p < 0.001$) between stations and depths (Table 5). No distinct patterns were evident in WAWW growth rates among stations and depths when the means were compared, suggesting that depth is not affecting WAWW growth rate the same way at each station during the 56-day deployment period.

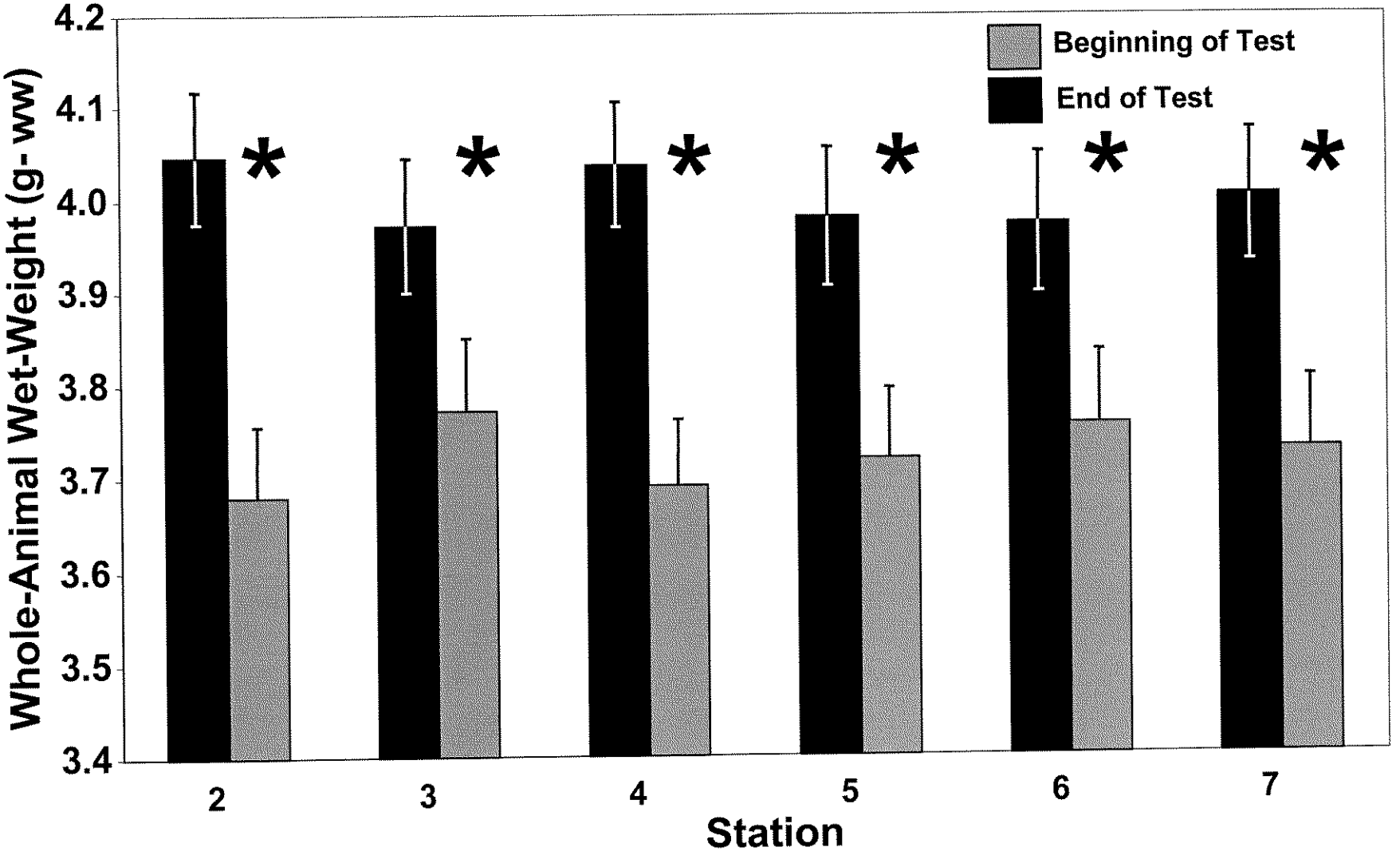


Figure 12. Comparison between BOT and EOT WAWW (±2SE). * = statistically significant difference.

4.3.3 Wet Tissue Weights

Mean wet tissue weight at the start of the test was estimated at 0.72 g-ww (Table 4) based on the tissue weights from the 300 baseline BOT measurements. EOT wet tissue weights ranged from 0.65 to 0.75 g (Table 4). The percentage change in wet tissue weight across stations varied from -10.4% to 3.8%, with a mean of -2.5% (Table 3). Although the estimated changes in wet tissue weights were relatively small (Figure 13), results of Dunnett's multiple range test comparing the baseline BOT wet tissue weight to EOT wet tissue weights showed that only at Station 6 was the estimated change considered statistically significant. The wet tissue weights for mussels at Station 6 had an estimated decrease of 10.4%. Since tissue weight measurements are destructive and the same individuals cannot be measured at both the beginning and end of test, the changes in tissue weight are less accurate than the changes in WAWW and shell length, which are made on the same individuals. Therefore, for wet tissue weights the comparisons across stations at the end of the test are usually more reliable than beginning versus end of test comparisons.

EOT wet tissue weight data were suitable for a parametric nested ANOVA without transformation. Results of the ANOVA indicated that there were significant differences among zones ($p = 0.01$), stations ($p < 0.001$) and depths ($p < 0.01$), and there was a significant interaction ($p < 0.001$) between stations and depths (Table 5). The results of the crosswise ANOVA show a significant interaction effect for EOT wet tissue weights. No distinct patterns were evident in EOT wet tissue weights among stations and depths when the means were compared, suggesting depth is not affecting EOT wet tissue weights the same way at each station.

4.3.4 Dry Tissue Weights

Mean dry tissue weight at the start of the test was estimated at 0.117 g-dw (Table 4) based on the tissue weights from the 300 baseline BOT measurements and the percent moisture determined during chemical analysis. Mean EOT dry tissue weights by station ranged from 0.103 to 0.119 g-dw; the overall range for individuals was 0.043 to 0.200 g-dw (Figure 14; Table 4). The percentage change in dry tissue weight across stations ranged from -0.16 to -11.66 g, with a mean loss of 3.46 g-dw. Although the estimated change in dry tissue weights were relatively small (Figure 14), results of Dunnett's multiple range test comparing the baseline BOT dry tissue weight to EOT dry tissue weights showed that only at Stations 4 and 6 was the estimated change considered statistically significant. The dry tissue weights for mussels at Stations 4 and 6 had estimated decreases of 4.99 and 11.66%

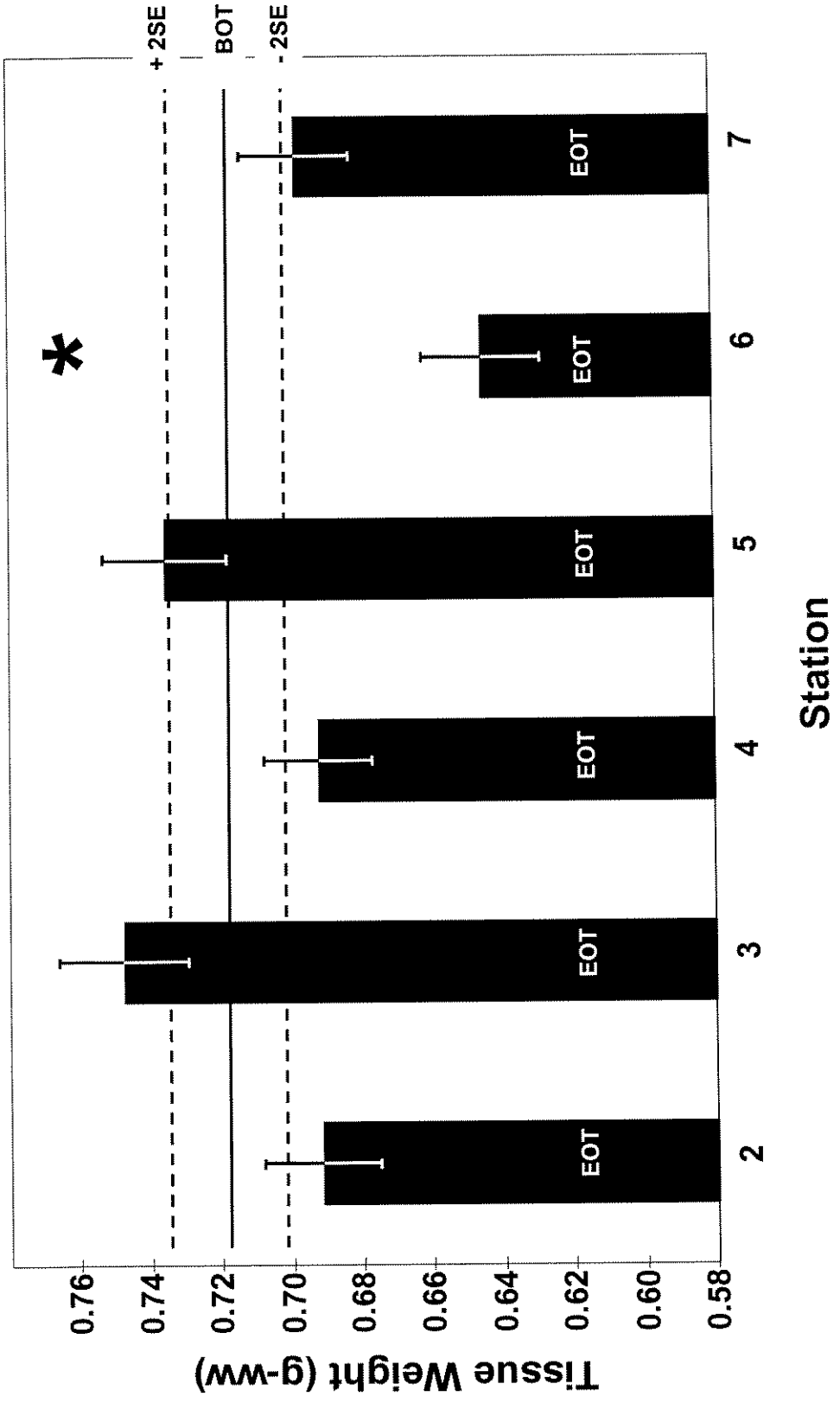


Figure 13. Comparison between BOT and EOT wet tissue weight ±2SE). * = statistically significant difference.

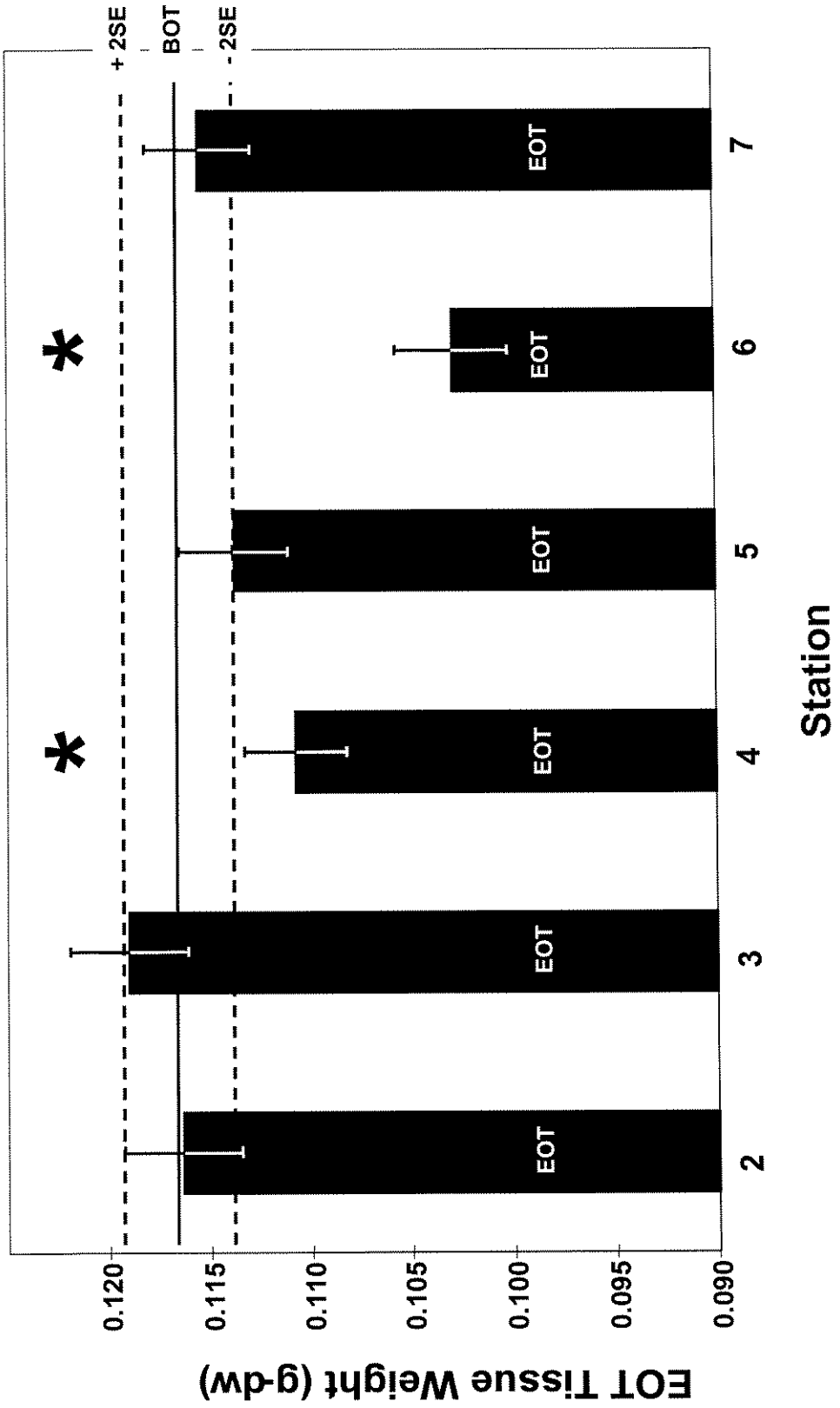


Figure 14. Comparison between BOT and EOT dry tissue weight (±2SE). * = statistically significant difference.

respectively. Because of the destructive nature of tissue weight measurements as discussed above, for dry tissue weights the comparisons across stations at the end of the test are usually more reliable than beginning versus end of test comparisons.

The EOT dry tissue weight data were suitable for a parametric nested ANOVA without transformation. Results of the ANOVA indicated that there were significant differences ($p < 0.001$) among zones, stations, and depths, and there were significant ($p < 0.001$) interactions (Table 5). The results of the nested ANOVA show a significant interactions between zone and depth and between station and depth. This is the only parameter for which the zone-depth interaction was significant. No distinct patterns were evident in EOT dry tissue weights among stations and depths when the means were compared, suggesting that depth is not affecting EOT dry tissue weights the same way at each station.

4.3.5 Shell Weights

Mean shell weight at the start of the test was estimated at 0.117 (Table 4) based on the shell weights from the 300 baseline BOT measurements. EOT mean shell weights by station ranged from approximately 1.64 to 1.69 g-ww; the overall range for individuals was 0.91 to 3.62 g-ww (Figure 15; Table 4). The percentage change in shell weight across stations ranged from 1.7% to 4.5% with a mean of 2.4% (Table 3). Shell weight was the only metric that suggested an apparent increase in mussel health at all stations. The estimated change in shell weights were relatively small (Figure 15), and results of the ANOVA comparing the baseline BOT shell weight to EOT shell weights showed no significant differences. Because of the destructive nature of tissue and shell weight measurements as discussed above, for shell weights the comparisons across stations at the end of the test are usually more reliable than beginning versus end of test comparisons. Results of the nested ANOVA indicated that EOT shell weights were statistically similar across all zones, stations, and depths (Table 5).

4.3.6 EOT Condition Index

Condition index was calculated as dry tissue weight divided by shell weight. Mean condition index at the start of the test was estimated at 0.074 (Table 4) based on the tissue and shell weights from the 300 baseline BOT measurements. EOT condition indices by station ranged from 0.063 to 0.072 (Table 4) and were all lower when compared to the BOT condition index. The percentage decrease in condition index across stations ranged from 2.4% to 14.6% with a mean of 6.3% (Table3). Condition index was the only metric that suggested an apparent decrease in mussel health at all stations. Although the

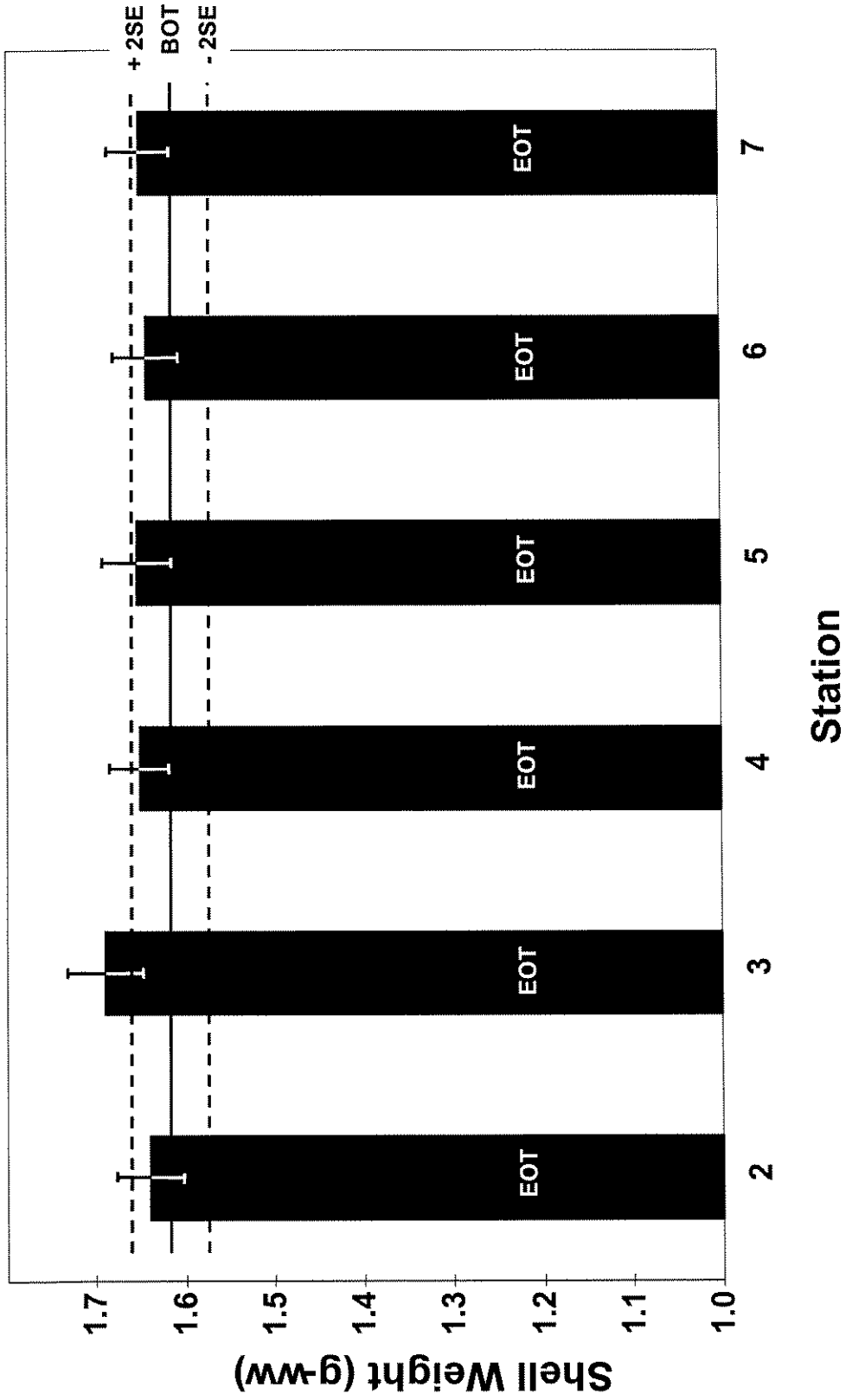


Figure 15. Comparison between BOT and EOT Shell Weight (±2SE). No statistically significant differences were found.

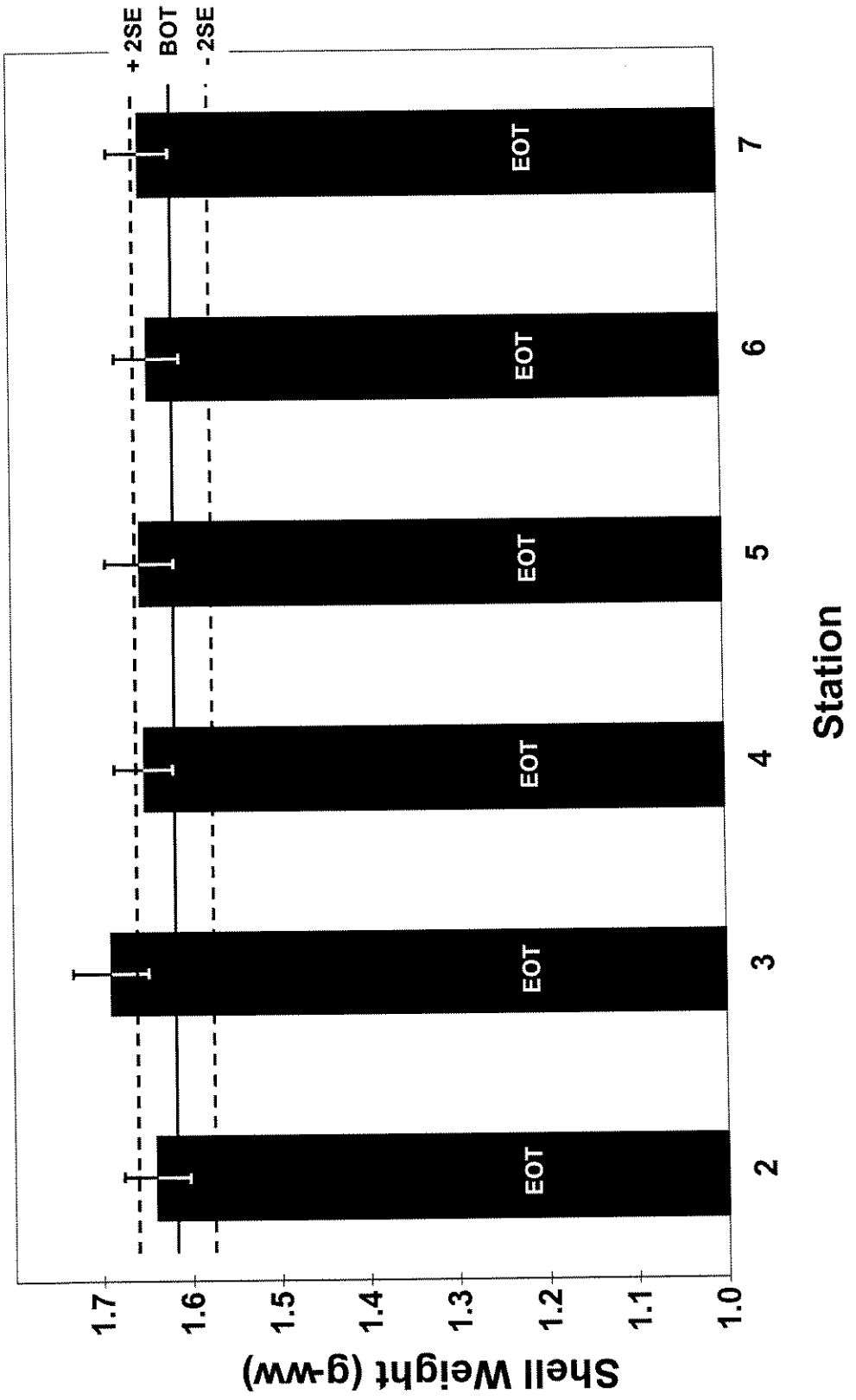


Figure 15. Comparison between BOT and EOT Shell Weight (±2SE). No statistically significant differences were found.

estimated change in condition indices were relatively small (Figure 16), results of the Dunnett's multiple range test comparing the baseline (BOT) condition index to end-of-test condition indices showed significant differences at Stations 4, 5, 6, and 7, stations furthest from the diffuser (Figure 16).

The EOT condition index data were suitable for a parametric nested ANOVA without transformation. Results of the ANOVA indicated that there were significant differences ($p < 0.001$) among zones, stations and depths, and there were significant interactions ($p < 0.001$) between stations and depths and between stations and depths (Table 5). It is not surprising that a significant interaction was found between zone and depth because condition index is a function of dry weight, and a similar interaction was found for this parameter as well.

The results of the crosswise ANOVA show a significant interaction effect for condition index. No distinct patterns were evident in condition indices among stations and depths when the means were compared, suggesting that depth is not affecting condition index the same way at each station.

4.3.7 Percent Lipids

Percent lipids were measured as part of the chemical analytical process. The baseline BOT mussels had a lipid concentration of 1.23%. Compared to initial percent lipids, EOT lipids decreased at all stations. At the end of the study, lipid concentrations ranged from 0.93 to 1.07 % (Table 4). The greatest loss was at Station 5 but this included an outlier of only 0.5% lipids which was 50% lower than all other measurements. Without that data point, Station 6 showed the greatest loss in lipids of approximately 16% compared to baseline BOT. There were no statistically significant differences between baseline BOT and EOT lipid concentrations.

4.3.8 Percent Water

Percent water in mussel tissues was measured as part of the chemical analytical process. The baseline BOT mussels had a water content of 83.8%. EOT water content ranged from 83.2 to 84.5% (Table 4). Mussels from Stations 2 and 7 exhibited the greatest decreases in percent water compared to baseline BOT and indicated the best health. All other stations increased in percent water, indicative of poorer health. Water losses at Station 6 were not greater than at other stations. There were no differences between initial and EOT percent water concentrations.

4.4 Mussel Tissue Chemistry

Tissue samples were analyzed for PAHs, selected metals, and TBT. Only the results for total PAH concentrations measured in mussel tissues, and results of statistical analyses comparing baseline BOT concentrations to EOT concentrations are presented below. Additional details for specific PAH compounds and their alkylated homologs are provided in Appendix B.

4.4.1 Total PAHs

EOT total PAH concentrations ranged from 1154 to 5644 μg/kg-dw (Table 6). The highest three highest concentrations of total PAHs in mussel tissues were all measured at Station 2; 5644, 4856, and 3993 μg/kg-dw at 9m, 7m, and 5m above the bottom respectively. The concentrations decreased with proximity to the bottom sediment. The tissue sample at Station 7, 9-m above the bottom was lost during the re-analysis process. This is indicated in each graph by a dashed circle at the 1200 m distance from the diffuser position. Table 6 also contains tissue chemistry data collected at the end of the test for the Anderson Bay and Saw Island locations. These data are provided for comparative purposes with other programs and will not be discussed here.

Mean total PAH concentration at the start of the test was 521 μg/kg-dw (Table 6) based on the 3 tissue replicate baseline BOT measurements. Mussels at all stations accumulated total PAHs and statistically significant differences between baseline BOT and EOT were found for all stations, except Station 6 ($p < 0.05$). This appears to be primarily attributable to the exceptionally low value reported for mussels at Station 6, 5-m above the bottom, and the variability in the replicates at the beginning of the test, as shown in Figure 17. Increases of approximately an order of magnitude were found at Station 2 nearest the diffuser.

Table 6. Total PAHs (μg/kg-dw) measured in mussel tissues

	Station									
Depth	2	3	4	5	6	7	Rep	AB BOT	AB EOT	Saw EOT
9-m	5644	2653	2155	1567	1502	na	1	672	708	543
7-m	4856	2394	1989	2223	1179	1154	2	455	745	903
5-m	3993	2628	1647	1754	598	2691	3	437	541	1000
Mean	4861	2559	1930	1848	1093	1923		521	665	815

na = not available

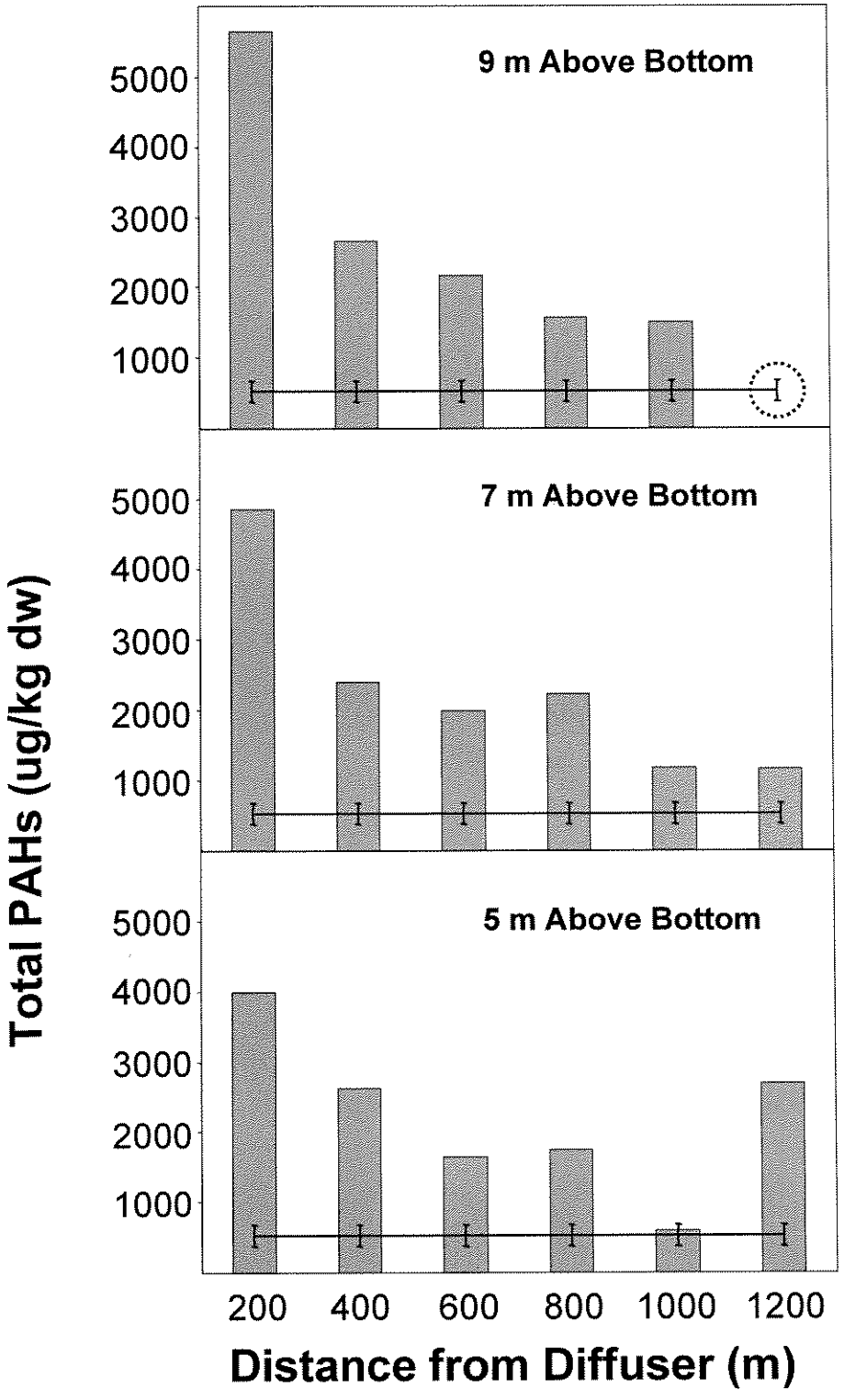


Figure 17. Total PAHs BOT vs EOT by station and distance above bottom sediment. ◌ = missing point.

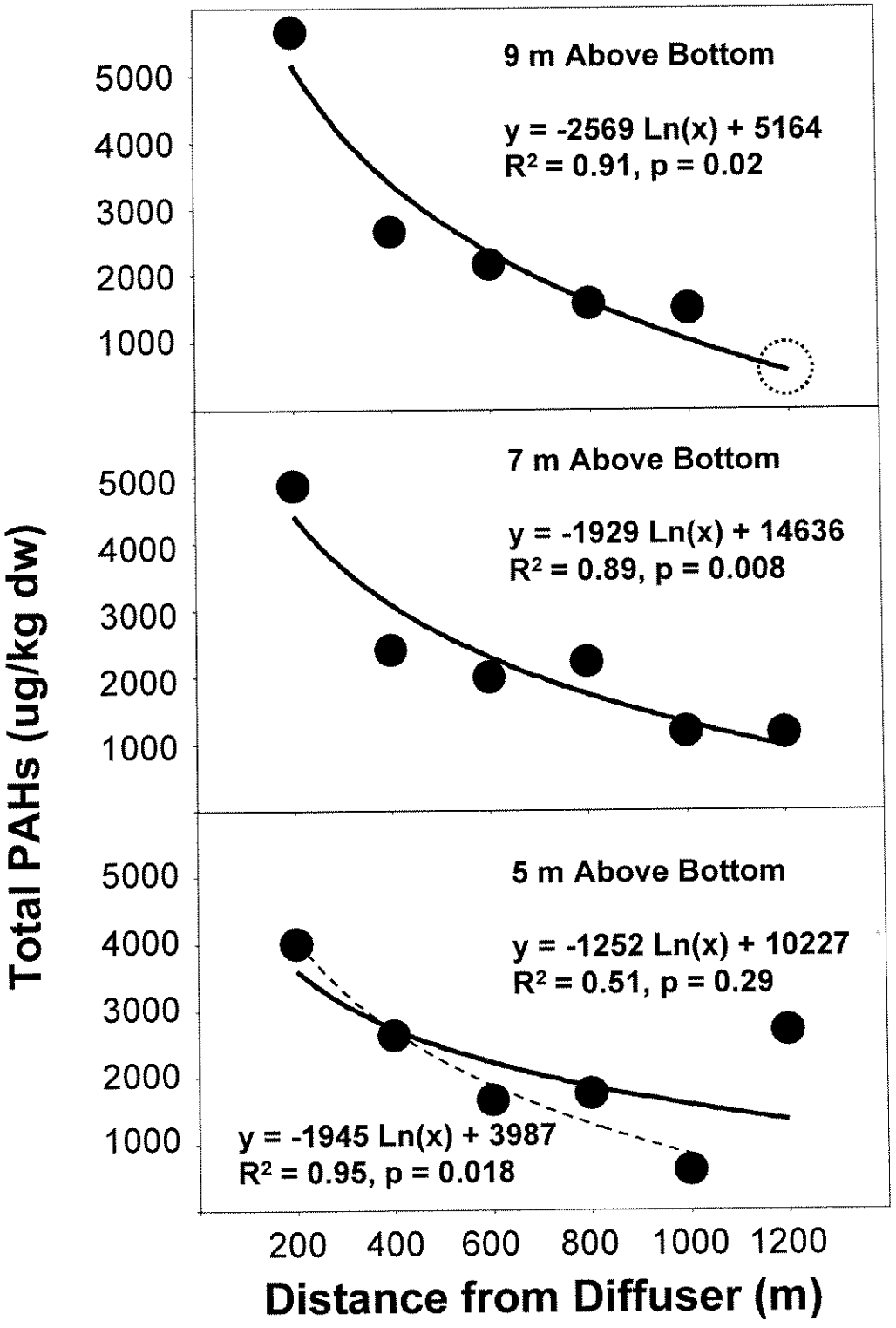


Figure 18. Total PAHs in mussel tissues at 5, 7, and 9 m above bottom. ◌ = missing point.

A decreasing gradient of total PAHs in mussel tissues was shown with distance from the diffuser (Figure 18). Regression analyses showed that these relationships were statistically significant at 9-m ($p = 0.02$) and 7-m ($P = 0.008$), but not 5-m ($p = 0.29$) above bottom sediments. However, by eliminating the data from the station furthest from the diffuser, where PAHs could have come from another source, the regression for the 5-m above bottom sediments data set becomes statistically significant ($p = 0.02$). Coefficients of determination for the significant regressions were 0.91, 0.89, and 0.95 at 9, 7, and 5-m above bottom sediments, respectively. Similar gradients were found for alkylated homologs and a similar indication of another source of PAHs in the vicinity of Station 7 (Appendix B).

4.4.2 Metals and TBT

Tissue chemistry results for metals and TBT did not show significant relationships with distance from the diffuser but are provided in Appendix B for comparative purposes.

4.5 Ancillary Measurements

Seawater samples were analyzed for chlorophyll, phaeopigments, total suspended solids and temperature. Results for these ancillary measurements are provided in Appendix D.

4.5.1 Water Temperature

Temperature at all stations displayed similar patterns with daily and seasonal cycles over the 56-day deployment period. Stations 6 and 7 had significantly higher temperatures throughout the exposure period than the other stations (Figure 19; Table 7) and Station 6 had higher temperatures than any other station. This provides corroborative evidence that Station 6 was in shallower water throughout the exposure period rather than just being moved at the end. For all stations, water temperatures at the time of deployment ranged from about 4.1 to 4.5°C and declined rapidly over the first day to between 4.0 and 4.3°C. This was followed by a steady increase over the next week; then several peaks and valleys over the next few weeks with the highest temperatures at each station recorded toward the end of March. This was followed by another temperature decrease toward the end of March and beginning of April when the six stations were closer in temperature for about one week than at any point in the study. This was followed by another rapid increase at Stations 6 and 7 while the other stations remained about the same. Stations 2, 3, 4 and 5 varied together, decreased, and then began to slowly increase toward the end of the test

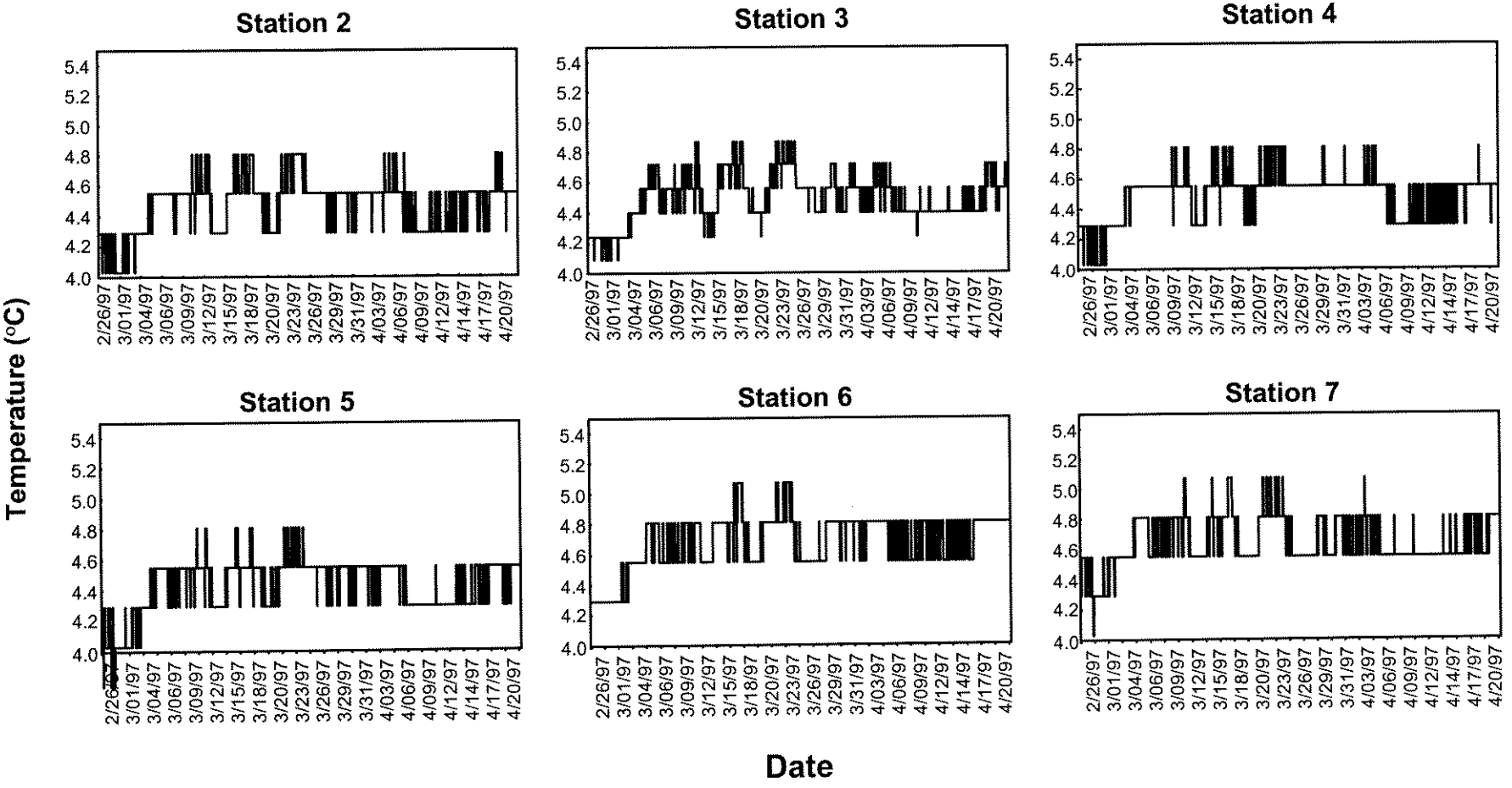


Figure 19. Actual temperature profiles by station using all data.

when they approached temperatures at Stations 6 and 7. The largest separation between temperatures at Stations 6 and 7 when compared to the other stations occurred during this period although temperatures at these stations were consistently higher than the other stations throughout the exposure period.

Table 7. Summary of overall temperature conditions by station: February 25 - April 23, 1997

Station	Minimum (°C)	Maximum (°C)	Average (°C)
2	4.03	4.81	4.48
3	4.09	4.87	4.50
4	4.29	4.81	4.51
5	4.29	4.81	4.44
6	4.55	5.07	4.71
7	4.55	5.07	4.62

Differences in Daily Average Temperature

Daily average temperatures for each station are presented in Figure 20. Results of the Kruskall-Wallis and Dunn's tests indicated statistically significant differences in daily average temperatures across stations at 7-meters above bottom sediments: Station 2 = 3 = 4 = 5 < Station 6 = 7.

Differences in Temperature Range

The results of the one-way ANOVA and Newman-Keuls Multiple Range tests indicated that the range of temperatures across stations was not significantly different.

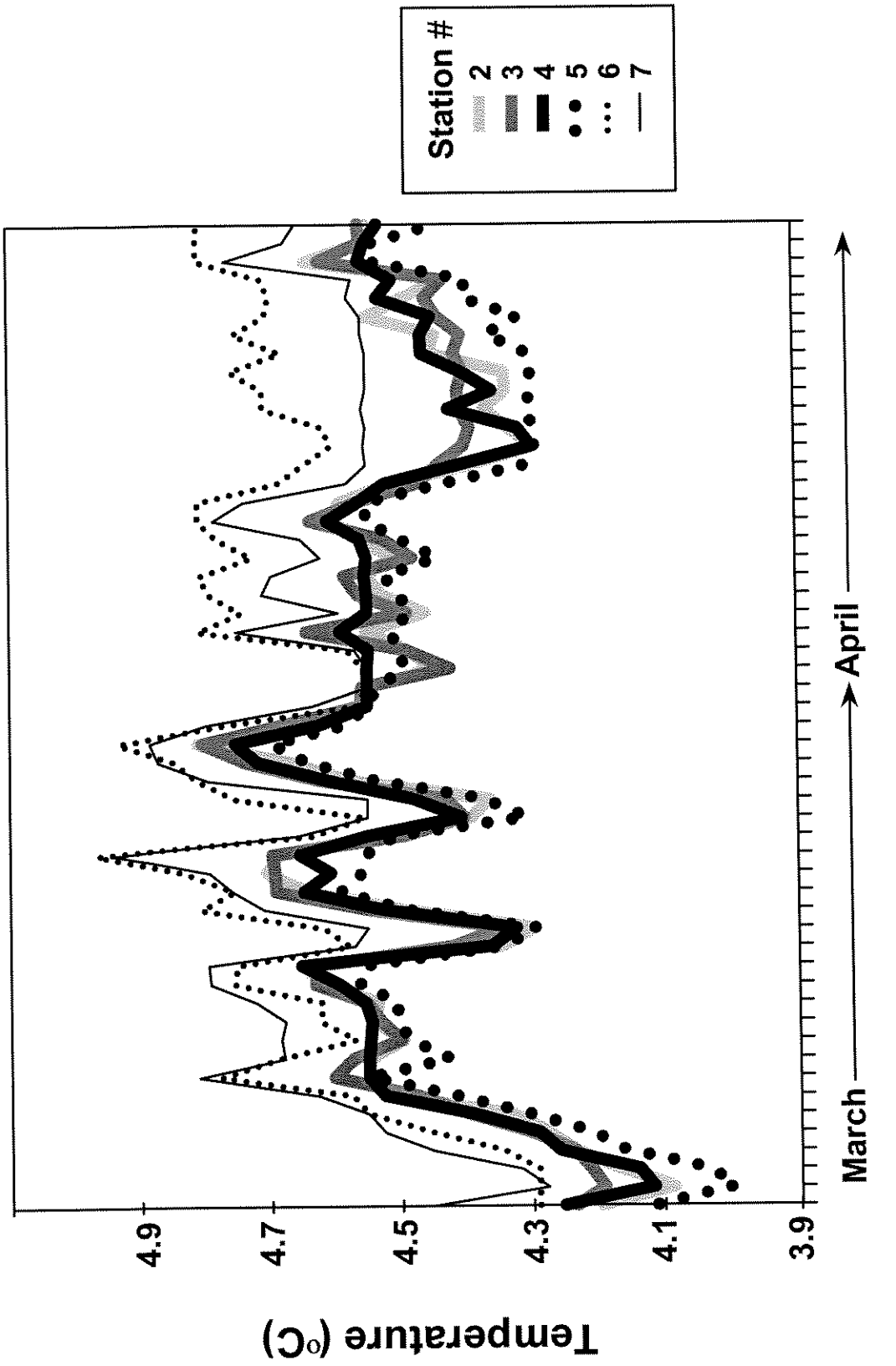


Figure 20. Temporal variation in daily average temperatures at the middle depth (7 m above bottom) by station.

5.0 DISCUSSION

Within the Regional Citizens Advisory Council (RCAC), the Terminal Oil Tanker Operations and Environmental Monitoring Committee has been given the responsibility of developing a monitoring strategy that will permit early detection of environmental effects associated with terminal operations. Within the Alyeska Marine terminal, the Ballast Water Treatment Facility (BWTF) is generally considered as having the most potential for impact on the marine environment. One of the least understood and most controversial aspects of the BWTF is the fate and effects of PAHs associated with effluents discharged to Port Valdez via the BWTF diffuser at a depth of 70 m. This is due in part to the difficulty in sampling at those depths, the uncertainty of traditional monitoring methods, and concerns expressed by local citizens. Regulatory concerns occur since the State of Alaska has an anti-degradation policy outside of the effluent mixing zone (State of Alaska, 1997).

A caged mussel pilot study was conducted to test this newer methodology as an alternative monitoring tool for the fate and effects of PAHs associated with the BWTF effluent. The study achieved the major objective: to evaluate the feasibility and scientific value of the method as a monitoring tool for evaluating chemical exposure and biological effects associated with the BWTF effluent which is discharged at a depth of 70 m in Port Valdez. Study results demonstrate that mussels can be readily collected, sorted, deployed, and retrieved at the 70-m study site, an area where these mussels are not normally found. The pilot study also demonstrated that bioaccumulation of chemicals in mussel tissues can be used to estimate exposure and that changes in growth, both in terms of shell and soft tissues, can be used to assess effects. Each of the three technical questions, identified as critical to conducting subsequent studies, were also answered: the mussels survived the 70-m depth with survival equivalent or greater to that achieved in intertidal studies, the mussels grew and met the growth criteria necessary to allow chemical analysis of soft tissues, and they accumulated chemicals known to have been associated with the BWTF. The high survival rate of 97% is clear evidence that the mussels can tolerate conditions at a depth of 70 meters, provide exposure and effects information using commonly accepted endpoints like bioaccumulation and growth, and that caged mussels are a viable tool for evaluating conditions associated with deeper waters in Port Valdez. High survival was expected based on the robust nature of most bivalves, but confirmation was required because none of the very few studies that have been conducted at these depths measured mussel growth (Karinen 1980; Forlin et al., 1996a,b). Caged mussel monitoring quantified exposure and effects over 3-dimensional space that would not have been possible using other traditional approaches.

The collection of multiple effects endpoints permitted a preponderance-of-evidence

approach to be used in evaluating conditions in the vicinity of the diffuser. Changes in shell length and WAWW, which involved making measurements both at the beginning and end-of-test on the same individual mussels, were more discriminating than either shell or soft-tissue weights. However, using each of these growth metrics, we were able to detect differences among stations along the 70-m contour, as well as differences between the mixing zone and non-mixing zone. Because these analyses are outside the original purpose of this pilot study, the station and zone comparisons are discussed in Appendix B.

In addition to documenting differences in growth metrics between BOT and EOT, among stations, depths, and zones, important information was gained regarding the bioavailability of chemicals associated with the BWTF effluent. When compared to the baseline BOT tissue weights determined for a surrogate number of mussels, there appears to have been a slight decrease in total soft tissue weight. However, it appears that these small losses in tissue mass did not affect bioaccumulation of PAHs. The concentrations of PAHs in mussel tissues increased significantly during the exposure period. After the 56-day exposure period at Station 2 nearest the diffuser, total PAHs increased by about an order of magnitude when compared with baseline BOT concentrations. A decreasing gradient was established between concentrations of total PAHs in mussel tissues and distance from the diffuser. A comparison of the ratio of parent compounds to their respective alkylated homologs provides evidence to show that these chemicals came from the diffuser (Appendix C).

The focus of the discussion will be on the generic results and how caged mussels could be used by RCAC as an early warning system consisting of *in-situ* sentinels for marine environmental monitoring in Port Valdez . The purpose of this report is not to evaluate the environmental effects of the BWTF effluent *per se*, but to demonstrate how the caged bivalve approach could be applied to a full-scale monitoring program. The data from this study and this report can be used to guide planning discussions at a later date regarding the possible objectives and design of future studies.

5.1 Feasibility and Scientific Value

The pilot study demonstrated that it is feasible to conduct caged mussel studies at depths of 70 meters in Port Valdez and to use this approach to monitor water and sediment quality in the vicinity of the BWTF diffuser. Logistical feasibility was demonstrated by successfully collecting, sorting, caging, deploying and retrieving mussels from the desired locations along the 70-m depth contour. Technical feasibility was demonstrated by establishing a statistically significant relationship between the concentration of PAHs in mussel tissues

and those known to have been associated with the BWTF effluent, and by detecting statistically significant differences in growth metrics among stations and between zones.

Other studies have demonstrated that caged bivalves are very useful as sentinels for many chemicals, including PAHs, from the intertidal zone (Salazar and Salazar, 1997a) to a depth of 650 meters in the Norway Trench (Forlin et al., 1996a,b) and even at a marine terminal (Widdows et al., 1987, 1995b). It can probably be assumed that environmental conditions at a depth of 70 meters represent was a worst-case scenario for Port Valdez waters in terms of stress on transplanted mussels. There was less concern as to whether mussels could be successfully transplanted at shallower depths although there could be other problems such as mooring logistics, freshwater input and siltation, and time of year. Based on other studies, it appears that mussels could be used as biological monitors at the greatest depths within in Port Valdez, approximately 230 m (Hood et al., 1973).

The scientific value of this approach lies in the ability to 1) monitor conditions and make predictions about the effects from exposure to those conditions, 2) identify differences among stations and zones, and 3) use mussels as surrogates to help understand and characterize ecological processes in Port Valdez. Using caged mussels to monitor environmental conditions and quantify exposure and effects on a site-specific basis will allow the RCAC to make management decisions on water and sediment quality in the vicinity of the BWTF effluent diffuser not possible with traditional approaches such as routine analyses of water chemistry, laboratory bioassays, or evaluation of benthic community structure. The information gained in this study quantified exposure and effects over 3-dimensional space that would not have been possible using other traditional approaches. The monitoring results from caged mussel studies can then be used to predict exposure and effects. Although predictions over space and time can be made with data collected from traditional approaches, such predictions made from data collected with field bioassays like caged mussels reduce the uncertainty in the predictions. This is because field studies are conducted under natural conditions and the effects in organisms represent an integration of all exposure conditions, natural and introduced. It should be emphasized that mussels are being used as surrogates for other species that are not as easy to collect, cage, and measure and do not have the same bioaccumulation potential.

5.2 Survival

The caged mussel pilot study was terminated after 56 days because of concerns that spawning was imminent and because if stresses at 70 meters were too severe, test animals might not survive a longer exposure period. High mortality would also preclude obtaining necessary growth and bioaccumulation information. Nevertheless, 56 days

represents a substantial exposure period to chemicals of concern and environmentally realistic, site-specific conditions. Most laboratory bioassays are conducted for only 4 days under environmentally unrealistic conditions. Therefore, survival results from this pilot study would tend to have more validity because of the realistic exposures. In this pilot study, survival served as an effects endpoint and as a criterion for a successful test. Survival in bivalves, or any other species, is generally not considered a very sensitive endpoint for evaluating effects although in a preponderance of evidence approach it sometimes provides useful corroborative information. Here, the high survival of 97% suggests that the mussels were in reasonably good condition after the 56-day deployment. The conservative survival criterion of 50% was exceeded and provided the first evidence that chemical analysis of soft tissues would be useful.

The results from many different studies suggest temperature and depth are not the primary limiting factors for mussel survival and for maintaining necessary physiological functions (Loo, 1992; Loo and Rosenberg, 1983; Wallace, 1980). These other studies indicate that food may be the limiting factor at low temperatures and great depths. As previously discussed, this pilot study was not conducted at the preferred season for growth or the optimum exposure period to maximize growth (Cooney and Coyle, 1988; Feder and Shaw, 1996). It was conducted during the winter when plankton and other food sources are probably at a minimum in Port Valdez. This suggests that studies conducted at other times of the year could be even more successful although avoiding the spawning season and still maximizing growth rates presents a major challenge.

5.3 Growth

In this pilot study, growth metrics served as an effects endpoint to estimate mussel health, as a criterion for a successful test, and to help calibrate bioaccumulation results. As expected, the growth metrics were more sensitive indicators of effects than the survival endpoint or the corroborative endpoints of % lipids and % water. Many differences in growth metrics were found among stations and zones that were not detectable using survival or the biochemical endpoints. Although most of the evidence suggests that a longer exposure period would not have affected bioaccumulation, it is likely that a longer exposure would have resulted in greater differences in growth among stations.

As with the survival criterion, conservative growth criteria were established due to the expected harsh conditions at 70 meters and since the test was not conducted during the optimum time of the year to maximize growth. Nevertheless, results from other studies show that these criteria were reasonable. During initial tests to evaluate the relationship between tissue mass and bioaccumulation, unfed mussels lost an average of 10% of their

tissue mass after four weeks and 21% after eight weeks (Ernst et al., 1991). Fed mussels lost an average of 8% and 4% after four and eight weeks, respectively. Collectively, these results show several things relative to the caged mussel pilot study. 1) Even the fed laboratory mussels lost a significant amount of weight after four to eight week exposures. 2) The relative loss in tissue mass in the most stressed mussels after eight weeks (12%) was about half that of the starved mussels after eight weeks and suggests they were deriving some nutrition from the external environment. 3) On the basis of relative bioaccumulation, the authors concluded that animals losing only 10% of their tissue mass accumulated chemicals as expected while those losing 20% of their tissue mass did not. This suggests that the original criteria of weight losses of less than 20% are reasonable and that new criteria will probably be somewhere between 10 and 20% loss in tissue mass.

It should be acknowledged that the growth results are not fully explainable nor exactly as expected. For example, it was not clear whether growth would be enhanced near the diffuser due to organic enrichment or reduced due to the presence of toxic chemicals. These interactions remain unclear. There are no other data available for mussel growth at 70 meters and the precise relationships between stress associated with both natural and chemical factors and mussel growth remains to be elucidated. Furthermore, it was unclear which mussel metrics would be most affected by chemicals associated with the diffuser. Previous work has suggested that PAHs affect tissue mass more than shell growth (Salazar and Salazar, 1998) and that when growth rates are low, the tissue weight metric is often the most discriminating, even though it may not be the most accurate due to BOT baseline comparisons (Salazar and Salazar 1997a, EVS 1996). Most importantly of all, however, the caged mussel pilot study demonstrated that the method could be used to detect statistically significant differences among stations and zones even though changes in most mussel metrics were small.

It is encouraging that similar trends were seen both inside and outside the mixing zone for most of the effects endpoints measured, including weight growth rate (mg/wk), length growth rate (mg/wk), EOT tissue weight (g-ww and g-dw), condition index (tissue dry wt/shell wt.) EOT percent lipids, and EOT percent water. For each of these endpoints, higher values were found inside the mixing zone than outside the mixing zone. This is not very surprising considering the potential for organic enrichment from the effluent in the mixing zone (Feder and Shaw, 1996). Changes in shell length was the only metric where estimated growth was higher outside than inside the mixing zone. The only increase that was not statistically significant was at Station 2, nearest the diffuser.

The strength of the procedures used in the caged mussel pilot study lies in the ability to make multiple measurements on the same individuals for some metrics (i.e., shell length,

WAWW) and to employ metrics that include both tissue weights and shell weights. Based on previous studies (Salazar and Salazar, 1998), it was not surprising to find that shell length and WAWW were the most discriminating. Statistically significant increases in shell length were found at all stations except Station 2, which was only 200 m from the diffuser. Statistically significant increases in WAWW were found at all stations. These mussel metrics exceeded the growth criteria and suggested mussels were in good health. However, the tissue weights provide a different perspective on mussel health. Wet tissue weights decreased at most stations, but the difference between BOT and EOT was statistically significant only at Station 6. Dry tissue weights, based on conversions using percent water in tissues, also decreased at most stations with statistically significant differences found at Stations 4 and 6. No statistically significant differences in shell weights were found when comparing BOT and EOT measurements. Surprisingly, a condition index, which combined dry tissue weights and shell weights into a single metric, was the most discriminating measure based on comparisons between end-of-test and the baseline BOT measurements made on 300 individuals. Statistically significant differences in condition index between baseline BOT estimates and EOT measurements were found at Stations 4, 5, 6, and 7. This is the first time that this metric has demonstrated this discriminating power using the methods employed here. It has been acknowledged that shell length and WAWW are generally more discriminating at higher growth rates and estimated changes in tissue weight become more important at low growth rates (Salazar and Salazar, 1998) such as found in the caged mussel pilot study in Port Valdez. Perhaps even more surprising, however, is that there were no statistically significant changes in shell weights but when combined with dry tissue weights the condition index metric was more discriminating. These data suggest that condition index may be more discriminating than previously believed. However, it should be emphasized that all of the statistics for the tissue-related measurements are based on differences between end-of-test measurements on individuals and one BOT baseline average determined from 300 surrogates. Based on the available data and supporting literature, it must be concluded that although the mussels deployed at 70 meters were under some stress, they maintained their tissue weights at a level which provided useful information, and these tissues could be analyzed for chemicals of concern with confidence. More importantly, the growth metrics have been quantified to a level that could permit re-analysis of the growth and bioaccumulation data if different information becomes available in the future.

Percent lipids and percent water were not intended to be primary metrics to estimate mussel health but rather provide additional evidence to either support or refute the mussel growth metrics. Both biochemical endpoints suggest better mussel health inside the mixing zone compared to outside the mixing zone. The higher percentage of water in the tissues of mussels from outside the mixing zone suggests that they were in poorer health than

those inside the mixing zone and support most of the other mussel metrics. As with the tissue chemistry data however, there were only three data points per station, and strong statistical inferences were not expected. The multiple metrics also provided another piece of evidence that mussels at Station 6 experienced a different exposure. This hypothesis was supported by retrieving the mussels for Station 6 at a different location than originally deployed, higher temperatures, almost no accumulation of PAHs, and the highest losses in tissue mass of any other station. If the lipid data are compared to the wet and dry tissue weight data this suggests that mussels losing approximately 10% tissue mass and approximately 16% lipids should probably not be used for chemical analysis in future monitoring.

5.4 Bioaccumulation

The chemistry data showed a gradient of decreasing concentrations of total PAHs in mussel tissues with increasing distance from the diffuser. This gradient is consistent with results from other studies, such as dye dispersion studies, plume modeling, sediment monitoring, and a previous caged mussel study conducted in the same area, which suggest a similar decrease in PAHs with distance from the diffuser (Karinen, 1980). The Karinen study and the caged mussel pilot study, two caged mussel studies conducted near the 70-m depth contour, are the only two studies demonstrating a decreasing gradient in PAHs for animal tissues with distance from the diffuser. Since the highest concentrations of total PAHs in mussels from the pilot study were generally found at the greatest distance from bottom sediment (i.e., the 9-m position), the data suggest that the diffuser was the major source of total PAHs in mussel tissues. It should be recognized that total PAHs only represent part of the summary of PAH exposure, and results of individual PAH compounds are provided in Appendix B. Based on consistently higher proportions of alkylated homologs relative to their respective parent compounds, these data also suggest that the effluent diffuser is the source of these PAHs. Additional confirmation of the diffuser as a source of the PAHs accumulated by mussels is provided by the application of a weathering model (Short and Heintz, 1997) and presented in Appendix B. Based on a preponderance of evidence, and in the absence of conflicting data, it appears that the diffuser is the source of the PAHs accumulated in mussel tissues.

Although the bioaccumulation portion of the study was the objective of Tier 2 and might be considered of secondary importance, it was imperative that this study demonstrate accumulation of PAHs associated with the diffuser to confirm the feasibility of the approach. The placement of mussels at various depths enhanced the possibility of exposure to PAHs associated with the BWTF effluent and/or bottom sediment. For some PAHs it was possible to provide first-order approximations regarding whether the source of

bioaccumulated PAHs was either the effluent itself or bottom sediment.

In the context of the exposure-dose-response triad used as a framework for the caged mussel pilot study, exposure is characterized primarily with the chemical measurements of mussel tissues. These chemical concentrations are referred to as the dose. Caged bivalves have been used to monitor trace metals and organic chemicals in marine, estuarine, and freshwater environments for a number of years (Salazar and Salazar, 1996). The advantages of using caged bivalves over in-place, resident populations have been summarized previously and the approach has been used to monitor PAHs from the intertidal zone to 750 meters (Salazar and Salazar, 1997a; Young et al., 1976, Forlin et al., 1996a,b). Interestingly, the concentrations of total PAHs in mussel tissues are similar in the pilot study described here, the Karinen (1980) study at a similar depth and distance from the BWTF diffuser in Port Valdez, and the Forlin et al. (1996a,b) study at 650 meters in the Norway Trench. The differences in bioaccumulation shown in the pilot study help clarify the fine structure of exposure and suggest that both BWTF effluent and contaminated sediments could be pathways of exposure for total PAHs and some of the individual PAHs. Other individual PAHs seem to be accumulated more from either the effluent or the bottom sediment. It should be acknowledged that direct exposure to effluent by transplanted mussels has not been unequivocally demonstrated. This could be confirmed however, if future monitoring studies were to be more directly tied to the effluent plume. During the time period when this study was conducted, the plume should have been relatively homogeneous from surface waters to the bottom. During other times of the year the plume is more stratified and intertidal mussels used in other monitoring programs are not exposed to the effluent plume.

Mussels are commonly used as a surrogate species for exposure and effects because of their ability to filter large quantities of water and extract food particles. Since mussels also extract food from sediment particles they can be used to evaluate sediment pathways of exposure. Mussels are a functional monitoring tool because both pathways of exposure can be simultaneously evaluated. Naes et al. (1995a) used both laboratory bioassays and measurements of natural mussel populations (*Mytilus edulis*) to examine the bioavailability of PAHs from contaminated sediment. Mussels continuously exposed to the water from the test sediments accumulated PAHs in a clear response to concentrations in sediments and water and to the degree of resuspension. After a 6-month exposure, there was a significant positive correlation between TOC in the sediments and PAH in the mussels across both fjords. Higher PAH concentrations in mussels exposed to sediments from one fjord compared to another suggest differences in PAH bioavailability due to TOC. Results further suggest that it may take up to three months to reach chemical equilibrium. Naes et al. (1995a,b) concluded that of the plants and animals compared in their studies, mussels

were the best indicators of potentially carcinogenic PAHs. A critical question that should be pursued is whether organic enrichment in general, as found in many eutrophic fjords and particularly in the vicinity of the BWTF diffuser, is making micropollutants such as PAH more readily available for uptake by filter-feeders, especially during winter with low availability of seston as food.

Caged bivalves have been used successfully in many different environments to quantify exposure. The level of sophistication in quantifying that exposure is dependent on the temporal and spatial coverage of the caged bivalve deployments. Short and Harris (1996) showed that particulate oil was biologically available 25 m below the surface after the Exxon Valdez spill. Short and Babcock (1996) also monitored pre- and post-spill concentrations of hydrocarbons in both mussels and sediments after the spill. Harris et al. (1996) were also able to show that underlying sediments were a source of oil to intertidal mussels long after the spill. This observation was confirmed by Shigenaka and Henry (1995). Young et al. (1976) showed that the concentration of both DDT and PCBs increased with increasing depth and proximity to contaminated sediment in the southern California Bight adjacent to a major municipal outfall and concluded that contaminated sediments were the major source of these chemicals. Conversely, Salazar and Salazar (1996) concluded that ship hulls were the major source of TBT accumulated in mussel tissues since higher concentrations were found near the surface rather than near the bottom. A similar trend was found in the Port Valdez caged mussel pilot study. In an intertidal study with oysters (Salazar and Salazar, 1997) transplanted oysters were collected at intervals after an experimental oil spill to determine rates of accumulation and depuration and a relationship was found between growth and tissue burdens of total PAHs. In the most recent comparable study, mussels were deployed at three depths across six stations to monitor bioaccumulation and effects of pulp and paper mill effluents (Applied Biomonitoring, 1997). This study showed a significant gradient in both exposure and effects.

These other studies are relevant to and support the concept of expanded caged bivalve monitoring in Port Valdez. They all demonstrate that sediment-sorbed PAHs were biologically available, and that correlations could be established between PAHs in mussel tissues, overlying water, and sediment (both bottom and suspended). These studies also show that differential accumulation of PAHs by mussels is site-specific. In addition, the organic carbon content of sediments seemed to enhance bioaccumulation rather than reduce bioaccumulation as suggested by many previous studies. Finally, PAH tissue burdens may not reach chemical equilibrium until three months of exposure which is consistent with the initial suggestion of a 90-day exposure period.

5.5 Karinen Study

Naphthalene and methyl-substituted naphthalenes made up almost 80% of the PAHs in the BWTF effluent (Karinen, 1980). Other compounds, in descending order of abundance were: dibenzothiophenes, phenanthrenes/anthracenes, pyrenes, fluoranthenes, and chrysenes. These compounds make up approximately 95% of all PAHs present in the effluent at concentrations greater than 1 ng/g-dw. The concentration of total PAHs in the BWTF effluent is relatively low, 0.213 μg/g-dw compared to 0.451 μg/g-dw measured in the particulates and 2.07 μg/g-dw (2,070 μg/kg-dw) measured in the mussels at the site 200 m away from the effluent diffuser and 2 m off the bottom sediment. Surprisingly, the sediments at the Karinen station with the mussels had a total PAH concentration of only 0.082 μg/g-dw. The relative percentages of the suspended particulates and bottom sediment were similar at that site. The concentration of total PAHs found in mussel tissues by Karinen (1980) of 2.07 μg/g-dw is very similar to the total PAH concentration of 4.01 μg/g-dw measured in mussels from Station 2 of this pilot study. In both cases, mussels were about 200 meters from the effluent diffuser. The differences are that Karinen made his measurements in 1980, his mussels were suspended only 2 meters off the bottom, and there was only an 8-day exposure period. Mussels in the pilot study at Station 2 were 5 meters off the bottom and were exposed for 56 days. These data suggest that, although the concentrations of PAHs in the effluent have declined significantly since 1985, the bioavailability of total PAHs may not have decreased concomitantly. These data also suggest that bivalves and sediments accumulate different fractions of the total PAHs. High molecular weight PAHs are accumulated more slowly, but to higher levels (BCFs) than low-molecular-weight PAHS. Therefore, a 56-day exposure would enhance the relative proportions of high molecular weight PAHs relative to low-molecular weight PAHs (BAP>phenanthrene/anthracene>naphthalene).

5.6 Ancillary Measurements

The model used to guide the pilot study emphasized the importance of toxic chemicals and natural factors in determining mussel growth rates in Port Valdez. In other words, it must be acknowledged that measured differences in mussel growth among stations, zones, and depths could be related to natural factors. It is also possible that using the few data points measured for some water quality parameters could lead to spurious correlations and ultimately unwarranted conclusions. Temperatures measured at Stations 6 and 7 were significantly higher than all other stations but this suggests higher growth rates and yet they were not significantly higher than other stations. In fact, most metrics suggested that mussels at Station 6 were more stressed than any other station. This could be accounted for in part, by the apparent movement of Station 6 shoreward into shallower water. Nevertheless, temperature differences among stations does not account for the measured differences in growth metrics.

Many have assumed that growth rates would decrease with increasing depth because of the low temperatures, low food supplies, and pressures associated with depths of 70 m. During the study period between 25 February and 23 April, temperatures at 7-m above bottom sediments ranged from 4.03 to 5.07°C with a mean near 4.5°C. Temperatures were significantly higher outside the mixing zone, particularly at Stations 6 and 7 which were furthest from the BWTF diffuser and closest to the mouth of Port Valdez. These temperature patterns are similar to those described previously (Cooney and Coyle, 1988). Based on temperature alone, higher growth rates would have been expected at these stations. This suggests that other factors contributed to the differences in growth measured in the pilot study. There are significant data available from laboratory and field studies to suggest that growth continues at these low temperatures. In one laboratory study (Loo, 1992), mussels successfully filtered natural seston and effectively absorbed food particles at temperatures between -1 and +4.8°C. The author pointed out that since the freezing point in his experiments was -1.46°C and filtration was only measured as low as -1°C, it is possible that mussels can effectively filter even at lower temperatures. Field studies in several Scandinavian countries have confirmed that growth occurs at low temperatures and that there is a synchrony of growth and reproduction similar to that described by Feder and his colleagues (Feder and Keiser, 1980; Gardner and Thomas, 1987; Loo and Rosenberg, 1983). Even at the very low temperatures found about 350 km north of the Arctic Circle, mussels are capable of growth, reproduction, and survival (Wallace, 1980).

Food, instead of temperature, is probably the growth-limiting factor at 70 meters in Port Valdez. In the Wallace (1980) study, food was the primary limiting factor. Wallace showed

that mussels in the vicinity of fish farms grew continuously throughout the year at twice the rate of animals at locations removed from fish farms.

Based on the preponderance of evidence however, and the most straightforward explanation of the data, it does appear that mussel growth is more affected by chemicals and nutrients associated with the BWTF effluent than physical-chemical factors associated with these depths. Neither temperature, chlorophyll, phaeopigments or total suspended solids across stations show the same relationships. However, it should be acknowledged that these measurements were not made at different depths and other than temperature, the other measurements were not made at a frequency commensurate with possible changes in mussel growth metrics during the exposure period.

5.7 Evaluating the Assessment Framework, Mussel Monitoring Model, and Characterizing Exposure and Effects over Space and Time

In order to properly assess ecological risk, one must understand the various pathways of chemical exposure and associate them with potentially adverse biological effects. The exposure-dose-response (EDR) model formalizes a template for that approach within the space and time model. Traditional approaches using laboratory bioassays and community assemblages have a relatively high degree of uncertainty because the dose has not been adequately characterized. Because of their mobility, uncertain exposure has been the most consistent problem with fish surveys. Caged bivalve monitoring can help to reduce some of that uncertainty through direct, integrated measurements to characterize exposure and effects over a well-defined time period. The natural factors/chemical monitoring model provides a template for integrated monitoring of the BWTF effluent that accounts for the effects associated with chemicals of concern, natural factors, and non-toxic, man made factors. Results demonstrate that using caged bivalves in sampling arrays that cover 3-dimensional space and time are a potentially powerful tool for monitoring the BWTF effluent. The fine structure of chemical exposure was quantified by depth and distance with mussel tissue chemistry and effects were quantified with various mussel growth metrics. This evaluation of water quality would not have been possible with any of the current approaches for monitoring the BWTF effluent. While the depth effects complicated the analyses and may not be necessary for future BWTF effluent monitoring, the fact that the mussels responded by altering growth rates along these gradients of depth and distance demonstrates the power of this approach. The depth deployments may have complicated the design and implementation, but they enabled the inferential distinction between BWTF effluent and bottom sediments. They also strengthened the apparent gradient relationship.

The main drawback of traditional field approaches is that they are all descriptive. The *in-situ* field methodology advocated as part of the EDR triad (Salazar and Salazar, 1998), is a manipulative field test that has more of the characteristics of a laboratory bioassay in terms of an experiment than the descriptive information associated with other *in-situ* studies advocated by Chapman et al. (1992). Descriptive approaches do not guarantee adequate characterizations of exposure or effects because the exposure period associated with bioaccumulation and bioeffects is largely undefined. Chapman (1995) has also emphasized the importance of measuring exposure and dose as a key issue in ecotoxicological approaches. Increasing emphasis is being placed on the use of tissue burdens associated with adverse biological effects and a new meaning to the term dose-response. Recently, Chapman et al. (1997) have suggested that bioaccumulation should be considered as part of the SQT if the beneficial uses are clear and consistent with monitoring goals. Increased emphasis is also being placed on the use of manipulative field bioassays such as those described here (Parrish et al., 1988; Green et al., 1985).

5.8 Possible Applications

If bivalve monitoring is undertaken in the future, the following design characteristics should be included. First, the objectives of the monitoring need to be clearly defined, performance criteria should be established for a successful test, and a framework established for how the data will be used. Second, replication should be maximized around the parameters most important to the study. For example, if depth is an issue, as shown in this study, more replicates should be included to statistically compare the results by depth. If the mixing zone is an issue, then more stations and more replicates should be included in the mixing zone. Third, bioaccumulation and growth should be measured to help characterize exposure and effects in a manner that is consistent with ecological risk assessment and the characterization of various exposure pathways.

Generic applications of this specific method quantifying bioaccumulation and growth have been described previously (Salazar and Salazar, 1991, 1995): 1) short-term and long-term trends; 2) temporal and spatial variability; 3) site-specific differences; 4) source identification; and 5) dose-response. In the Port Valdez pilot study, the bioaccumulation gradient was much stronger than the growth gradient. Therefore, based on the results of the pilot study, it appears that monitoring tissue chemistry may have more direct applications for evaluating the BWTF than monitoring growth. Growth measurements should still be included in any monitoring program however for the reasons previously described (e.g., effects endpoint, calibrating tissue chemistry burdens and meeting performance criteria).

Short-term trends can be estimated by comparing measurements made during one monitoring event with subsequent monitoring events. Long-term trends can be estimated by serial transplants over time and evaluating the time-series data for temporal trends. In the Port Valdez pilot study for example, it was interesting to note that the concentrations of total PAHs and some individual PAHs were very similar to those measured by John Karinen in 1980. Since it is generally believed that total emissions and flow rate from the diffuser have decreased, decreased tissue burdens were expected. This approach gives a quantifiable concentration with which to compare over time. These comparisons can be made over space and time to identify temporal and spatial variability. This information can also be used to identify site-specific differences in bioaccumulation that may be associated with oceanographic and physical-chemical differences at each site. This is not possible using traditional laboratory bioassays. Several relationships were established using the tissue chemistry data and future studies could be used to clarify those relationships. This is another advantage of the caged mussel approach. Since it is essentially an experiment in the field, the approach facilitates hypothesis testing and additional studies can be used to verify or refute hypotheses that are developed.

Even though the pilot study was not designed to reach conclusions about the mixing zone and impacts to the benthos, the results indicate that this method can be used to answer longstanding questions about the BWTF's potential impact on Port Valdez. Furthermore, this approach of using caged mussels as sentinels of potential exposure and effects is consistent with the RCAC objective of developing a monitoring strategy that will permit early detection of environmental impacts associated with BWTF operations. Results of the pilot study will be used to evaluate the possible use of caged bivalves as an environmental monitoring tool in Port Valdez. It should be stressed that mussels are being used as surrogates for other species because they are relatively easy to collect, cage, and measure. Mussels have frequently been used as models to help understand and characterize ecological processes under real-world, site-specific conditions such as those within Port Valdez. Collecting these types of data is not possible with traditional approaches and possible future monitoring will be discussed. Mussels are also being used as a surrogate for both water column and sediment exposure pathways.

A conceptual diagram (Figure 21) that is not to scale, and is not meant to portray the actual position of the plume, is included to help visualize the scope of the ecological processes involved and the scale of the necessary measurements to adequately characterize exposure and effects in Port Valdez. First it seems obvious from the diagram that

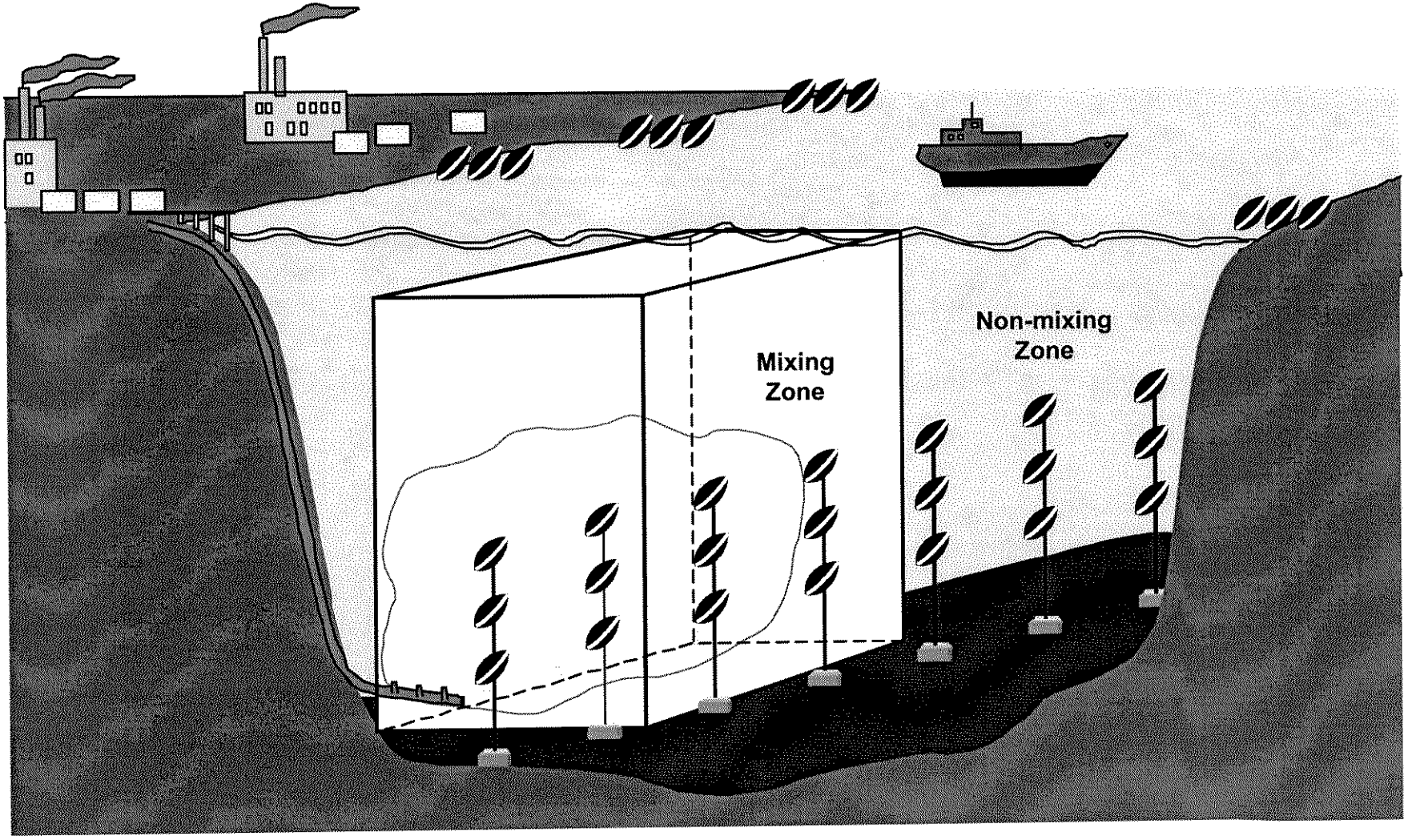


Figure 21. Visual perspective of possible future monitoring (not to scale).

measuring bioaccumulation and growth in natural populations of mussels in the intertidal zone and the distribution of bottom species does not provide a direct measurement of either exposure of effects associated with the effluent plume. Second, it also seems obvious that placing caged mussels along a suspected chemical gradient could serve as a method for mapping bioavailable chemicals and associated effects in the water column. Third, because the placement of caged mussels is under experimental control, it is also easy to see how placement could be altered to facilitate hypothesis testing and identification of potential sources of PAHs in addition to the effluent diffuser. Fourth, it is easy to visualize a string of caged mussels throughout the fjord that serve as sentinels to permit early detection of potential environmental effects.

Possible specific applications include 1) mapping the effluent plume for biologically available PAHs, 2) mapping the appropriateness of the mixing zone, 3) mapping biologically available PAHs outside of the mixing zone and in the vicinity of the benthos, and 4) fjord-wide monitoring. These issues are particularly important due to citizens' concerns that traditional methods may not have appropriately sensitive and due to the state policy of allowing no degradation outside of the mixing zone. More precise characterizations of exposure and effects will help resolve these issues. Furthermore, since the caged bivalve methodology is a manipulative field experiment, it is relatively easy to test different hypotheses based on the available data.

Possible design criteria include 1) use of dispersion models to more accurately place caged mussels within the plume, 2) temperature monitors on all cages, 3) more replication at various depths to evaluate the depth effect, 4) conducting the test during the maximum growth period, 5) conducting the test for 60 to 90 days, if possible, 6) more frequent ancillary measurements, 7) possible use of sediment traps, 8) greater emphasis on engineering design, and 9) development of a more open planning process that includes more participation from the various stakeholders.

Currently there are three different approaches to predicting adverse effects based on tissue burdens (dose response): (1) Quantitative Structure-Activity Relationships (QSARs); (2) Equilibrium Partitioning Theory; and (3) Actual measurements of PAH tissue burdens associated with physiological alterations. McCarty (1991) and McCarty and Mackay (1993) have suggested that 2-4 μmol/g for nonionic organic chemicals with a non-specific narcotic action are associated with acute toxicity and roughly 0.2-0.4 μmol/g are associated with chronic toxicity. Widdows and Donkin (1989, 1992) have synthesized tissue concentrations measured around the world with scope for growth in mussels. This includes areas with significant reductions in scope for growth, control areas and even oil terminals. Neff and Burns (1996) have used tissue burdens of PAHs to predict water column

concentrations after the Exxon Valdez oil spill. All of the preceding approaches could be used to predict the environmental significance of the Port Valdez mussel tissue chemistry but those predictions are beyond the scope of the Draft Final Report.

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