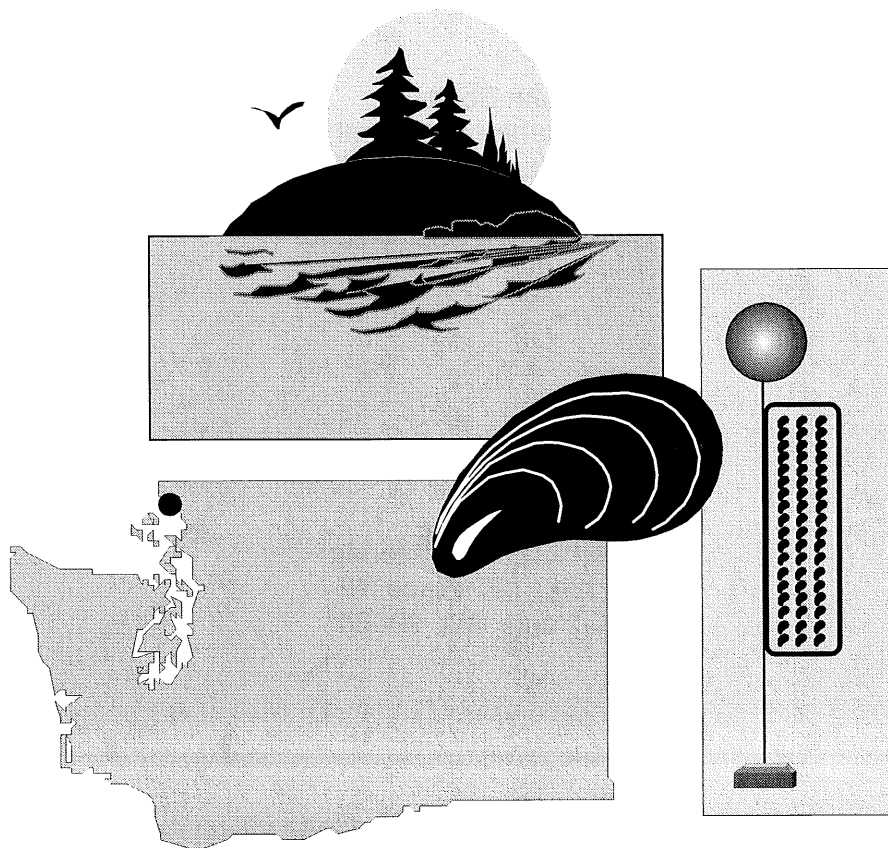


1999 Cherry Point Mussel Study



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Cherry Point: 1999 Caged Mussel Study

DRAFT FINAL REPORT

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LIST OF ACRONYMS

| | |
|--------|---|
| ANOVA | Analysis of variance |
| BOT | beginning-of-test |
| dw | dry weight |
| EOT | end-of-test |
| EqP | equilibrium partitioning theory |
| MLLW | mean lower low water |
| NOAA | National Oceanic and Atmospheric Administration |
| PAH | polynuclear aromatic hydrocarbon |
| PVC | polyvinyl chloride |
| QA/QC | quality assurance/quality control |
| QSAR | quantitative structure-activity relationship |
| SE | standard error |
| TBT | tributyltin |
| US EPA | U.S. Environmental Protection Agency |
| UW | University of Washington |
| WAWW | whole-animal wet-weight |
| WDNR | Washington Department of Natural Resources |
| WDFW | Washington Department of Fish and Wildlife |
| ww | wet weight |

1.0 EXECUTIVE SUMMARY

A caged mussel study was conducted between 14 April and 17 June 1999 along the Cherry Point reach on the coast of Washington state (a similar study was conducted in the spring of 1998). The primary purpose of this study was to use mussels for estimating polycyclic aromatic hydrocarbon (PAH) exposure to caged herring eggs that were deployed in a concurrent study at the same locations. The herring egg study was intended to estimate effects from PAH exposure. Herring eggs were considered the primary receptors of concern for three reasons: 1) herring stocks have declined throughout Puget Sound in recent years, 2) the Cherry Point stock appears to be particularly vulnerable, and 3) previous studies have shown that herring eggs are sensitive to PAHs. Mussels were used as surrogates for herring eggs to estimate the bioavailability of PAHs because of the difficulty in acquiring sufficient tissue masses of herring eggs for chemical analyses, the mussels' ability to integrate and concentrate PAHs in their tissues, and previous experience with resident mussel populations to estimate herring exposure to PAHs. Although mussels were primarily intended to estimate exposure, potential effects were evaluated using mussel growth. The primary difference in experimental design between the 1998 and 1999 studies was that more stations were used in 1999 and these stations were grouped in areas of concern across the Cherry Point Reach. Temperature was measured with in-situ monitors at 15-minute intervals to clarify the potential affects of temperature as a stressor to herring eggs.

The study was designed to answer three questions:

- What is the potential PAH exposure to herring eggs in the Cherry Point reach?
- Are there any effects of PAH exposure on mussel growth?
- Is temperature another potential stressor for herring eggs?

Emphasis was placed on a regional analysis of PAHs and temperature with clusters of approximately seven stations each in the following locations from north to south: Point Whitehorn, Cherry Point, Gulf Road, Intalco Pier, Mid-Pier, and Tosco Pier.

The most important findings of the 1999 study were the following:

- 1) PAHs and temperature were confirmed as potentially significant stressors for herring egg development;
- 2) Mussels accumulated PAHs to concentrations shown to affect herring egg development in previous studies;
- 3) The Cherry Point reach should be evaluated in terms of regions rather than gradients, particularly between the Intalco and Tosco Piers.
- 4) PAH exposure was highest at Gulf Road and lowest at the Tosco Pier;
- 5) Nearly all effects indicators for mussels (i.e., shell growth in length, whole-animal wet-weight growth, increases in tissue weight) were lowest at the Intalco Pier and suggested that mussels there were under more stress than at other sites;

- 6) Significant differences in absolute water temperature and ranges in water temperature were found between 1998 (the El Nino year) and 1999 that could affect the Cherry Point and other herring stocks;
- 7) Based on stressors and effects measurements (i.e., mussel growth) there are significant differences in the microhabitats in the vicinity of the piers at Intalco, Arco, and Tosco that are consistent with the relative volume of discharges at those piers;
- 8) In-situ field studies provided valuable information with respect to monitoring and assessments of stressors to herring in the Cherry Point reach that could not have been achieved with traditional methods; and
- 9) Although credible evidence has been provided by the in-situ herring egg deployments conducted by Kocan and Hershberger to suggest that stock effects are the major causes for the decline of herring stocks in Puget Sound, the data collected from the caged mussel study suggest that site effects may be equally or more important than stock effects.

A total of 2244 mussels (*Mytilus galloprovincialis*) were transplanted from the culture rafts of the Taylor United mussel farm in Shelton, WA to 44 stations along the subtidal zone of the Cherry Point reach in approximately 18 feet of water. Since mussel size has a significant affect on bioaccumulation and growth, the size range was limited to 38 to 46 mm to minimize variability in measurement endpoints. The 44 stations were divided over four monitoring areas referred to as sites: Point Whitehorn, Cherry Point, Gulf Road, and the Intalco-Tosco stretch. One cage holding 51 mussels was deployed at each of the 44 stations. All cages were retrieved after a 61-day exposure period. Statistical analysis of exposure and effects data demonstrated that there were no clear gradients in the Intalco-Tosco reach and that it was more appropriate to consider this area as three separate regions. Therefore, all the data were re-analyzed using six separate regions of approximately seven stations each: Point Whitehorn, Cherry Point (Arco Pier), Gulf Road, Intalco, Mid-Pier, and Tosco.

To estimate initial tissue weights and establish a baseline tissue concentration of PAHs in mussel tissues before deployment, an additional 151 mussels in the identical size range as the mussels deployed at the 44 stations were weighed at the beginning of the test and stored for chemical analysis. At the beginning of the test, there was no statistically significant difference in the size of the mussels (i.e., shell length and whole-animal wet-weight) among stations or monitoring areas, including mussels used for the baseline measurements.

Compartmentalized cages were used to facilitate repeated measurements on the same individual mussels at the beginning and end of the test. Multiple measurements on the same individuals improved the confidence in shell length and weight measurement data and the discriminating power of the test for effects and detecting differences among stations. Growth of individual mussel shells and tissues were used to characterize biological effects associated with exposure to PAHs). Bioaccumulation of PAHs was used to characterize potential chemical exposure along the Cherry Point reach. For each of the 44 stations, mussel tissues from a

single cage were pooled to provide one sample per station for the 61-d exposure period. Only soft tissues from live mussels were used.

Average mussel survival was 57% over the 61-d exposure period. Survival ranged from 18% at Station IT-01 to 96% at Station CP-07. Increases in average shell lengths and whole-animal wet-weights were relatively small but consistent across sites, approximately 7% and 27%, respectively. Average tissue and shell weights increased 78 and 59%, respectively, when compared to the beginning-of-test (BOT) estimates. Even though changes in tissue and shell weights for mussels deployed at all 44 stations were based on comparison to the baseline mussels and there is more uncertainty in these results, these metrics were the most sensitive indicators.

Although the PAH data were evaluated four different ways, emphasis for the regional analysis was placed on PAH comparisons made on a non-lipid-normalized, dry-weight basis using "0" for non-detects. For total PAHs (TPAHs), the concentration was determined by summing the concentrations of the individual compounds; a value of "0" was used for non-detects. Tissues of mussels taken directly from the culture rafts at the mussel farm had a TPAH concentration of about 91 ug/kg-dw. Most mussels accumulated PAHs, based on comparisons of concentration and content, after the 61-day exposure period. Maximum tissue concentration of TPAHs was measured at GR-01 (526 ug/kg-dw); the minimum tissue concentration of PAHs, 0 ug/kg-dw, was measured at GR-06, IT-15 and IT-19.

The study is considered successful because important new information was provided regarding potential exposure to PAHs, the potential for temperature effects was identified, and the utility of using caged bivalves for monitoring PAHs in the Cherry Point reach was documented. Mussels accumulated PAHs to concentrations shown to affect herring egg development in previous studies. There was no obvious effect of these accumulated PAHs on mussel growth at particular sites, but some correlations were established with lower growth rates at the southernmost stations. Temperature was identified as a potentially significantly stressor for herring egg development. This approach of using mussels as sentinels of potential exposure and effects is consistent with the Washington Department of Fish and Wildlife (WDFW) objective of developing a monitoring strategy that will permit early detection of environmental impacts associated with man-made environmental stressors and distinguishing the difference between those and natural stressors like temperature.

2.0 INTRODUCTION

Within the Washington State regulatory agencies, the Washington Department of Natural Resources (WDNR) and the Washington Department of Fish and Wildlife (WDFW) have been given the responsibility of evaluating the status of herring stocks in Puget Sound. Implicit in that responsibility is a need to develop a monitoring strategy to quantify the potential environmental effects associated with potential natural and man-induced stressors such as the oil refineries and associated terminal operations in the Cherry Point reach. Recently, a screening level risk assessment has been conducted to evaluate potential stressors (EVS Environment 1999). Among the possible chemical stressors within the Cherry Point reach, the oil terminals are generally considered as having the most potential for impact on the marine environment. One of the least understood and most controversial aspects of these terminals is the fate and effects of PAHs associated with effluents and terminal operations in the Cherry Point reach. This is due in part to the difficulty in taking representative water and sediment samples, the uncertainty associated with traditional monitoring methods, and difficulty in establishing causality. Water temperature was identified as a potential physical stressors in the screening level risk assessment. The 1998 study with caged mussels documented extreme shifts in temperature range as well as extended periods of extremely high water temperature that could have significant effects on herring spawning and development. The 1998 study provided important new information regarding potential exposure to PAHs and quantified the potential for temperature effects. Mussels accumulated PAHs to concentrations shown to affect herring egg development in previous studies.

Historically, the Cherry Point reach has been a primary spawning ground for Pacific herring. However, the herring (*Clupea palassi*) population at Cherry Point has been steadily decreasing since 1977. In 1976, the spawning deposition at Cherry Point was approximately 12,000 tons, but by 1998 the total spawn deposition was 1,213 tons. These continued declines have become a major concern to both the WDNR and the WDFW, which have proposed to list the Cherry Point herring stock on the state Endangered, Threatened and Sensitive species list.

In the spring of 1998, WDNR and WDFW initiated the first caged mussel pilot study to help determine if polycyclic aromatic hydrocarbon (PAHs) associated with the oil terminals and other industries may be adversely affecting the survival and development of herring eggs. A direct and practical assessment of these potential effects on herring eggs was conducted using survival, growth, development, and genotoxic (teratogenic) evaluations of herring eggs deployed in-situ at 12 stations along the Cherry Point reach (Kocan et al. 1998). A caged mussel study accompanied the caged herring egg study as a means of identifying PAH bioavailability and other possible stressors for the observed effects on herring egg survival and development. Dick Kocan, University of Washington (UW), has used the caged herring egg approach successfully in previous studies in the Cherry Point reach (Kocan et al. 1998) and in

studies associated with the Exxon Valdez oil spill in Alaska (Brown et al. 1996). The second caged mussel study conducted in 1999 had a modified experimental design to answer slightly different questions and refocus the effort on bioaccumulation and exposure endpoints to support the effects endpoints of the in-situ herring egg study.

2.1 Physical Setting

Cherry Point is a small promontory of land located in northern Washington along the Strait of Georgia, approximately 12 miles northwest of the city of Bellingham. The 9-mile region between Birch Bay and Sandy Point (Figure 1) is known as the Cherry Point reach (EVS Environment 1999). The area is moderately developed and it supports three major industrial sites which are potential sources of PAHs: the ARCO oil refinery north of Cherry Point and the Intalco aluminum plant and Tosco oil refinery to the south. Piers are present at each of the facilities for the loading and unloading of cargo vessels. These facilities each have National Pollution Discharge Elimination System permits to discharge effluents into the Strait of Georgia along the Cherry Point reach. Other facilities also permitted to discharge into the area include the Birch Bay Sewage Treatment Plant, the Chemco wood treating facility, and Paraxair, Inc., a carbon dioxide liquefaction facility.

The shoreline along Cherry Point reach is one of the most important herring spawning areas in the state (Whatcom County 1996); it supports a rich and diverse macroalgal community as well as scattered eelgrass beds. Vegetation and substrate types in the Point Whitehorn to Sandy Point nearshore area have been described in a number of studies. According to WDFW, only 5 to 10% of the available spawning habitat along the Cherry Point reach is being used, with 90% of the spawning occurring between Point Whitehorn and Birch Beech State Park in Birch Bay (EVS Environment 1999). Herring spawn in the intertidal and shallow subtidal habitats of Cherry Point depositing their adhesive eggs on eelgrass and marine macroalgae or any smooth, firm substrate.

2.2 Study Objectives

The primary objective of this study was to use PAH bioaccumulation in mussel tissues to estimate PAH exposure to herring eggs deposited along the Cherry Point reach. Exposure conditions were evaluated during May and June, the months during which herring spawn and egg development occurs for the Cherry Point stock. Herring eggs were not used for the exposure assessment because of the limited amount of egg biomass available and the short exposure period fertilized eggs would experience prior to hatching. By ensuring that the mussel study enveloped the in-situ herring egg study (i.e., the mussels were deployed prior to deployment of the herring egg cassettes and retrieved after the cassettes were collected), it was possible to acquire exposure information that was representative of exposure conditions

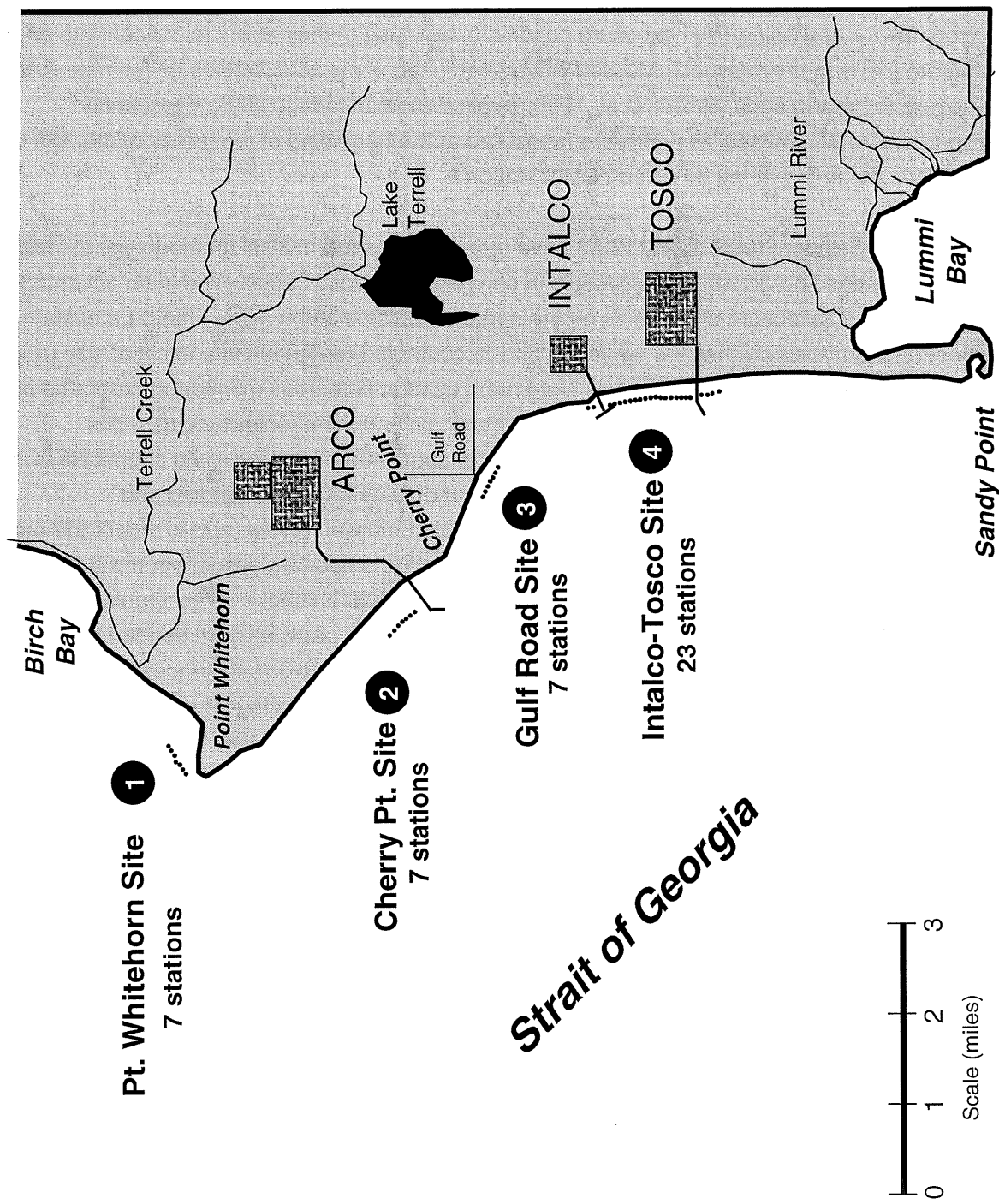


Figure 1. Map of Cherry Point reach showing four mussel deployment sites and 44 station locations.

during the early phases of herring development. Although potential effects on the herring stock from exposure to site-specific conditions were determined by the in-situ herring egg study, potential effects were also evaluated from the caged mussels. Mussels were considered good surrogates for assessing PAH exposure conditions because of their ability to concentrate and integrate PAHs in their tissues. Mussels have been used in previous studies to estimate PAH exposure to herring eggs (Brown et al. 1996; Applied Biomonitoring 1999). To quantify exposure, PAHs in mussel tissues were measured at the beginning of the test and after the 61-day exposure period at the 44 Cherry Point stations.

Salazar and Salazar (1996, 1998, 1999) have refined the caged mussel methodology to include bioaccumulation and growth on individuals in compartmentalized cages. The main advantages of caging are 1) exposure and effects on the same organisms at the same time, 2) measuring exposure and effects over space and time, and 3) controlled replication in a minimal size range. In-situ field studies with caged mussels have been used in numerous monitoring programs as a way to evaluate exposure to bioaccumulative chemicals and the effects caused by this exposure. The ability of bivalves to bioaccumulate, concentrate, and integrate chemicals from their surrounding environment makes them good candidates for understanding and characterizing ecological processes. 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. Mussels are sessile filter feeders and have been shown to accumulate bioavailable chemicals from the water column and suspended particles from bedded sediments. Their ability to accumulate and integrate concentrations of trace toxic substances has made them highly preferred biomonitoring organisms for coastal water quality. They have also been used extensively as model organisms in many scientific studies and a vast literature exists from basic physiological, biochemical, genetic, and toxicological investigations (Gosling, 1992). *Mytilus galloprovincialis* from the Taylor United Mussel Farm in Shelton, WA were used in both the 1998 and 1999 caged mussel studies in the Cherry Point reach.

Effects on mussels were quantified using a preponderance-of-evidence approach that involved several different growth metrics previously demonstrated to be appropriately sensitive indicators of mussel health: whole-animal wet-weight (WAWW), shell length, tissue weight, shell weight, percent lipids, and percent water. 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 (Salazar and Salazar 1998). 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 exposure period to properly interpret the tissue chemistry data. Knowing how tissue masses have changed helps interpret apparent "increases" or "decreases" in tissue concentrations. Comparing end-of-test (EOT) and baseline beginning-of-test (BOT) tissue weights helps calibrate measured tissue concentrations and explain the portion of the change associated with change in tissue mass and the portion associated with the change in chemical mass.

2.3 Report Focus and Organization

This report summarizes the methods used to conduct the study, the results, problems encountered during the study, and recommendations for future work. This report is divided into eight sections. An Executive Summary (Section 1) provides a detailed overview of the in-situ study with caged mussels. Section 2, this Introduction, describes the needs of WDNR and pertinent background information, and identifies project objectives. Section 3 describes the study methods, including the experimental design and specific methods used in conducting the in-situ field study. Results are provided in Section 4. Section 5 is the discussion of test results and compares data among stations and sites for the 1999 study, and compares the 1998 and 1999 results. The Discussion also includes problems encountered during the study and lessons learned that are important for successfully completing future studies. Applications to future work are provided in Section 6. Acknowledgments are given in Section 7; References in Section 8.

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 Study Design

The study was designed to address the following questions:

1. will mussels deployed along the Cherry Point reach accumulate PAHs,
2. will these mussels demonstrate adverse effects due to exposure to natural and chemical conditions along the Cherry Point reach, and
3. is temperature a stressor on mussel bioaccumulation and growth.

The study design allows exposure and effects measured for mussels to help explain effects on herring egg development.

The approach for this mussel study involved collecting mussels from the Taylor United mussel farm, Shelton, WA, sorting mussels into size groups and assigning them to cages, and transplanting the caged mussels to stations in the subtidal zone along the 9-mile stretch of Cherry Point reach (Figure 1). One cage of 51 mussels was deployed at each of 44 stations. These stations were divided among four monitoring areas: Point Whitehorn (7 stations); Cherry Point (7 stations), Gulf Road (7 stations), and the Intalco-Tosco reach (23 stations). To ensure that mussels would always be submerged in approximately 18 feet of water during the entire exposure period, the cages were attached to the deployment line 6 feet above the cement anchor (Figure 2). All of the transplanted mussels were retrieved after a 61-day exposure period. Following retrieval, the mussels were measured for changes in whole-animal weight, shell length, shell weight, and tissue weight. The in-situ mussel study was conducted according to the following schedule:

- 14 April 1999: Mussel collection, initial sort, and transportation to Samish Island for overnight holding.
- 15 April 1999: Measurement and distribution of mussels to mesh bags, attachment of mussel bags to PVC frames, overnight holding
- 16 April 1999: Deployment of all caged mussels at the 44 Cherry Point Stations (Note: herring egg cassettes deployed on 14 May 1999 and retrieved on 19 May 1999)
- 16 June 1999: Retrieval of all cages for assessment of 61-d exposure and effects; cages transported to Samish Island for overnight holding. Additional temperature monitors deployed.
- 17 June 1999: Measurement of all mussels, removal of tissues, tissue samples

frozen for chemical analysis

- 23 June 1999: tissue samples delivered to the US EPA Laboratory, Manchester, WA for chemical analysis
- 16 September 1999: 1st retrieval effort for post-test temperature monitors
- 12 November 1999: 2nd retrieval effort for post-test temperature monitors

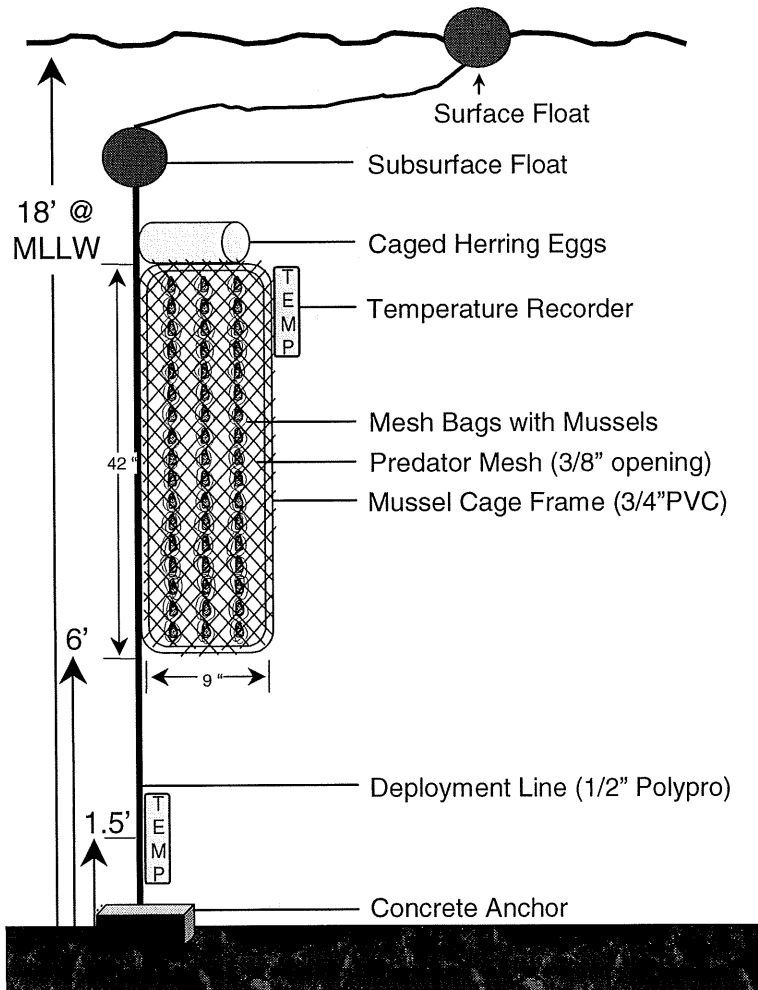
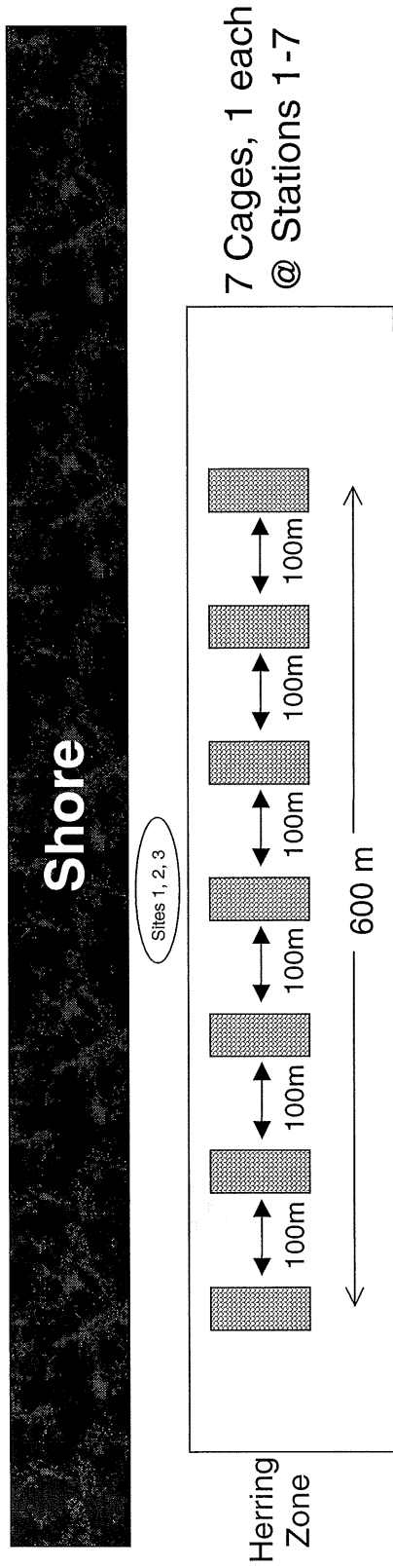


Figure 2. Deployment configuration used for the 1999 Cherry Point Mussel study.

The statistical model (Figure 3) used in the effects portion of the study was an ANOVA. The model shows the overall study design and the level of replication for the effects measurements. The intent was to test for differences among the four sites — Point Whitehorn, Cherry Point, Gulf Road, and Intalco-Tosco, and to determine if there were any trends or gradients within a given site.

Deployment configuration at each of the northernmost sites: Point Whitehorn, Cherry Point and Gulf Road



Deployment configuration for stations in the vicinity of the Intalco and Tosco Piers

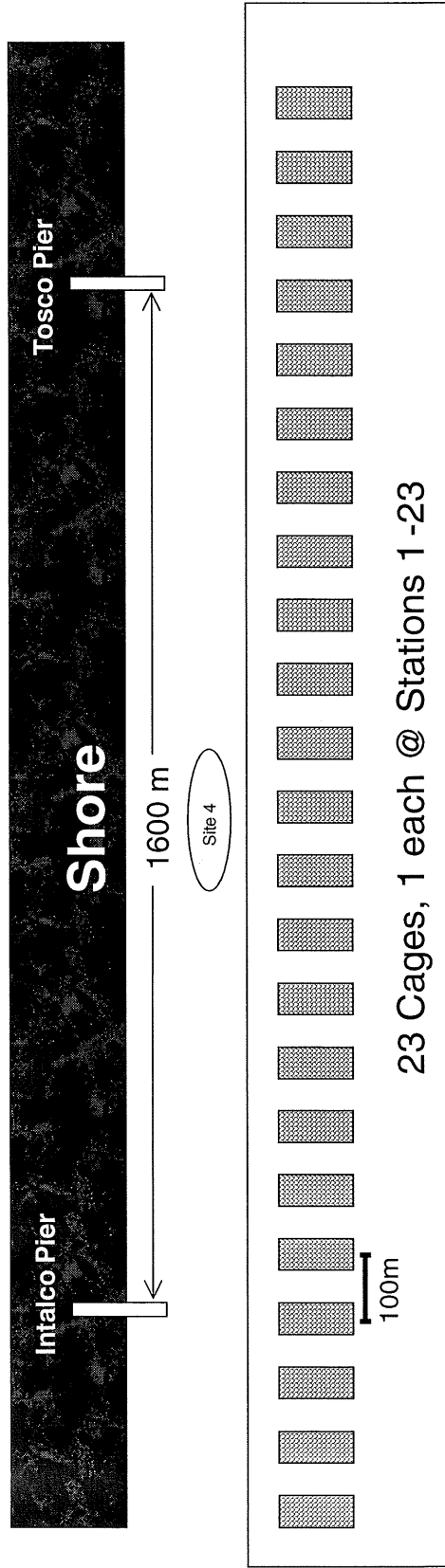


Figure 3. Schematic of experimental design and deployment configurations for mussels deployed at 4 sites (44 stations) along the Cherry Point reach.

For the effects characterization portion of the study, the level of replication was the individual mussels. Power analyses performed on data from other similar studies conducted in Alaska (EVS, 1996, 1997) indicate that between approximately 100 mussels per station are sufficient to detect differences in weight on the order of 0.2 g. By placing a minimum of 7 cages, each containing 51 mussels, at each station, there was sufficient replication to test for such differences.

For the exposure characterization portion of the study, the analytical samples were created by combining the soft tissues of all living mussels from a given cage at a particular station. One chemistry sample was prepared for each station. A total of 7 tissue samples each were prepared for the Point Whitehorn, Cherry Point, and Gulf Road sites; 21 replicate tissue samples were prepared for the Intalco-Tosco stations (caged mussels at two of the IT stations were not found at the end of the test, yielding only 21 of 23 cages collected at that site).

The parameters used in assessing mussel health and mussel responses to exposure conditions were WAWW, shell length, growth rates based on weight and shell 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 (Applied Biomonitoring 1999).

The following null hypotheses were developed for characterizing effects:

- There is no significant difference in whole-animal weight or shell length among stations (1 cage) or among sites (7 cages pooled for the Point Whitehorn, Cherry Point and Gulf Road sites; 23 cages pooled for the Intalco-Tosco site) at the beginning of the test.
- There is no significant mortality in mussels among sites after the 61-d exposure period.
- There is no significant change in mussel metrics among sites after the 61-d exposure period.
- There is no significant difference in end-of-test mussel survival, whole-animal wet weight, shell length, shell weight, growth rate, tissue weight, or condition index among sites.

The following null hypothesis was developed for characterizing exposure:

- There is no significant accumulation of total PAH (TPAH) concentrations in mussel tissue after the 61-d exposure period.
- There is no significant difference in TPAH concentration in mussel tissue among sites.

The following null hypotheses were developed for comparing the potential effects of temperature among stations:

- There is no significant difference in daily average temperature among sites.
- There is no significant difference in daily temperature ranges among sites.

All null hypotheses were tested at the 95 percent confidence level ($\alpha = 0.05$).

3.2 Species Selection and Justification

The blue mussel (*Mytilus galloprovincialis*) was selected as the test species because: 1) of 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. *Mytilus galloprovincialis* was selected over other mussel species because it is cultured in Puget Sound at several commercial culturing facilities, providing a continued source of uncontaminated test animals, and it could be easily used in future monitoring studies. *M. galloprovincialis* grows rapidly and does not experience neoplasia during the summer as does the native mussel, *Mytilus trossulus*.

3.3 Mussel Collection

Mussels (*Mytilus galloprovincialis*) were collected for the study on 14 April 1999 from the Taylor United mussel farm in Shelton, WA. Four lines of cultured mussels were selected from a raft identified by Gordon King of Taylor United as containing mussels that had previously spawned. These mussels were placed in plastic buckets and returned to the shore.

3.4 Mussel Sorting and Distribution

The detailed methods used for mussel sorting and distribution are described in Salazar and Salazar (1999). In summary, shell length (longest axis, generally from the anterior end near the beak to the leading posterior end, as determined with vernier calipers) was used to select mussels for this study. Mussels were first sorted into 1-mm size groups. Initially, all mussels between 35 and 45 mm in shell length were retained; this size group combines attributes of the smallest mussels with the highest growth rates and attributes of the largest mussels with the most tissue for chemical analysis. After this initial sort, the number of mussels per size category was determined. A final size range of 38.0 to 46.0 mm provided enough mussels to satisfy the requirements of the test and the largest number of individuals of the most uniform size.

The initial sorting process required approximately 3.5 hours. During the entire sorting process, all mussels (i.e., unsorted and sorted) were kept in the shade. Shade was provided by a 10 ft x 10 ft screen gazebo erected adjacent to the sorting area. All mussels appeared wet and moist throughout the sorting process.

To minimize the effects of temperature stress on the mussels, approximately half way through the sorting process the sorted mussels were placed into mesh bags, each size group in its own bag. The bagged mussels were then placed in the water in the intertidal zone to maintain temperature and provide flowing seawater for mussel respiration and ventilation. After the initial sort was completed, all mussels were bagged according to size and held in the intertidal zone for another 60 minutes. Following this temperature and food maintenance period, the bagged mussels were placed in an ice chest containing plastic bags filled with wet ice. Paper towels were placed between the bags of ice and the mussels to minimize the possibility of contamination from the ice. The mussels were transported by auto to the work area on Samish Island, WA. Immediately after arrival at the work area, the bags of mussels were attached to polyvinyl chloride (PVC) frames. The frames with the mussels were then wrapped with predator mesh and suspended from a log raft situated in Samish Bay approximately 100 yards off shore. Transportation time from Shelton to the Samish Bay facility was approximately 4.5 hours. The total time mussels were out of the water was approximately 8 hours. The mussels were retrieved the next morning just prior to initiating length and weight measurements. Samish Bay was used as the holding area for both beginning and end-of-test activities.

Final measurement and distribution was accomplished by two teams of three persons each. Prior to distributing mussels to the mesh bags, each individual mussel was remeasured for shell length (to the nearest 0.1 mm with electronic vernier calipers) and whole-animal wet-weight (to the nearest 0.01 g with a portable electronic balance). The procedure for making these measurements and the specific distribution process were done according to Salazar and Salazar (1999) to ensure an even distribution of mussels across stations based on size (Figure 4). Only live animals that were fully closed, or those that closed immediately upon light physical stimulation were used. The measurements and distribution included the 2244 mussels to be deployed at the 44 stations and the 153 mussels used to establish baseline beginning-of-test (BOT) conditions in tissue chemistry, shell weight, and tissue weight. The largest mussels (i.e., the 45-mm increment) were measured and distributed first (Figure 4). When all mussels in this size group were measured and distributed, mussels in the next smaller size group (i.e., 44 mm) were distributed. This process was repeated until all the bags were filled, with the 38 mm size group distributed last.

Due to the potential stress of elevated temperatures during the measurement process, water temperatures in the sorting trays were maintained between 8 and 10°C with ice contained in plastic bags. Aquarium thermometers were used to obtain approximate water temperatures;

more accurate temperature readings were made with *in situ* temperature monitors. Water was changed in the plastic holding tub after distributing all mussels of each particular size increment. No more than 350 mussels were held in the water at one time. The unmeasured mussels were held on ice in an ice chest until needed for distribution.

Following distribution, the 153 mussels identified for baseline BOT measurements were removed from their compartmentalized trays and the soft tissues were removed. Two of these mussels were dead; the shells were stuck together with sediment. Therefore, 151 mussels were processed for baseline measurements. Soft tissue and empty shell wet weights measurements were made on each of these 151 mussels. Three replicate samples for chemical analysis were prepared; two replicates contained soft tissues from 51 mussels, the third contained soft tissues from 49 mussels. 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 17 animals per tube (Figure 2). Nylon cable ties were used to separate mussels and create the individual cells. The mesh netting facilitated water circulation and even 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. Three tubes were prepared for each PVC frame for a total of 51 mussels per cage.

After BOT processing, the mesh tubes containing mussels were fastened to rigid PVC frames (approximately 9 x 42") by knotting the plastic mesh and securing the knots with nylon cable ties. The PVC frames, or mussel cages, were wrapped with heavy-duty plastic mesh (approximately 2.5 cm mesh size) to discourage predators. A temperature monitor was attached to the top of selected PVC frames before wrapping with predator mesh (See Section 3.8 for additional details regarding placement of temperature monitors). The completed cages (i.e., PVC frame, bagged mussels, and temperature monitor all wrapped with predator mesh) were then transported by small boat and attached to the log raft in Samish Bay for overnight holding. Mussels were completely submerged during the holding period.

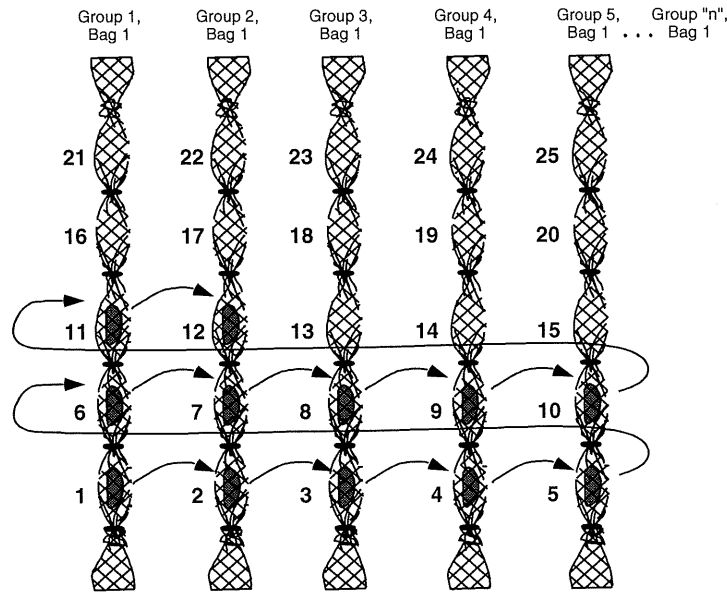


Figure 4. Distribution process for caging mussels

3.5 Deployment and Location of Sites and Stations

Sites were selected during consultation with WDNR, WDFW, and industry representatives. Primary areas of concern with respect to herring spawning are the industrial areas (i.e., piers) and points of discharge for upland runoff. The exposure period in the 1998 study was too short to clearly identify areas where PAH concentrations were most elevated. To better define chemical exposure along the Cherry Point Reach, the 1999 study focused on the following areas:

- Point Whitehorn: it served as a possible control site and other potential sources
- Cherry Point: the location of the ARCO Pier
- Gulf Road: a discharge point for upland runoff
- The Intalco-Tosco Reach: the area immediately north of the Intalco Pier to immediately south of the Tosco Pier. The piers are separated by approximately 1 mile and the area is considered one monitoring unit.

These sites were selected to determine exposure and effects in the vicinity of the primary industrial effluents along the Cherry Point Reach. Representatives of the Washington State Department of Ecology had expressed special interest in the Gulf Road site because of suspected anthropogenic chemical stressors in the area. Within each of these sites, stations were established along a transect parallel to the shore. At Point Whitehorn, Cherry Point, and Gulf Road, seven stations were established. Twenty-three stations were established along the

Intalco-Tosco Reach. Station coordinates (Table 1) were determined by WDNR staff. All reported depths were referenced to Mean Lower Low Water (MLLW).

Table 1. Station coordinates for the caged mussel study

| <u>Site/Station Number</u> | <u>Latitude</u> | <u>Longitude</u> |
|----------------------------|-----------------|------------------|
| POINT WHITEHORN | | |
| 1 | N48° 54.028 | W122° 47.114 |
| 2 | N48° 53.993 | W122° 47.168 |
| 3 | N48° 53.954 | W122° 47.236 |
| 4 | N48° 53.946 | W122° 47.335 |
| 5 | N48° 53.906 | W122° 47.375 |
| 6 | N48° 53.870 | W122° 47.441 |
| 7 | N48° 53.839 | W122° 47.510 |
| CHERRY POINT | | |
| 1 | N48° 52.178 | W122° 45.769 |
| 2 | N48° 52.149 | W122° 45.715 |
| 3 | N48° 52.102 | W122° 45.668 |
| 4 | N48° 52.065 | W122° 45.612 |
| 5 | N48° 52.019 | W122° 45.557 |
| 6 | N48° 51.979 | W122° 45.503 |
| 7 | N48° 51.934 | W122° 45.476 |
| GULF ROAD | | |
| 1 | N48° 51.387 | W122° 44.289 |
| 2 | N48° 51.365 | W122° 44.202 |
| 3 | N48° 51.355 | W122° 44.131 |
| 4 | N48° 51.334 | W122° 44.053 |
| 5 | N48° 51.323 | W122° 43.981 |
| 6 | N48° 51.306 | W122° 43.916 |
| 7 | N48° 51.277 | W122° 43.841 |
| Intalco-Tosco | | |
| 1 | N48° 50.629 | W122° 43.058 |
| 2 | N48° 50.564 | W122° 43.045 |
| 3 | N48° 50.368 | W122° 42.995 |
| 4 | N48° 50.277 | W122° 42.996 |
| 5 | N48° 50.261 | W122° 42.993 |
| 6 | N48° 50.213 | W122° 42.977 |
| 7 | N48° 50.178 | W122° 42.938 |
| 8 | N48° 50.119 | W122° 42.944 |
| 9 | N48° 50.071 | W122° 42.930 |
| 10 | N48° 50.022 | W122° 42.935 |
| 11 | N48° 49.999 | W122° 42.939 |
| 12 | N48° 49.947 | W122° 42.942 |
| 13 | N48° 49.900 | W122° 42.934 |
| 14 | N48° 49.858 | W122° 42.919 |
| 15 | N48° 49.793 | W122° 42.918 |
| 16 | N48° 49.756 | W122° 42.911 |
| 17 | N48° 49.715 | W122° 42.896 |
| 18 | N48° 49.656 | W122° 42.872 |
| 19 | N48° 49.625 | W122° 42.862 |
| 20 | N48° 49.537 | W122° 42.875 |
| 21 | N48° 49.460 | W122° 42.875 |
| 22 | N48° 49.402 | W122° 42.849 |
| 23 | N48° 49.321 | W122° 42.820 |

The caged mussels were deployed at these 44 stations on 16 April 1999. A random number table was used to assign cages to stations. For each station, one cage of 51 mussels was attached to the deployment line with large nylon cable ties. The deployment array was completed by adding an anchor and a subsurface buoy. This subsurface float maintained vertical position of the caged mussels in the water column after deployment (Figure 2). Concrete anchors were used to maintain station position. A small surface buoy was used to identify each station. Mussels were deployed at the 44 stations (Figure 1) between 9:00 am and 12:30 pm on 16 April 1999.

3.6 Mussel Retrieval and EOT Mussel Measurements

3.6.1 Retrieval

The mussel cages were retrieved by boat on 16 June 1999. Of the 44 cages originally deployed, a total of 42 mussel cages were retrieved. Cages deployed at Stations IT-22 and IT-23 could not be found during the collection effort; it was assumed that these cages were either stolen during the 61-day period or moved by strong currents and/or tides. All cages were heavily fouled by barnacles. The barnacles primarily settled on the exterior surfaces of the mussels, often completely covering each valve of the mussel shell. None of the fouling organisms were removed from the cages during the retrieval operation. After retrieving the 42 mussel cages, the cages were transported to the marina, placed in automobiles, and transported to the Samish Bay work area.

During the retrieval effort, the mussels were exposed to air for approximately 7 hours. Upon arrival at the Samish Island facility, the caged mussels were hand carried over the tidal flats to the log raft in Samish Bay. The cages were tethered to this raft and immersed in seawater. They were held in Samish Bay overnight for elimination of sediment-associated PAHs from the gut.

3.6.2 EOT Mussel Measurements

On 17 June 1999, the mussels were collected from Samish Bay, removed from the mesh bags and placed in compartmentalized trays, and the number of dead and missing animals was recorded for each station. A considerable effort was required to remove the mussels from the mesh bags due to the substantial amount of barnacle growth surrounding each mussel. In many cases, the barnacles were so dense that the space within the "compartment" was completely utilized. Mussels in the queue for processing were held in an ice chest to reduce the potential for heat stress.

All live mussels were then processed according to Salazar and Salazar (1999) for WAWW, shell length, tissue weight, shell weight. After making the shell length and weight measurements on surviving mussels, the tissues were carefully removed according to Salazar and Salazar (1999) so that the internal tissues did not come in contact with the external shell. 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 according to the following process before processing a new cage of mussels: wash with Liquinox[®], rinse with hot tap water, rinse with deionized/distilled water. Thin-bladed stainless steel knives were used to penetrate the gap on the hinged side of the shell and cut the adductor muscle. Once the adductor muscles were severed, the valves were separated and the soft tissues removed. Gloves were not worn during the shucking process to reduce the potential for injury as handling and shucking wet mussels causes the latex 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.

After all the tissues from surviving mussels at a particular station were weighed, the tissues were transferred from the weigh pan to a certified clean sample jar. 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 mussels from the next station. These procedures were followed to avoid cross contamination among stations.

3.7 Mussel Tissue Chemistry

All frozen mussel tissue samples were transferred from Mike Salazar of Applied Biomonitoring to Stuart Magoon, US EPA Manchester Laboratory, on 23 June 1999, using the appropriate chain-of-custody forms. The Manchester Laboratory was selected by WDNR to conduct the chemical analyses. At the Manchester Laboratory, mussel tissues were homogenized and analyzed for PAH compounds (including homologs), selected metals, percent lipids, and percent water. All PAH analyses were conducted according to procedures developed by the National Oceanographic and Atmospheric Administration (NOAA) Auke Bay Lab in Alaska, as provided by Jeff Short. Metals analyses were performed using the following procedures: arsenic - SW7060; mercury - EPA245.5; lead, cadmium, copper and zinc - ICPMS EPA 200.8;

selenium - SW7740. PAH analyses were conducted between 10 August and 6 September 1999. Metals analyses were conducted between 13 October 13 and 20 December 1999. The PAH results were supplied to Applied Biomonitoring by 1 September 1999. The metals results were supplied to Applied Biomonitoring by 26 January 2000.

All tissue chemistry data were reported in terms of wet weight. Dry weight conversions were made according to the following formula:

$$\text{Chemical}_{\text{dry}} = (\text{chemical}_{\text{wet}}) / (\text{percent solids as decimal equivalent; e.g. 20\% as 0.20})$$

To determine whether growth dilution occurred during the study, the content of both PAHs and metals was determined according to the following formulas:

$$\text{Tissue}_{\text{dry}} = (\text{tissue}_{\text{wet}}) * (\text{percent solids as decimal equivalent; e.g. 20\% as 0.20})$$

$$\text{Content (amount/Animal)} = (\text{Tissue}_{\text{dry}} * \text{Concentration}_{\text{dry}}) / 1000$$

Lipid-normalized PAH concentrations were determined according to the following formula:

$$\text{ug PAH/g lipid} = (\text{PAH ug/kd-dw}) / (\text{percent lipid as decimal equivalent; e.g. 5\% as 0.05})$$

The concentrations of the individual PAH compounds presented in this report are in units of ug/kg-dry weight. TPAH concentrations were calculated four ways:

- ug/kg-dry weight; using ½ the detection limit for non-detects
- ug/kg-dry weight; using "0" for non-detects
- ug PAH/g lipid-dry weight; using ½ the detection limit for non-detects
- ug PAH/g lipid-dry weight; using "0" for non-detects

A large percentage of the PAH compounds analyzed for were reported as undetected. By using a value of ½ the detection limit for these compounds in the totaling process, the TPAH concentrations became biased on the high side. The lipid-normalized TPAH concentrations were also biased because of changes in the relationship between total lipids and TPAHs. The most relevant comparisons are made using "0" for non-detects.

3.8 Temperature Measurements

One set of temperature monitors (HoboTemp®, Onset Instruments) was attached to selected deployment arrays at the beginning of the caged mussel study. A second set was attached at the end of the test when the mussels were retrieved on 19 June 1999. For all phases of the study, each monitor was set to record temperatures at 15 minute intervals over the duration of the test.

At the beginning of the test, a total of 32 temperature monitors were attached to 17 of the 44 deployment arrays (Table 2): 17 monitors were attached to the top of the PVC frame to collect near surface water temperature data and 15 monitors were attached to the deployment lines

approximately 1.5' above the concrete anchor to collect bottom water temperature data (Figure 2). Two stations (i.e., IT-03 and IT-23) only had temperature monitors at the surface position. The surface water temperature monitors were attached to the mussel cages before attaching the protective predator mesh and before overnight holding at Samish Island (See Section 3.4). The bottom water temperature monitors were attached to each mooring line as the cages and anchors were being deployed. The two temperature monitors were attached to the cages identified for deployment at Stations 1, 4, and 7 for each of the three sites north of Intalco. Temperature monitors were also deployed at the following locations for stations between the Intalco and Tosco piers: IT-03 and IT-23 surface; IT-04, IT-07, IT-10, IT-13, IT-16, and IT-19 surface and bottom.

After the mussel test was already started, the decision was made to collect post-caged-mussel-study water temperature data during the summer when temperatures were highest. This was accomplished by (1) downloading temperature data from selected monitors during the retrieval process and returning these monitors to the field, and (2) adding additional monitors at stations where none was in use during the mussel deployment (Table 2). A total of 45 monitors were deployed for post-test temperature recording.

The monitors deployed at the beginning of the test were checked during mussel retrieval on 16 June 1999. Of the original 32 monitors deployed in April, one monitor was lost (Station IT-23 Surface), one monitor was damaged (IT-19 Bottom) and returned to shore, and a third monitor was returned to shore for downloading (IT-13 Surface). While in the field, a data shuttle was used to download temperature data for the period from 16 April to 16 June 1999 only from 10 monitors at the Intalco-Tosco site:

- IT-03 Surface,
- IT-04 Surface and Bottom,
- IT-07 Surface and Bottom,
- IT-10 Surface and Bottom,
- IT-13 Bottom,
- IT-16 Bottom, and
- IT-19 Surface.

These 10 monitors were reattached to their original deployment lines to collect water temperature data during the summer. The remaining 19 monitors were left in place because they could not be easily downloaded in the field. The original monitor deployed at IT-16 surface was moved to IT-03 bottom prior to downloading any collected data. Additional temperature monitors were placed at stations IT-01, IT-02, IT-17, IT-18, IT-20, and IT-21 surface and bottom; IT-13 and IT-16 surface; and IT-19 bottom. A total of 45 monitors were deployed for post-test water temperature recording. This includes the single monitor at IT-23 which could not be located during mussel retrieval; we hoped to find the cage at the end of the summer.

Two end-of-summer retrieval efforts were made by Department of Natural Resources staff for the 45 temperature monitors. During the first effort on 16 September 1999, a total of 12 temperature monitors were retrieved. During the second effort on 12 November 1999, a total of 9 temperature monitors were retrieved. The data were downloaded from these logging devices using the instruments' data recovery software. Table 2 summarizes the distribution of temperature monitors and retrieval dates.

3.9 Data Analysis and QA Measurements

The original study design was to assess PAH exposure over four general sites: Point Whitehorn, Cherry Point, Gulf Road, and the Intalco-Tosco reach. The individual stations within each of these sites allow for statistical comparisons to be made on a site-by-site basis. To facilitate comparison with the 1998 data, the Intalco-Tosco reach will be subdivided into three regions: Intalco (Stations 1-8), Mid-pier (Stations 9-14), and Tosco (Stations 15-21). These three regions have corresponding monitoring stations in the 1998 study. All data will first be presented in terms of the original four sites identified at the start of this study. An Analysis by Region section is provided at the end of the Results Section that discusses the PAH exposure and associated effects on a finer scale, where the Intalco-Tosco site is divided into three regions.

3.9.1 Analytical Procedures: Survival and Mussel Growth Metrics

A convention was applied to the analysis of the 1998 Cherry Point data of not analyzing the tissues of mussels where survival was <50%. This convention was based on acceptance criteria established for the Port Valdez study (Applied Biomonitoring 1999). The same convention was not applied to analyzing the survival data in this study because, whereas the low survivals appeared sporadic in the 1998 study, low survival was consistent throughout the southern portion of the test area. All data were included in the analysis of survival, effects, and exposure data.

Effects from exposure to PAHs and temperature were assessed by evaluating survival, comparing 61-d mussel metrics to BOT measurements, and comparing both survival and changes in mussel metrics across monitoring areas. In addition, separate analyses were conducted on the stations within the Intalco-Tosco stretch to identify trends in this specific sampling area.

Table 2. Temperature monitor distribution and retrieval schedule

| | 4/16/99 | | 6/16/99 | | 6/16/99 | | | | Final Retrieval Date | |
|--|---------|---|-----------------|-----------------|---------------------------|----|----------------------|---|--------------------------------------|--|
| | Deploy | | Down Load Data | | Left in Place/ Redeployed | | New Monitor Deployed | | Surface | Bottom |
| | S | B | S | B | S | B | S | B | | |
| PW-01 | ● | ● | | | L | L | | | NR | NR |
| PW-04 | ● | ● | | | L | L | | | NR | NR |
| PW-07 | ● | ● | | | L | L | | | NR | 10/12/99 (memory chip ran out 9/28/99) |
| CP-01 | ● | ● | | | L | L | | | 9/16/99 | 9/16/99 (memory chip ran out 7/5/99) |
| CP-04 | ● | ● | | | L | L | | | 9/16/99 | 9/16/99 |
| CP-07 | ● | ● | | | L | L | | | 9/16/99 (memory chip ran out 7/5/99) | 9/16/99 (memory chip ran out 7/5/99) |
| GR-01 | ● | ● | | | L | L | | | NR | NR |
| GR-04 | ● | ● | | | L | L | | | 9/16/99 | 9/16/99 |
| GR-07 | ● | ● | | | L | L | | | NR | NR |
| IT-01 | | | | | | | ● | ● | 9/16/99 | 9/16/99 |
| IT-02 | | | | | | | ● | ● | 10/12/99 | 10/12/99 |
| IT-03 | ● | | DL | | RD | | | M | 10/12/99 | 10/12/99 (memory chip ran out 7/5/99) |
| IT-04 | ● | ● | DL | DL | RD | RD | | | NR | NR |
| IT-07 | ● | ● | DL | DL | RD | RD | | | NR | NR |
| IT-10 | ● | ● | DL | DL | RD | RD | | | NR | NR |
| IT-13 | ● | ● | DL ^A | DL | | RD | ● | | NR | NR |
| IT-16 | ● | ● | | DL | (M) | RD | ● | | NR | NR |
| IT-17 | | | | | | | ● | ● | NR | NR |
| IT-18 | | | | | | | ● | ● | 10/12/99 | 10/12/99 |
| IT-19 | ● | ● | DL | DL ^B | RD | | | ● | 10/12/99 | 10/12/99 |
| IT-20 | | | | | | | ● ^C | ● | 9/16/99 ^C | 9/16/99 |
| IT-21 | | | | | | | ● | ● | NR | NR |
| IT-23 | ● | | | | Lost | | | | NR | NR |
| Total number deployed | 32 | | | | | | | | | |
| Total number downloaded in field | | | 10 | | | | | | | |
| Total number returned to shore for downloading | | | 2 | | | | | | | |
| Total number out for post-test | | | | | 45 | | | | | |
| Total number monitors found at end of summer | | | | | | | | | 21 | |

A = old style probe; returned to shore for downloading

B = damaged in field; returned to manufacturer for downloading

C = prob set improperly; no data collected

M = monitor originally deployed at IT-16 surface was moved to IT-03 bottom on 6/16/99, prior to downloading any data

L = Left in Place; DL = Downloaded; RD = Redeployed; NR = Not Retrieved

The Simes method (Piegorisch and Bailer 1997) was used to evaluate differences in survival among stations because it allows greater flexibility in multiple comparisons among binomial populations than the traditional contingency table approach. Survival was calculated as initial number deployed minus number dead. For this 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.

Six metrics were used to assess growth and thereby animal health: shell length, WAWW, wet tissue weight, shell weight, shell length growth rate, and WAWW growth rate. 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, and were calculated as:

$$\text{Growth Rate (mg/wk)} = (\text{EOT Measurement} - \text{BOT measurement})/\text{time},$$

where time = 61 days (8.7 weeks).

Because of the even 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 EOT tissue weights and shell weights were compared to the BOT estimate and evaluated for statistical differences among stations as were the EOT shell lengths and WAWWs. Any differences between EOT and BOT were assumed to have occurred during the test period.

Descriptive summary statistics (e.g., mean and standard error, standard deviation) were calculated for all growth metrics. These statistics were used to prepare graphs showing the overall mean, plus or minus two standard errors ($\pm 2SE$) by station for each parameter measured. Two standard errors are presented because they approximate the 95th 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 3.1:

Hypothesis (H_0 : There is no significant...):

- Difference in whole-animal weight or shell length among cages or among sites (7 cages pooled) at the beginning of the test.

Statistical Process ($\alpha = 0.05$):

- ANOVA

- Change in mussel metrics after the 61-d exposure period (compare BOT to EOT) at any site
- For WAWW and shell length, t-test. For tissue weight and shell weight, ANOVA and Dunnett's multiple comparison test
- Difference in mussel survival among sites after 61 days
- Simes Binomial Multiple Comparison
- Difference in mussel whole-animal wet weight, shell length, shell weight, growth rate, tissue weight, %lipids or %water among sites
- One-way ANOVA, Tukey-Kramer multiple comparison test

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 using the Kolmogorov Smirnov test and common variances using Bartlett's test. The parametric one-way ANOVA was run on all data sets to test for differences among stations. The Tukey-Kramer multiple comparison test was used to identify where those differences occurred. Non-parametric tests were used if the data failed to meet the normality or homogenetic requirements.

An ANOVA was used to compare EOT measurements to BOT for tissue weight and shell weight, followed by Dunnett's multiple comparison test. Dunnett's compares all data sets to a single value (i.e., the BOT baseline). To compare EOT measurements to BOT for WAWW and shell length, individual t-tests were run comparing the individual measurements made at the end-of-test to those made at the beginning. It was not possible to use this approach for tissue and shell weights because the baseline measurements were not made on the same individuals.

All statistical analyses were run at the 95 percent confidence level ($\alpha = 0.05$).

3.9.2 Bioaccumulation Data

The TPAH and metals bioaccumulation data were evaluated several ways to allow the identification of trends and groupings. A one-way ANOVA, followed by the Tukey-Kramer multiple comparison test, were used to determine if differences exist among the exposure periods:

Hypothesis (H₀: There is no significant...):

- difference in mussel tissue PAH or metals concentrations among sites after the 61-day exposure period
- difference in EOT mussel tissue PAH or metals concentrations compared to BOT at any site

Statistical Process ($\alpha = 0.05$):

- ANOVA; Tukey-Kramer multiple range test
- ANOVA; Dunnett's multiple comparison test

3.9.3 Water Temperature

Minimum, maximum, and mean temperatures were calculated for each station. Temperatures were analyzed in terms of during mussel deployment (i.e., days 0-61), during herring deployment (i.e., days 28-33 of mussel deployment), and post mussel deployment (i.e., days 62-retrieval date). Temperature profiles based on all the temperature data collected during the field deployment were generated for each station and used to identify overall temperature trends. To reduce variability and autocorrelation in the temperature data for statistical analyses, the temperature series for each station was reduced to average daily temperatures. The following 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.

Hypothesis (H₀: There is no significant...):

- difference in average daily temperatures among stations
- difference in daily temperature ranges among sampling areas

Statistical Process ($\alpha = 0.05$):

- ANOVA, Tukey-Kramer multiple comparison test
- ANOVA, Tukey-Kramer multiple comparison test

Testing for Differences in Average Daily Temperature

Average daily temperatures were calculated for each station. These data were normally distributed and had common variances. Differences in daily average temperatures among stations were determined using a one-way ANOVA ($\alpha = 0.05$). A Tukey-Kramer multiple comparison test was used to identify which stations were significantly different.

Testing for Differences in Temperature Range

The minimum and maximum daily temperatures were first determined for each station, then the

minimum was subtracted from the maximum temperature at each station to determine the range in daily temperature. The daily temperature range data were normally distributed and the variances were approximately equal across stations. The daily temperature ranges at each station were statistically analyzed using a one-way ANOVA.

3.9.4 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) twice during the measurement process. 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. In previous in-situ field studies with caged bivalves, precision was assessed by performing multiple measurements of shell length and WAWW on the same individuals. Errors in shell length measurements are most likely due to misplacement of the calipers along the longest axis of the mussel shell, or pressing too hard on the calipers causing the mussel shell to be squished. Errors in weight measurements are most likely due to the loss of water from the outside of the mussel shell or from the inside between the two valves, or to improper taring of the balance prior to making the weight measurements. Results of the remeasurement process indicated that field staff were consistent in the measurement technique and that any error associated with those measurements would not significantly compromise the quality of the data.

4.0 RESULTS

The caged mussel study was completed as proposed and considered successful because it accomplished its major objectives, most cages were retrieved, PAH exposures from the previous year were corroborated, and new insight was gained into temperature as a significant stressor to herring eggs in the Cherry Point reach. After the 61-d deployment period, 42 of the 44 mussel cages were retrieved. Survival was lower than expected due to substantial fouling by barnacles. Survival and the concentration of TPAHs in mussel tissues were generally lower at stations in the vicinity of the Intalco-Tosco piers. Growth rates, based on changes in whole animal wet-weights and shell length, were also lowest for mussels deployed within the Intalco-Tosco area. Differences in survival in caged bivalves were not a sensitive indicator of environmental stress.

The study accomplished its three major objectives: 1) Potential chemical exposure to herring eggs was quantified through mussel bioaccumulation; 2) Potential associated effects were quantified through mussel growth metrics; and 3) Potential stress associated with temperature was quantified. The specific questions identified by WDNR regarding applicability of this monitoring approach were also answered:

- *Would the mussels survive and provide sufficient tissue for chemical analysis?* Yes, overall survival was 57%
- *Would the mussels grow?* Yes, whole-animal wet-weights and shell lengths increased by 27 and 7%, respectively, at every station. Based on comparisons with baseline mussel tissue weights, increases were estimated at approximately 78%.
- *Would the mussels accumulate chemicals?* Yes, tissue chemistry data after the 61-d exposure period show that, for nearly all stations, the TPAHs (ug/kg-dw) in mussel tissues were higher than concentrations measured in mussels collected from the Taylor United Mussel Farm.

4.1 Data Quality Review

Survival was lowest at Stations IT-01 (17.6%) and IT-09 (19.6%). Both of these stations were located at the north end of the IT site. Tissues from the surviving mussels at these two stations were chemically analyzed, although there is greater uncertainty in these results to the limited amount of tissue mass available for the analyses. Due to low survival and the uncertainty of the resulting tissue chemistry data on the small sample size, Stations IT-01 and IT-09 were excluded from the statistical analysis of the tissue chemistry results.

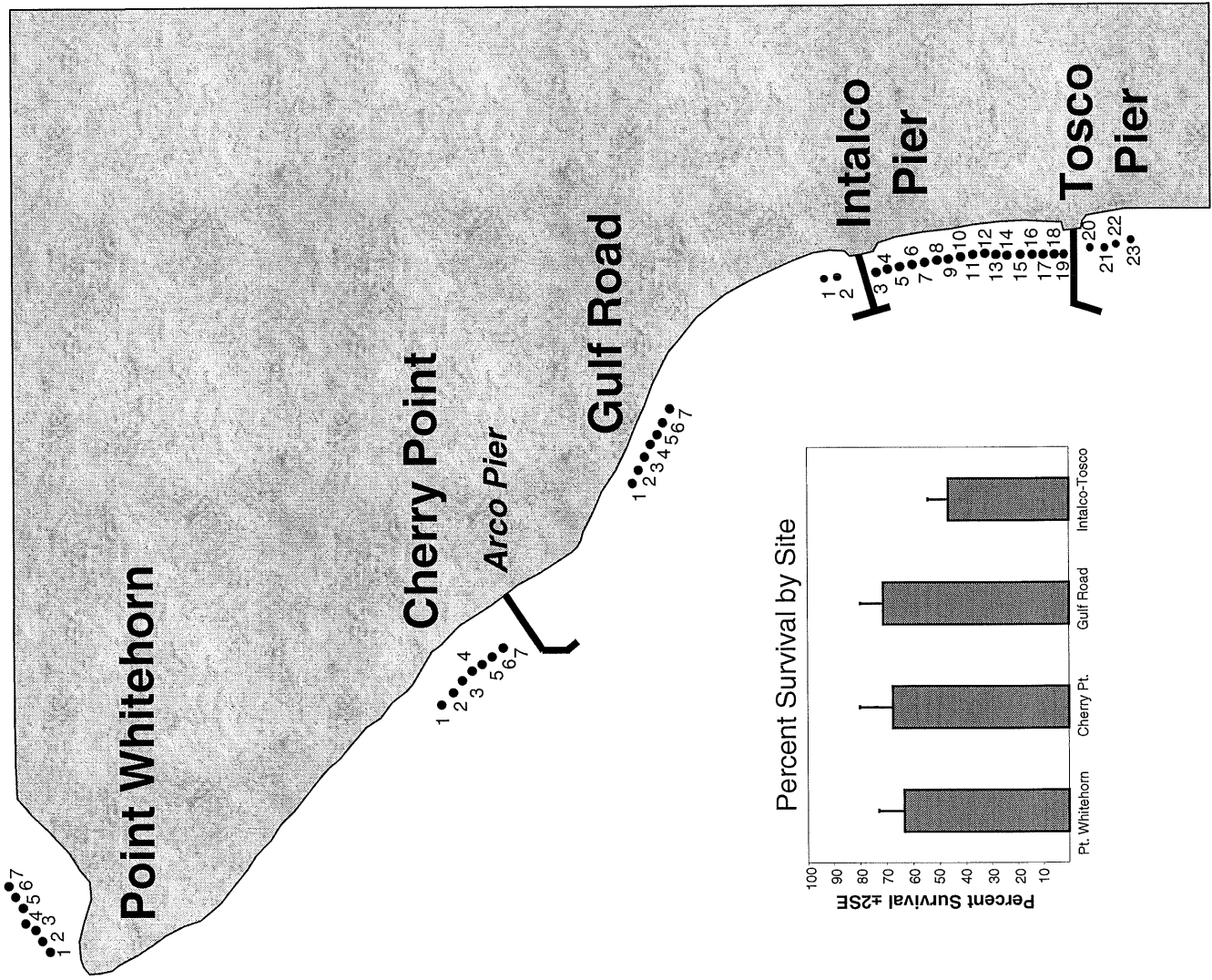
Although survival was considered good at Stations IT-08, IT-13 and IT-16, ranging from 45% to 71%, the Manchester Laboratory reported 0% lipids for mussels deployed at these stations. These data were verified by calling the lab, but the reasons for undetectable lipids is unclear. To facilitate calculating lipid-normalized total PAH (TPAH) concentrations for mussels at these stations, an estimated value of 0.93%-wet was used. This value represents the average of all other samples.

A "0" was used for PAH compounds reported as undetected to calculate total PAHs.

In addition to the quantifiable mussel growth metrics, the appearance of the shell and tissues are always examined as a general indicator of animal health. The external appearance of many mussel shells could not be assessed until the barnacles that had attached during the exposure period were removed from the mussel shells. Once the barnacles were removed, we observed that most mussels grew during the exposure period, based on the small, thin leading edge indicating recent growth. Based on the appearance of internal tissues, most tissues appeared normal with the presence of reproductive tissues in some individuals. In some cases it was not possible to determine if the mussels were truly alive until the tissues had been extracted. All mussel growth data are considered usable for the purpose of this report. No data were considered outliers and none were excluded from the data set.

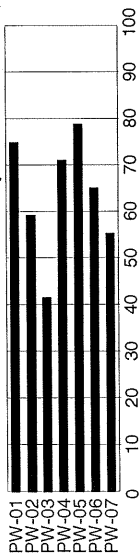
4.2 Mussel Survival

Mean survival ranged from 17.6 to 96% for individual stations after the 61-day exposure (Table 3; Figure 5) and the two stations with the lowest survival (IT-01 and IT-09) were both in the vicinity of the Intalco Pier and the seven lowest percent survivals were measured in the Intalco-Tosco vicinity. Average survival by site ranged from 46.6 to 71.1% (Table 3). The Simes multiple comparison test on mean survival by site indicated that survival in the Intalco-Tosco reach was significantly lower than survival at the other sites; there was no difference in survival among the Point Whitehorn, Cherry Point, or Gulf Road sites (Table 4). Interestingly, survival increased slightly from north to south between Point Whitehorn, Cherry Point and Gulf Road, but then decreased dramatically in the vicinity of Intalco-Tosco.

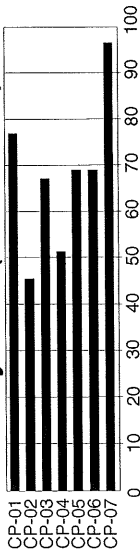


Percent Survival by Station

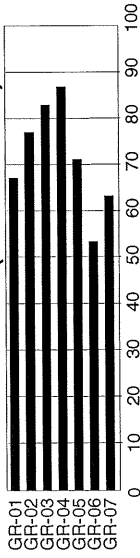
Pt. Whitehorn ($\bar{x} = 63\%$)



Cherry Pt. ($\bar{x} = 68\%$)



Gulf Road ($\bar{x} = 71\%$)



Intalco-Tosco ($\bar{x} = 47\%$)

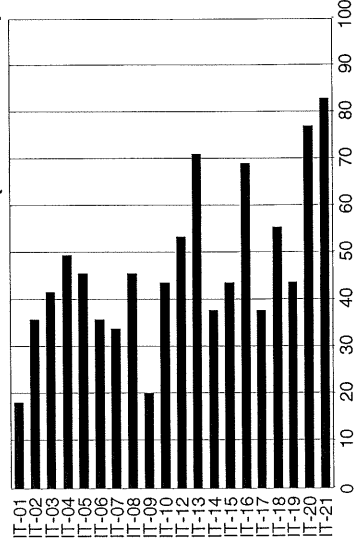


Figure 5. Percent survival by station and site.

Table 3. Percent survival for mussels

| | Pt. Whitehorn | Cherry Pt. | Gulf Road | Intalco-Tosco |
|-----------------|---------------|------------|-----------|---------------|
| Station 1 | 74.5% | 76.5% | 66.7% | 17.6% |
| Station 2 | 58.8% | 45.1% | 76.5% | 35.3% |
| Station 3 | 41.2% | 66.7% | 82.4% | 41.2% |
| Station 4 | 70.6% | 51.0% | 86.3% | 49.0% |
| Station 5 | 78.4% | 68.6% | 70.6% | 45.1% |
| Station 6 | 64.7% | 68.6% | 52.9% | 35.3% |
| Station 7 | 54.9% | 96.1% | 62.7% | 33.3% |
| Station 8 | | | | 45.1% |
| Station 9 | | | | 19.6% |
| Station 10 | | | | 43.1% |
| Station 11 | | | | na |
| Station 12 | | | | 52.9% |
| Station 13 | | | | 70.6% |
| Station 14 | | | | 37.3% |
| Station 15 | | | | 43.1% |
| Station 16 | | | | 68.6% |
| Station 17 | | | | 37.3% |
| Station 18 | | | | 54.9% |
| Station 19 | | | | 43.1% |
| Station 20 | | | | 76.5% |
| Station 21 | | | | 82.4% |
| Station 22 | | | | na |
| Station 23 | | | | na |
| Average | 63.3% | 67.5% | 71.1% | 46.6% |
| N (out of 357) | 226 | 240 | 254 | -- |
| N (out of 1173) | | | | 474 |

**Table 4. Cherry Point 1999
Results of Simes Test on Survival Data by Site**

| | z | p | k (rank) | P | Result |
|-----------------|--------------|---------------|----------|--------------|-------------------------------|
| PW vs CP | 0.954 | 0.3401 | 5 | 0.025 | No significant difference |
| PW vs GR | 1.820 | 0.0688 | 4 | 0.02 | No significant difference |
| PW vs IT | 4.263 | 0.0000 | 3 | 0.015 | Significant Difference |
| CP vs GR | 0.868 | 0.3853 | 6 | 0.03 | No significant difference |
| CP vs IT | 5.543 | 0.0000 | 2 | 0.01 | Significant Difference |
| GR vs IT | 6.736 | 0.0000 | 1 | 0.005 | Significant Difference |

4.3 Mussel Growth

Growth was consistently higher at the northern stations Point Whitehorn, Cherry Point, and Gulf Road than at Intalco-Tosco, the southernmost site. Summaries of the mussel growth metrics used to evaluate mussel health and effects after the 61-day exposure period are presented in Tables 5 and 6. Table 5 summarizes the data by station and Table 6 summarizes the data by site. Table 7 summarize results of statistical analyses on the mussel growth metrics. 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 generally provide the most accurate assessment of effects because they represent paired data for individual mussels. Growth rates were calculated from these EOT and BOT measurements. All other comparative metrics represent a comparison between EOT measurements on individual mussels and a BOT estimate determined by measuring these parameters on a separate group of mussels at the beginning of the test that are within the same size range as mussels deployed. Tissue weight and shell weight data indicated the greatest number of statistically significant differences among sites. Shell length, length growth rate, and WAWW growth rate data indicated no differences among sites.

Table 8 provides an overall site ranking based on the ranks of the individual growth metrics and tissue chemistry results. Although Intalco-Tosco had the lowest ranking for every growth metric, the highest rankings for each metric were not found at any particular site. Mussels at Point Whitehorn had the largest EOT tissue and shell weights; those at Cherry Point had the highest EOT WAWWs and WAWW growth rates; and mussels at Gulf Road had the highest EOT shell lengths and length growth rates. After ranking the various mussel growth parameters across sites and summing the ranks, the lowest rank sum (6) was found for the Intalco-Tosco site; the rank sum was much higher and very similar among the northern sites, ranging from 17 to 19.

Table 5. Summary by Station: Mussel Metrics used to Quantify Effects

| | EOT Shell Percent Survival | Length Length (mm) | EOT Growth Rate (mm/wk) | WAWW WAWW (g-wet) | EOT Growth Rate (mg/wk) | EOT Tissue Wt. (g-wet) | EOT Shell Weight (g-wet) | Percent Lipids (dw) | Percent Water |
|-------|----------------------------------|--------------------------|-------------------------------|-------------------------|-------------------------------|------------------------------|--------------------------------|---------------------------|------------------|
| PW-01 | 74.5% | 45.09 | 0.35 | 9.47 | 242 | 2.92 | 3.18 | 7.14 | 79 |
| PW-02 | 58.8% | 44.93 | 0.26 | 9.26 | 209 | 3.03 | 2.87 | 5.00 | 78 |
| PW-03 | 41.2% | 45.10 | 0.34 | 9.43 | 242 | 3.41 | 3.09 | 5.00 | 78 |
| PW-04 | 70.6% | 44.76 | 0.24 | 9.05 | 200 | 3.08 | 3.26 | 3.33 | 79 |
| PW-05 | 78.4% | 45.44 | 0.39 | 9.59 | 260 | 3.23 | 3.03 | 3.33 | 79 |
| PW-06 | 64.7% | 45.27 | 0.31 | 9.23 | 203 | 2.81 | 3.23 | 5.00 | 80 |
| PW-07 | 54.9% | 44.79 | 0.27 | 8.92 | 180 | 2.91 | 3.23 | 6.00 | 80 |
| CP-01 | 76.5% | 44.91 | 0.34 | 9.37 | 263 | 3.16 | 2.71 | 7.08 | 76 |
| CP-02 | 45.1% | 44.49 | 0.27 | 8.65 | 168 | 2.79 | 2.72 | 4.21 | 81 |
| CP-03 | 66.7% | 45.59 | 0.35 | 9.84 | 262 | 2.94 | 3.28 | 4.29 | 79 |
| CP-04 | 51.0% | 44.57 | 0.28 | 8.84 | 184 | 2.74 | 2.99 | 8.50 | 80 |
| CP-05 | 68.6% | 45.13 | 0.31 | 9.01 | 212 | 2.36 | 3.59 | 5.00 | 80 |
| CP-06 | 68.6% | 44.95 | 0.29 | 8.95 | 204 | 2.73 | 2.59 | 5.24 | 79 |
| CP-07 | 96.1% | 45.98 | 0.42 | 10.24 | 306 | 3.58 | 3.26 | 5.00 | 78 |
| GR-01 | 66.7% | 44.97 | 0.30 | 8.99 | 176 | 2.98 | 3.07 | 3.00 | 80 |
| GR-02 | 76.5% | 45.70 | 0.36 | 9.48 | 224 | 2.60 | 3.09 | 5.00 | 78 |
| GR-03 | 82.4% | 45.52 | 0.37 | na | na | 3.03 | 3.13 | 5.91 | 78 |
| GR-04 | 86.3% | 45.37 | 0.40 | 9.50 | 267 | 3.10 | 3.02 | 5.45 | 78 |
| GR-05 | 70.6% | 45.24 | 0.38 | 9.41 | 230 | 2.75 | 3.21 | 3.50 | 80 |
| GR-06 | 52.9% | 44.86 | 0.32 | 8.88 | 209 | 2.76 | 2.90 | 3.00 | 80 |
| GR-07 | 62.7% | 44.98 | 0.31 | 9.08 | 184 | 2.73 | 3.03 | 5.00 | 80 |
| IT-01 | 17.6% | 43.95 | 0.34 | 9.05 | 207 | 2.84 | 2.83 | 1.46* | 59* |
| IT-02 | 35.3% | 44.44 | 0.20 | 8.91 | 167 | 2.50 | 2.94 | 3.33 | 79 |
| IT-03 | 41.2% | 46.22 | 0.39 | na | na | 2.65 | 3.27 | 13.50 | 80 |
| IT-04 | 49.0% | 43.62 | 0.24 | 8.49 | 184 | 2.83 | 2.88 | 2.50 | 80 |
| IT-05 | 45.1% | 43.60 | 0.25 | 8.74 | 219 | 2.54 | 2.70 | 2.00 | 80 |
| IT-06 | 35.3% | 44.73 | 0.32 | 9.05 | 255 | 2.45 | 2.97 | 4.00 | 80 |
| IT-07 | 33.3% | 44.27 | 0.24 | 9.09 | 206 | 2.85 | 3.03 | 2.22 | 73 |
| IT-08 | 45.1% | 44.20 | 0.25 | 8.89 | 212 | 2.30 | 2.86 | 4.89 | 81 |
| IT-09 | 19.6% | 45.66 | 0.33 | 8.63 | 154 | 2.53 | 3.10 | 1.67* | 70* |
| IT-10 | 43.1% | 45.29 | 0.36 | 8.99 | 184 | 2.81 | 3.09 | 3.68 | 81 |
| IT-11 | Cage not retrieved | | | | | | | | |
| IT-12 | 52.9% | 44.37 | 0.26 | 8.79 | 180 | 2.24 | 3.10 | 3.21 | 72 |
| IT-13 | 70.6% | 45.36 | 0.39 | 9.35 | 249 | 2.82 | 2.81 | 4.43 | 79 |
| IT-14 | 37.3% | 45.63 | 0.42 | 9.47 | 246 | 2.80 | 3.30 | 8.50 | 80 |
| IT-15 | 43.1% | 44.06 | 0.21 | 8.48 | 138 | 2.71 | 2.97 | 3.89 | 82 |
| IT-16 | 68.6% | 45.37 | 0.38 | 9.44 | 248 | 3.31 | 2.83 | 4.89 | 81 |
| IT-17 | 37.3% | 45.07 | 0.37 | 9.20 | 194 | 3.08 | 3.11 | 3.68 | 81 |
| IT-18 | 54.9% | 44.63 | 0.31 | 9.13 | 232 | 2.87 | 2.85 | 3.00 | 80 |
| IT-19 | 43.1% | 43.82 | 0.20 | 8.48 | 159 | 2.43 | 2.92 | 3.89 | 82 |
| IT-20 | 76.5% | 45.07 | 0.30 | 9.33 | 231 | 3.10 | 2.81 | 2.50 | 80 |
| IT-21 | 82.4% | 45.07 | 0.30 | 9.27 | 212 | 2.61 | 2.84 | 2.86 | 79 |
| IT-22 | Cage not retrieved | | | | | | | | |
| IT-23 | Cage not retrieved | | | | | | | | |

*Extremely low survival and limited tissue mass likely biases these values.

Grey cells = samples reported as 0% lipids; average wet-weight value for all other tissue samples used to calculate % lipids dry-weight.

Table 6. Summary by Site: Mussel metrics used to Quantify Effects

| | <u>Pt. Whitehorn</u> | <u>Cherry Pt.</u> | <u>Gulf Road</u> | <u>It-Tos</u> | <u>T₀</u> | <u>Grand Mean</u> |
|---|----------------------|-------------------|------------------|---------------|----------------------|-------------------|
| Percent Survival | 63.3% | 67.2% | 71.1% | 46.5% | NA | 57% |
| % Change Weight | 26.3% | 28.7% | 26.1% | 25.5% | NA | 27% |
| % Change Length | 6.3% | 6.8% | 7.2% | 6.3% | NA | 7% |
| Est % Change in Tissue Weight | 86.6% | 81.4% | 75.5% | 68.1% | NA | 78% |
| Est % Change in Shell Weight | 62.8% | 58.6% | 59.9% | 52.9% | NA | 59% |
| <u>Initial Length (mm)</u> | | | | | | |
| Mean | 42.4 | 42.4 | 42.3 | 42.3 | 42.3 | 42.4 |
| Min | 38.0 | 38.1 | 38.0 | 38.0 | 38.1 | 38.0 |
| Max | 46.0 | 46.0 | 45.9 | 46.0 | 45.9 | 46.0 |
| StDev | 2.0 | 2.1 | 2.1 | 2.1 | 2.0 | 2.1 |
| N | 357 | 357 | 357 | 1173 | 151 | 2395 |
| 2SE | 0.2 | 0.2 | 0.2 | 0.1 | 0.3 | 0.2 |
| <u>EOT Length (mm)</u> | | | | | | |
| Mean | 45.07 | 45.18 | 45.27 | 44.76 | NA | 45.01 |
| Min | 38.85 | 39.27 | 38.94 | 39.31 | NA | 38.85 |
| Max | 53.23 | 51.59 | 53.43 | 53.56 | NA | 53.56 |
| StDev | 2.5 | 2.4 | 2.5 | 2.4 | NA | 2.5 |
| N | 226 | 240 | 254 | 474 | NA | 1194 |
| 2SE | 0.3 | 0.3 | 0.3 | 0.2 | NA | 0.1 |
| <u>Growth Rate Length (mm/wk)</u> | | | | | | |
| Mean | 0.311 | 0.334 | 0.352 | 0.305 | NA | 0.322 |
| Min | -0.082 | -0.040 | -0.160 | -0.075 | NA | -0.160 |
| Max | 0.964 | 1.162 | 1.446 | 1.194 | NA | 1.446 |
| StDev | 0.205 | 0.200 | 0.236 | 0.196 | NA | 0.208 |
| N | 226 | 240 | 254 | 474 | NA | 1194 |
| 2SE | 0.027 | 0.026 | 0.030 | 0.018 | NA | 0.027 |
| <u>Initial Weight (g-wet)</u> | | | | | | |
| Mean | 7.40 | 7.37 | 7.40 | 7.35 | 7.38 | 7.37 |
| Min | 4.55 | 4.83 | 5.10 | 4.34 | 4.73 | 4.34 |
| Max | 10.31 | 10.39 | 10.04 | 11.93 | 11.15 | 11.93 |
| StDev | 1.12 | 1.10 | 1.05 | 1.13 | 1.05 | 1.11 |
| N | 357 | 357 | 357 | 1173 | 151 | 2395 |
| 2SE | 0.12 | 0.12 | 0.11 | 0.07 | 0.17 | 0.05 |
| <u>EOT WAWW (g-wet)</u> | | | | | | |
| Mean | 9.29 | 9.38 | 9.26 | 9.04 | NA | 9.20 |
| Min | 5.11 | 5.97 | 5.67 | 5.45 | NA | 5.11 |
| Max | 14.44 | 14.26 | 13.51 | 15.36 | NA | 15.36 |
| StDev | 1.58 | 1.52 | 1.51 | 1.53 | NA | 1.54 |
| N | 226 | 240 | 212 | 453 | NA | 1131 |
| 2SE | 0.21 | 0.20 | 0.21 | 0.14 | NA | 0.09 |
| <u>Growth Rate WAWW (mg/wk)</u> | | | | | | |
| Mean | 221 | 238 | 218 | 209 | NA | 219 |
| Min | -145 | -142 | -186 | -221 | NA | -221 |
| Max | 658 | 643 | 625 | 693 | NA | 693 |
| StDev | 135 | 126 | 134 | 120 | NA | 127 |
| N | 226 | 240 | 212 | 453 | NA | 1131 |
| 2SE | 18 | 16 | 18 | 11 | NA | 8 |
| <u>EOT Wet Tissue Weight (g-wet)</u> | | | | | | |
| Mean | 3.04 | 2.96 | 2.86 | 2.74 | 1.63 | 2.87 |
| Min | 1.35 | 1.04 | 1.27 | 1.30 | 0.75 | 1.04 |
| Max | 6.07 | 6.47 | 5.84 | 5.27 | 2.45 | 6.47 |
| StDev | 0.69 | 0.82 | 0.67 | 0.67 | 0.26 | 0.72 |
| N | 226 | 241 | 254 | 475 | 151 | 1196 |
| 2SE | 0.09 | 0.11 | 0.08 | 0.06 | 0.04 | 0.09 |
| <u>EOT Shell Weight (g-wet)</u> | | | | | | |
| Mean | 3.13 | 3.05 | 3.07 | 2.94 | 1.92 | 3.02 |
| Min | 1.46 | 1.37 | 1.38 | 1.66 | 0.74 | 1.37 |
| Max | 4.69 | 5.06 | 4.24 | 5.31 | 2.73 | 5.31 |
| StDev | 0.51 | 0.60 | 0.47 | 0.45 | 0.30 | 0.51 |
| N | 226 | 241 | 254 | 475 | 151 | 1196 |
| 2SE | 0.07 | 0.08 | 0.06 | 0.04 | 0.05 | 0.07 |
| <u>Percent Lipids</u> | | | | | | |
| Wet Basis | 1.04 | 1.19 | 0.93 | 0.87 | 1.03 | 0.97 |
| Dry Basis | 4.97 | 5.62 | 4.41 | 4.28 | 5.74 | 4.67 |
| <u>Percent Water</u> | | | | | | |
| | 79.00 | 79.00 | 79.14 | 79.44 | 82.00 | 79.2 |

Note: Values highlighted in grey were NOT included in the calculation of Grand Means

Table 7. Summary of Statistical Analyses on Mussel Metrics

| Metric | Common | | | | Station Order (Mean) | | | | Comments |
|------------------------------|--------|-----------|----------|----------------|----------------------|---------------|---------------|---------------|-----------------------------------|
| | Normal | Variances | Analyses | p | | | | | |
| Survival (%) | na | na | Simes | see Table 4 | IT (46.5) | PW (63.3) | CP (67.5) | GR (71.1) | (GR=CP=PW) ≠ IT |
| EOT Length (mm) | Y | Y | ANOVA | 0.0678 | IT (44.8) | PW (45.1) | CP (45.2) | GR (45.3) | No sign difference among sites |
| Length GR (mm/wk) | Y | N | Kruskal | 0.0789 | IT (0.304) | PW (0.311) | CP (0.332) | GR (0.352) | No sign difference among sites |
| EOT WAWW (g-wet) | Y | Y | ANOVA | 0.0374 | IT (9.03) | GR (9.26) | PW (9.29) | CP (9.36) | IT ≠ CP |
| WAWW GR (mg/wk) | Y | Y | ANOVA | 0.0653 | IT (208) | GR (218) | PW (221) | CP (236) | No sign difference among sites |
| EOT Tissue Wt. (g-wet) | Y | N | ANOVA | <0.0001 | IT (2.74) | GR (2.86) | CP (2.96) | PW (3.04) | PW ≠ GR PW ≠ IT CP ≠ IT |
| Shell Wt (g-wet) | Y | N | ANOVA | <0.0001 | IT (2.94) | CP (3.05) | GR (3.07) | PW (3.13) | (GR=CP=PW) ≠ IT |
| % Lipids-dry | N | Y | Kruskal | 0.0335 | IT (4.28) | GR (4.41) | PW (4.97) | CP (5.62) | CP ≠ IT |
| % Water | N | N | Kruskal | 0.1647 | IT (79.44) | GR (79.14) | CP (79.00) | PW (79.00) | No sign difference among sites |

**Table 8A. Ranked Growth Metrics by Site: Survival and PAH Tissue Chemistry
(1 = lowest; 4 = highest)**

| Site ID | PW | CP | GR | IT |
|-------------------------------------|-----------|-----------|-----------|----------|
| Mussel Metric | | | | |
| Shell length (mm) | 2 | 3 | 4 | 1 |
| Length GR (mm/wk) | 2 | 3 | 4 | 1 |
| WAWW (g-wet) | 3 | 4 | 2 | 1 |
| WAWW GR (mg/wk) | 3 | 4 | 2 | 1 |
| Tissue (g-wet) | 4 | 3 | 2 | 1 |
| Shell (g-wet) | 4 | 2 | 3 | 1 |
| Sum Ranks Growth Metrics | 18 | 19 | 17 | 6 |
| Survival (%) | 2 | 3 | 4 | 1 |
| Sum Ranks All Mussel Metrics | 20 | 22 | 21 | 7 |
| TPAH _{0-ND} -dw | 2* | 2* | 4 | 2* |

*For purposes of ranking, the TPAH_{0-ND} concentrations at IT, PW and CP are considered the same.

Table 8B. Ranked Growth Metrics by Region: Survival and PAH Tissue Chemistry
(1 = lowest; 6 = highest)

| Region ID | PW | CP | GR | IT | MP | TOS |
|-------------------------------------|-----------|-------------|-----------|----------|-------------|-----------|
| Shell Length | 3 | 4.5 | 6 | 1 | 4.5 | 2 |
| Length GR (mm/wk) | 3 | 4 | 6 | 1 | 5 | 2 |
| WAWW (g-wet) | 5 | 6 | 4 | 1 | 2 | 3 |
| WAWW GR (mg/wk) | 5 | 6 | 4 | 1 | 3 | 2 |
| Tissue (g-wet) | 6 | 5 | 3 | 1 | 2 | 4 |
| Shell (g-wet) | 6 | 4 | 5 | 2 | 3 | 1 |
| Sum Ranks Growth Metrics | 28 | 29.5 | 28 | 7 | 19.5 | 14 |
| Survival (%) | 4 | 5 | 6 | 2 | 1 | 3 |
| Sum Ranks All Mussel Metrics | 32 | 34.5 | 34 | 9 | 20.5 | 17 |
| TPAHs | 2 | 3 | 6 | 5 | 4 | 1 |

4.3.1 Shell Length

At the start of the test, individual mussel shell lengths ranged from 38.00 to 46.00 mm, a range of 8.00 mm. Mean shell length by station (i.e., cage, since one cage was deployed at each station) was between 42.14 and 42.60 mm (Table 5); mean shell length at each of the 4 sites was between 42.2 and 42.4 mm (Table 6). There were no statistically significant differences in mean shell lengths among individual cages ($p = 1.000$) or among sites ($p = 0.909$) at the beginning of the test. Mean shell length increased at all stations during the 61-day exposure period. The percentage increase in shell length across sites ranged from 6.3 to 7.2% with a mean of 7% (Table 6). Although the 61-d increases in shell length across sites were relatively small, $\bar{x} \approx 3$ mm, results of t-tests comparing BOT and EOT shell lengths at each site showed that the increases in shell length were statistically significant at all sites (Figure 6A). To account for the high mortality at some stations and the possibility that only the smaller individuals died, a comparison was also made between BOT and EOT using only individuals at BOT that survived the study. This comparison also showed significant increases in growth in the surviving individuals.

Although the differences were not great, average EOT shell lengths and increases in shell length were the lowest at Intalco-Tosco and highest at Gulf Road. ANOVA results indicated the differences in shell length among sites were not quite significant ($p = 0.0678$; Table 7). Length growth rates were calculated to facilitate comparisons with literature values that are commonly expressed in terms of shell length increase per unit time. Average length growth rates among stations ranged from 0.20 to 0.42 mm/wk (Table 5; Figure 7); average length growth rates among sites ranged from 0.305 to 0.352 mm/wk with an overall mean of 0.322 mm/wk (Table 6,

Figure 7). The highest length growth rates were found for mussels at Gulf Road and the lowest at Intalco-Tosco. Results of the one-way ANOVA indicated that there were no significant differences ($p = 0.0789$) among sites (Table 7).

4.3.2 Whole-Animal Wet-Weight (WAWW)

At the start of the test, individual WAWWs ranged from 4.34 to 11.93 g, a range of 7.59 g. Mean WAWW by station (i.e., cage) ranged from 7.11 to 7.62 g (Table 5); mean WAWW by site ranged from 7.35 to 7.40 g (Table 6). There were no statistically significant differences in mean WAWWs among individual cages ($p = 0.999$) or among stations ($p = 0.791$) at the beginning of the test. WAWW increased at all stations during the 61-d exposure period. The percentage increase in WAWW across sites ranged from 25.5 to 28.7%, with a mean of 27% (Table 6). At the end of the test, mean WAWWs by cage (i.e., station) ranged from 8.48 to 10.24 g (Table 5); mean WAWW by site ranged from 9.04 to 9.38 g (Table 6). Although the increases in WAWW across sites were small, $\bar{x} \approx 2$ g, results of t-tests comparing beginning- and end-of-test WAWW showed that the increases were statistically significant at all sites (Figure 6B). The largest increase in WAWW occurred in mussels at Cherry Point survival was the second highest of all. Although the differences were not great, the highest average WAWW was found at Cherry Point and the lowest at Intalco-Tosco.

Results of the one-way ANOVA indicated that there were significant differences in EOT WAWW among sites ($p < 0.0374$, Table 7); the multiple range test showed:

Cherry Point \neq Intalco-Tosco.

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. Mean weight growth rates by cage (i.e., station) ranged from 138 to 263 mg/wk (Table 5; Figure 8); mean weight growth rates by site ranged from 209 to 238 mg/wk (Table 6; Figure 8). The highest growth rates were found for mussels at Cherry Point. The lowest growth rates were found at Intalco-Tosco. Results of the one-way ANOVA indicated that the differences in weight growth rates among sites were not quite significant ($p < 0.0653$; Table 7).

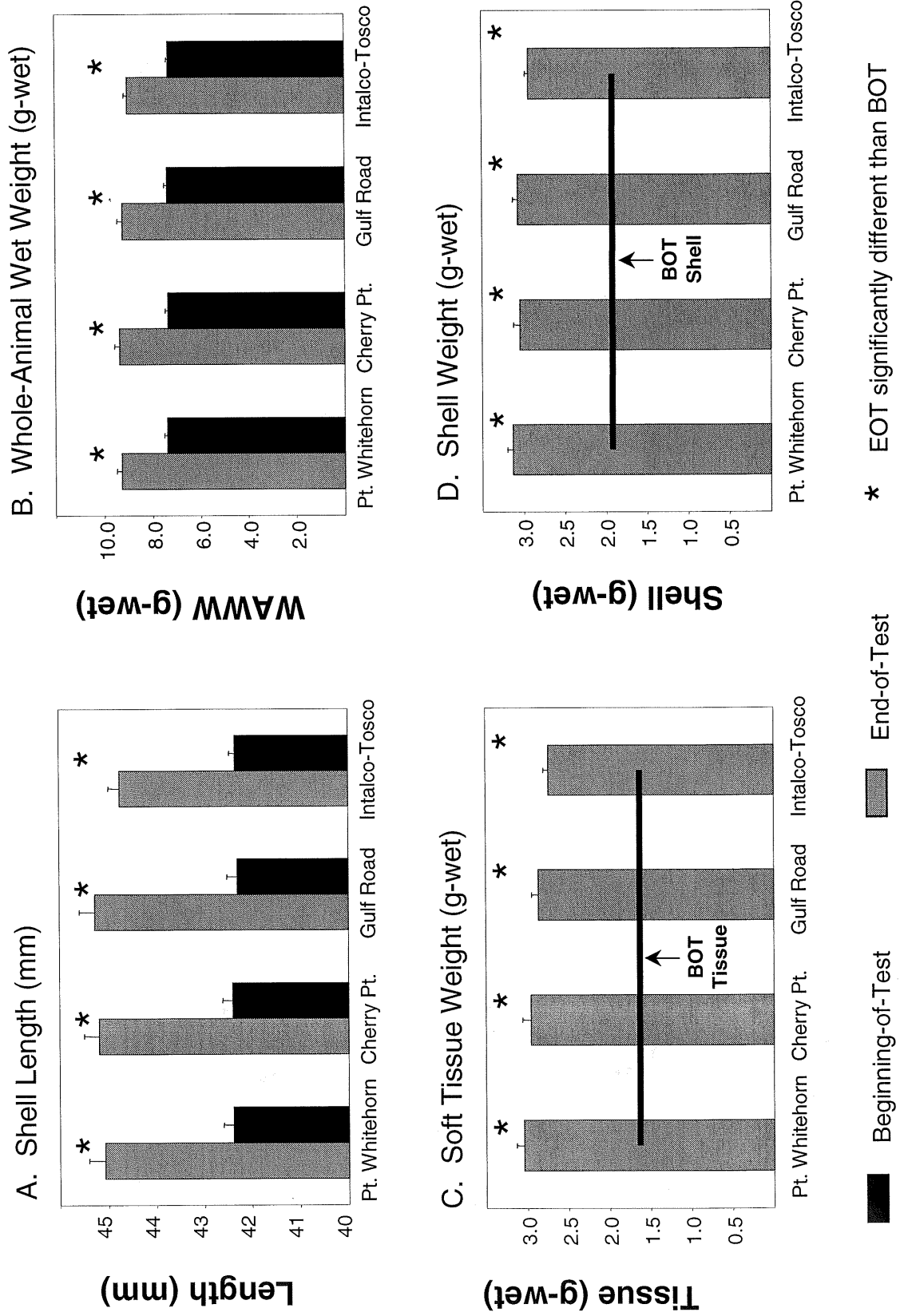
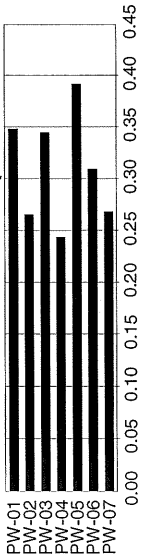


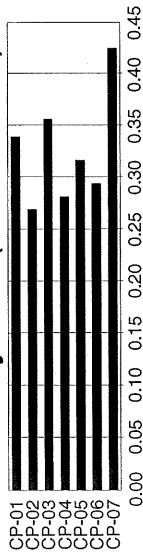
Figure 6. Mussel growth metrics $\pm 2SE$ --- Beginning vs End-of-Test

Length Growth Rate by Station

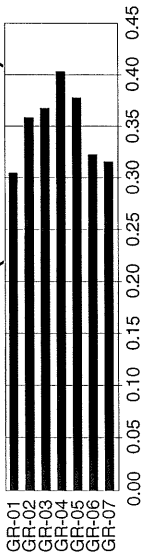
Pt. Whitehorn ($\bar{x} = 0.311$)



Cherry Pt. ($\bar{x} = 0.334$)



Gulf Road ($\bar{x} = 0.352$)



Intalco-Tosco ($\bar{x} = 0.305$)

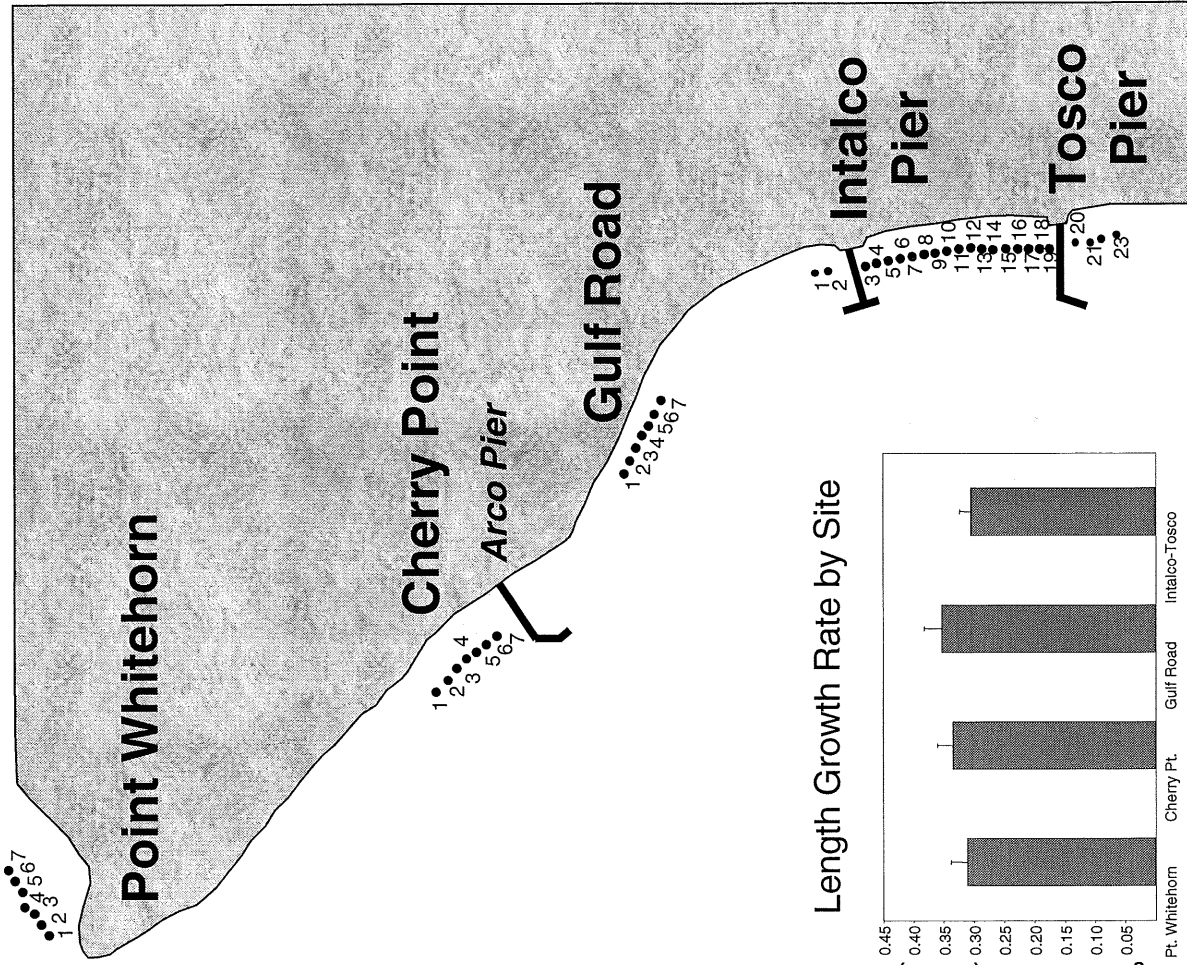
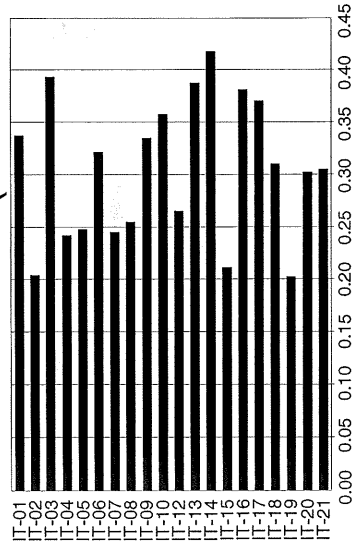


Figure 7. Mussel length growth rates (mm/wk) by station and site.

4.3.3 Wet Tissue Weights

Mean wet tissue weight at the start of the test was estimated at 1.63 g-wet (Table 6) based on the tissue weights from the 151 baseline BOT measurements. Mean EOT wet tissue weights by station (i.e., cage) ranged from 2.24 to 3.58 g (Table 5; Figure 9); mean EOT wet tissue weights by site ranged from 2.74 to 3.04 g (Table 6; Figure 6C). The percentage change in wet tissue weight across sites varied from 68.1 to 86.6%, with a mean of 78% (Table 6). The lowest EOT tissue weights were measured for mussels at IT-12, a station midway between the Intalco and Tosco piers. The highest tissue weights were measured for mussels at CP-07. Results of Dunnett's multiple range test comparing the baseline BOT wet tissue weight to EOT wet tissue weights showed that the increase in tissue weights was considered statistically significant at all stations (Figure 6C). 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. Results of the one-way ANOVA on EOT tissue weights indicated that there were significant differences ($p = <0.0001$) among sites (Table 7); the multiple range test showed the following differences:

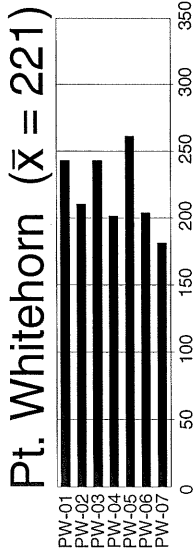
PW \neq GR and PW \neq IT and CP \neq IT

4.3.4 Shell Weights

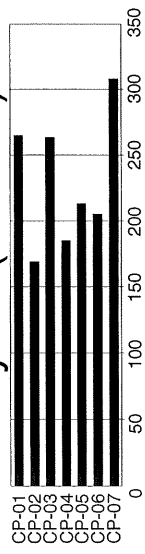
Mean shell weight at the start of the test was estimated at 1.92 g (Table 6) based on the shell weights from the 151 baseline BOT measurements. The overall range in EOT shell weight for individuals was 1.37 to 5.31 g-ww (Table 6); mean by cage (i.e., station) ranged from 2.70 to 3.59 g-wet (Table 5; Figure 10). Mean EOT shell weight by site ranged from 2.94 to 3.13 g-wet (Table 6; Figure 6D). A moderate change in shell weight was measured at all stations. The lowest percent change, 52.9%, occurred at the Intalco-Tosco site; the largest percent change, 62.8%, occurred at the Point Whitehorn site. This amount of increase in shell weight suggested an apparent increase in mussel health at all stations. Results of the ANOVA comparing the baseline BOT shell weight to EOT shell weights showed statistically significant increases at all stations (Figure 6D). Because of the destructive nature of tissue and shell weight measurements as discussed above, the comparisons of shell weights across stations at the end of the test is usually more reliable than beginning versus end of test comparisons. Results of the one-way ANOVA indicated significant differences ($p = <0.0001$) in EOT shell weights among sites (Table 7); the multiple range test showed the following differences:

PW \neq CP and PW \neq IT and GR \neq IT

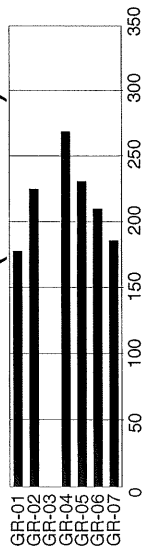
WAWW Growth Rate by Station



Cherry Pt. (x̄ = 238)



Gulf Road (x̄ = 218)



Intalco-Tosco (x̄ = 209)

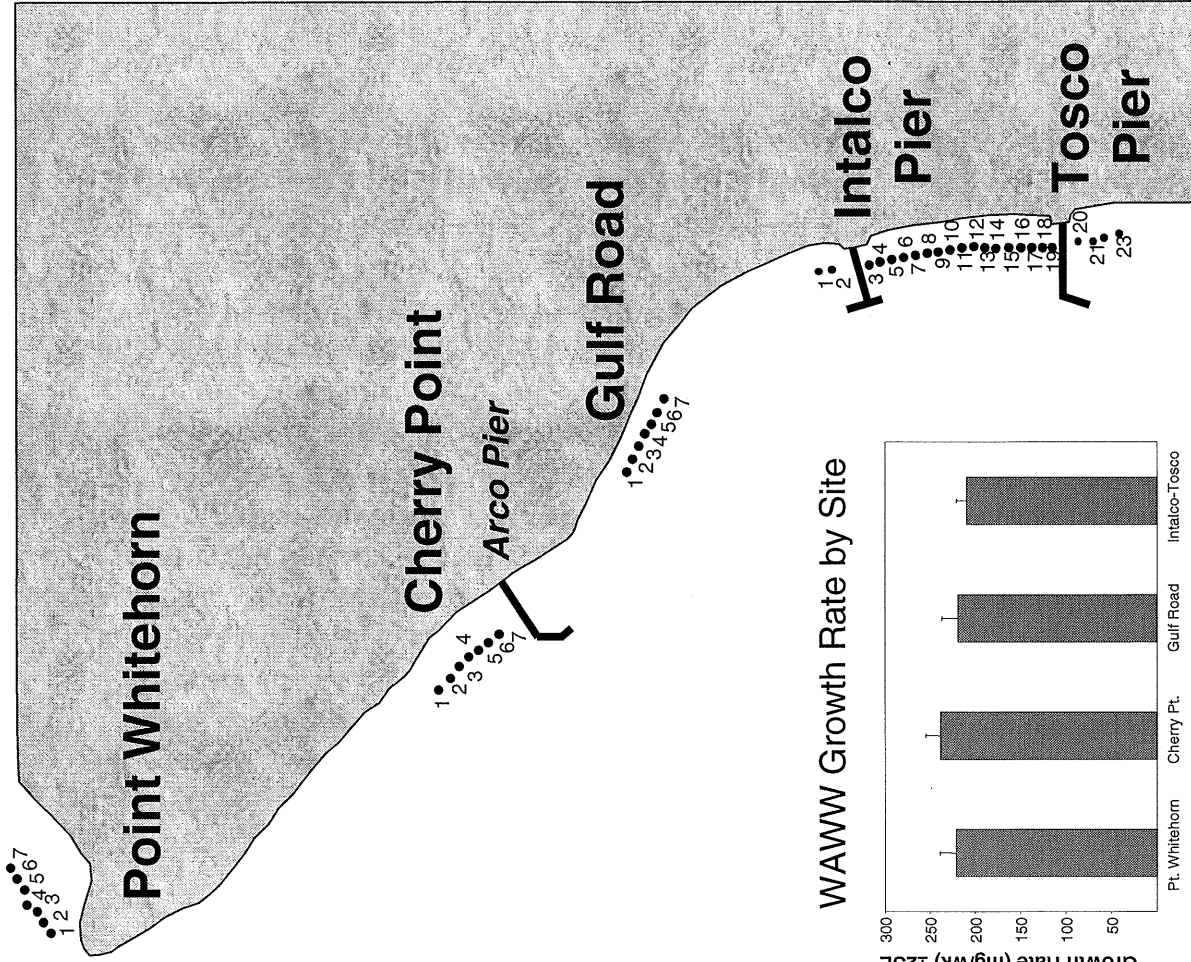
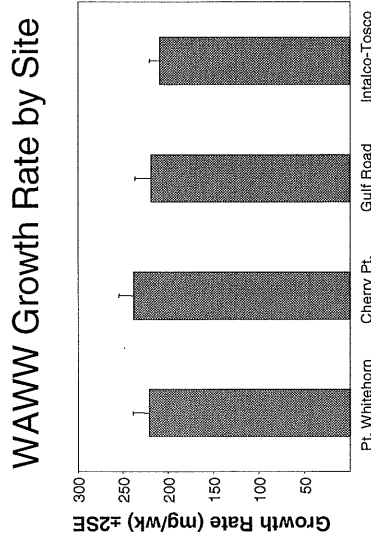
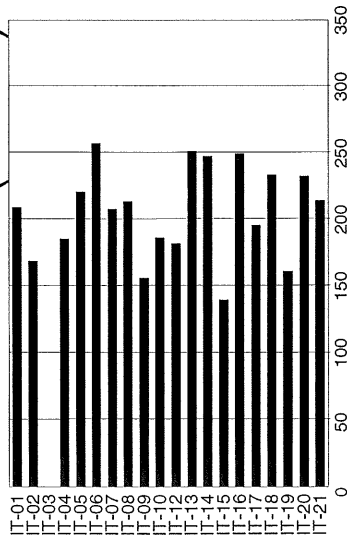


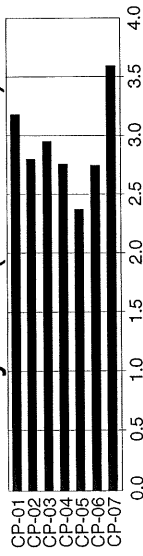
Figure 8. Mussel whole-animal wet-weights growth rates by station and site. No data available for IT-03 because tissues were weighed before making WAWWs.

Soft Tissue Weight (g-wet) by Station

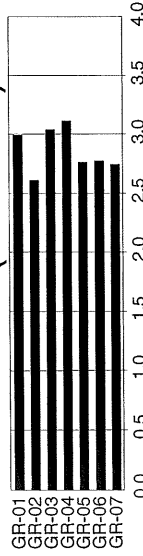
Pt. Whitehorn ($\bar{x} = 3.04$)



Cherry Pt. ($\bar{x} = 2.96$)



Gulf Road ($\bar{x} = 2.86$)



Intalco-Tosco ($\bar{x} = 2.74$)

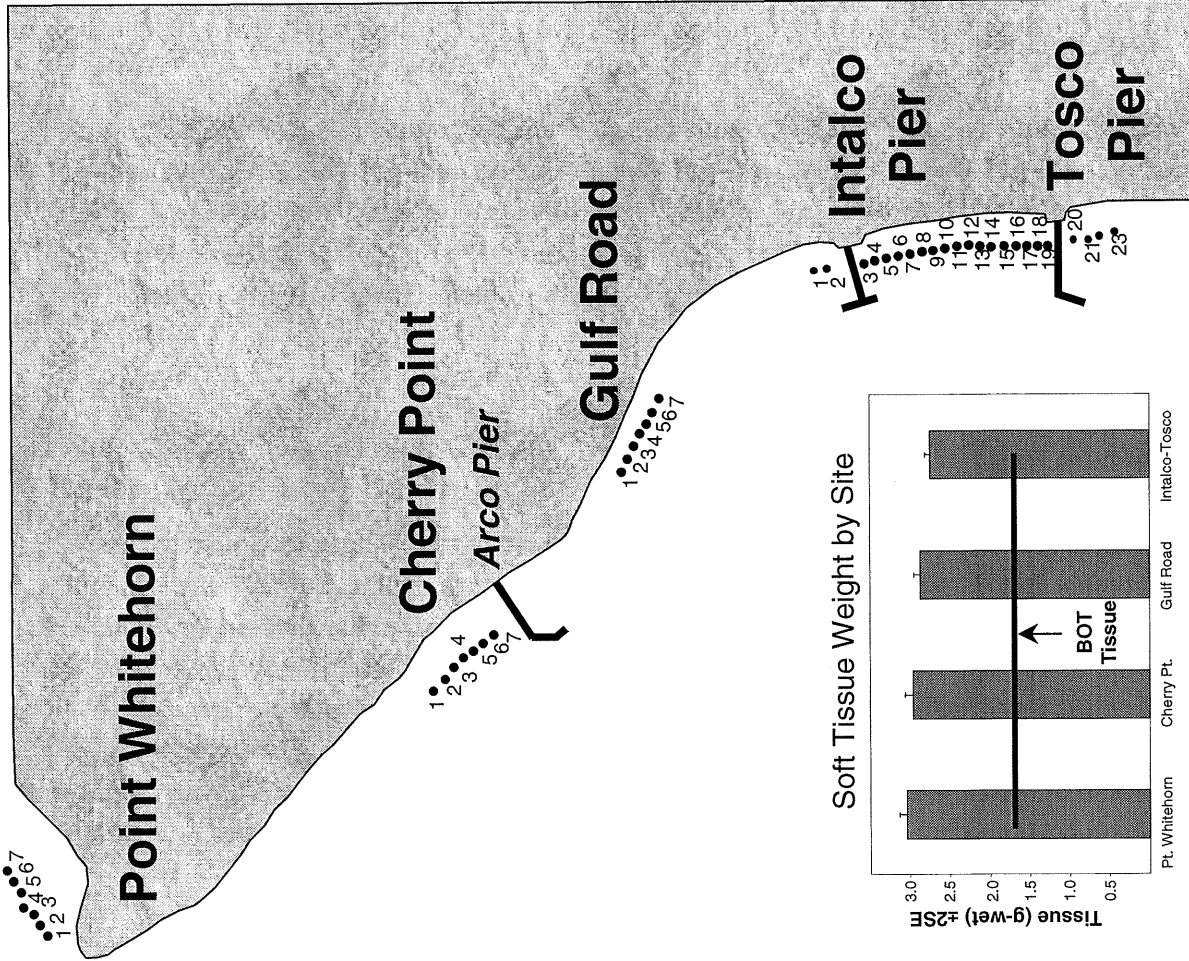
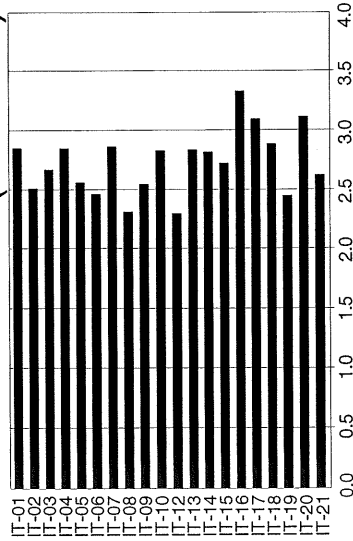


Figure 9. End-of-Test mussel tissue weights by station and site.

4.3.5 Percent Lipids

Percent lipids were measured as part of the chemical analytical process. The mussels collected at the Taylor United mussel farm had a mean lipid concentration of 5.74%-dry (Table 6; Figure 11). After 61-d exposure, lipid concentrations by station ranged from 2.22 to 13.50%-dry (Table 5); average concentrations by site ranged from 4.01 to 5.62%-dry (Table 6; Figure 11). There was a significant difference in percent lipids-dry between sites ($p = 0.0335$; Table 7):

Cherry Point \neq Intalco-Tosco.

4.3.6 Percent Water

Percent water in mussel tissues was measured as part of the chemical analytical process. As with the percent lipid data for Stations IT-01 and IT-09, the results of percent water analysis on mussel tissues for these stations were excluded for the statistical comparisons because of the poor survival and limited amount of tissue mass which may have compromised the chemical analyses. Mussels collected from the Taylor United Mussel Farm had a water concentration of 82% (Table 6; Figure 12). After 61-d exposure, water concentrations by station ranged from 72 to 82% (Table 5); average concentrations by site ranged from 79.00 to 79.44% (Table 6; Figure 12). There is no significant difference in percent water concentration among sites ($p = 0.1647$; Table 7).

4.4 Mussel Tissue Chemistry

Tissue chemistry results are based on mussel bioaccumulation at 39 stations. Of the 44 mussel cages deployed, three were lost and survival was too low (17.6 and 19.6%, respectively) at two stations to be reliable indicators of PAH accumulation.

4.4.1 Total PAHs

Tissue samples were analyzed for PAHs and their alkylated homologs. Only the results for TPAH concentrations measured in mussel tissues, and results of statistical analyses comparing TPAH concentrations in mussels collected from Taylor United mussel farm and after 61-d exposure are presented below. The concentrations of the individual PAH compounds presented in Table 10 are as reported by the analytical laboratory, converted to dry weight.

Four different sets of analyses were conducted on the TPAH data:

- TPAH in units of ug/kg-dw, non-detects at $\frac{1}{2}$ detection limit
- TPAH in units of ug PAH/g lipid-dw, non-detects at $\frac{1}{2}$ detection limit
- TPAH in units of ug/kg-dw, "0" for non-detects
- TPAH in units of ug/PAH/g lipid-dw, "0" for non-detects

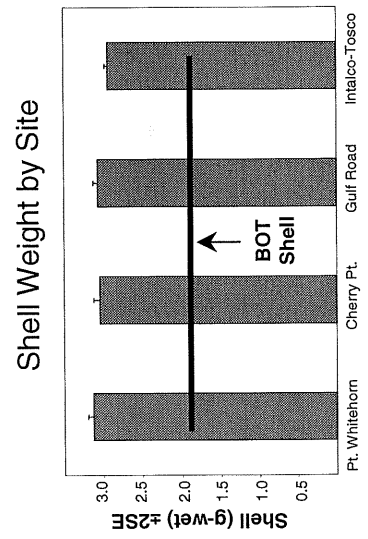
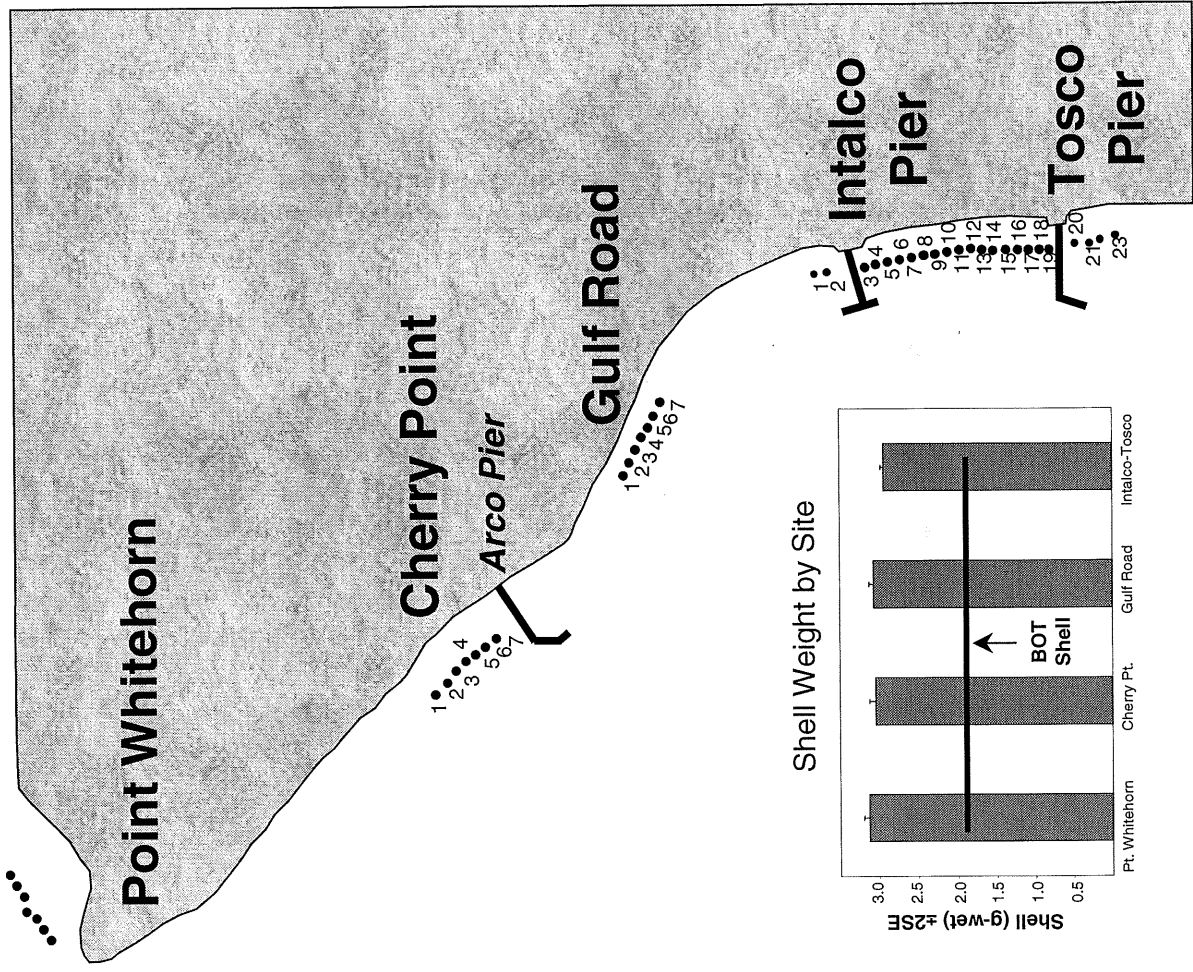
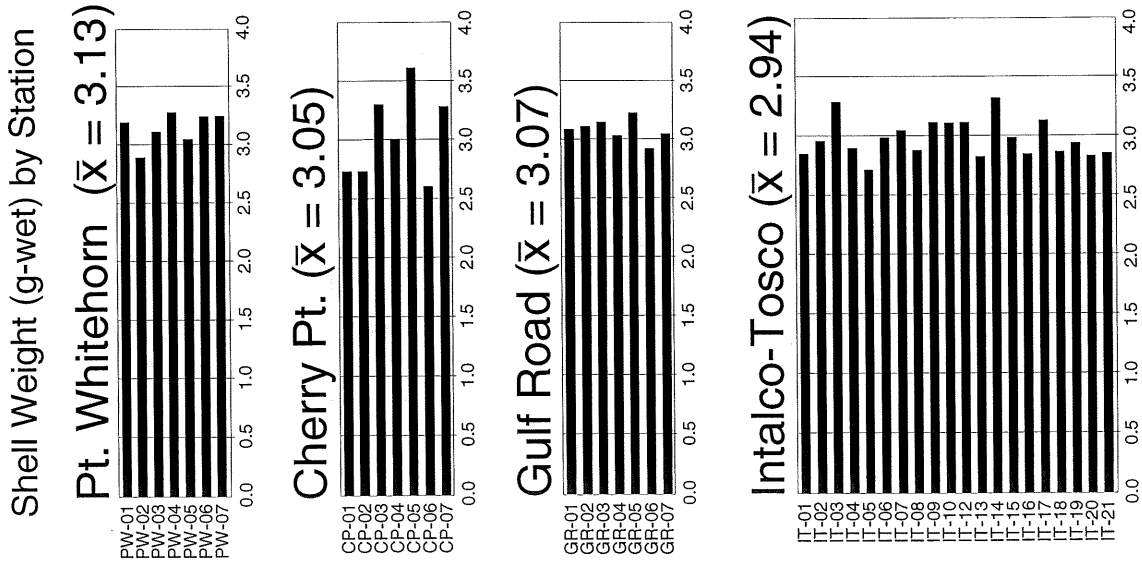


Figure 10. End-of-Test mussel shell weights by station and site.

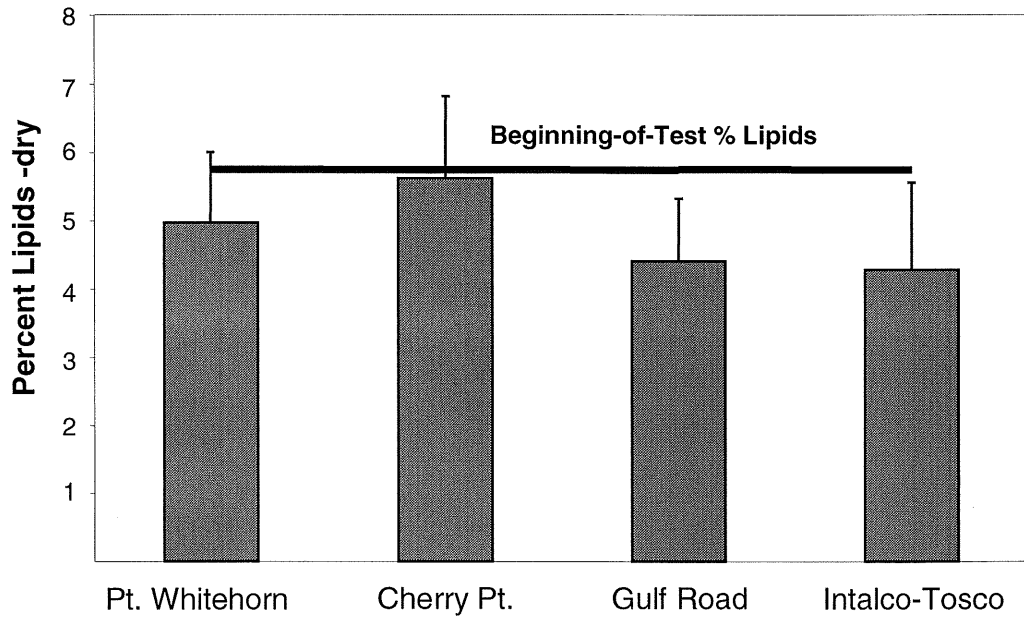


Figure 11. Percent Lipids-dry $\pm 2SE$ in mussel tissues by Site – BOT vs EOT

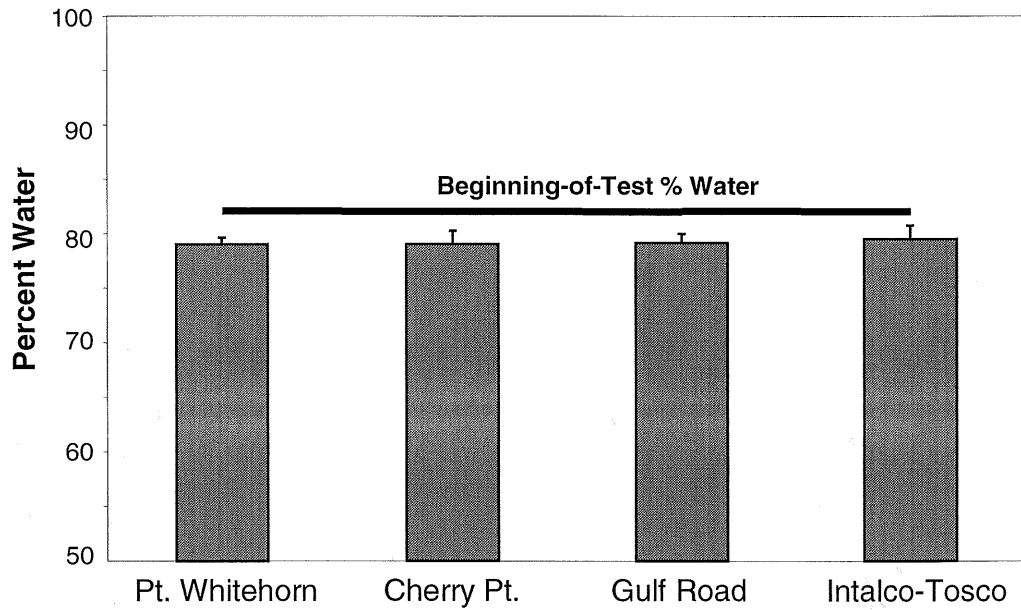


Figure 12. Percent Water $\pm 2SE$ in mussel tissues by Site – BOT vs EOT

The average calculated TPAHs dry-weight concentrations by site, for the four conditions shown above are summarized in Table 9. As indicated earlier, the tissue chemistry data for Stations IT-01 and IT-09 were excluded from average calculations and statistical comparisons because of the low survival and limited tissue mass. Most of the report and statistical analyses will be based on TPAHs using "0" for all non-detects. There are precedents for this approach and we feel it is the most meaningful. However, to provide different perspectives on these data as well as comparisons with the 1998 report and other data sets, Figure 13 compares BOT TPAH concentrations using the four different conditions. The concentrations of the individual PAH compounds measured in mussel tissues at the start of the test (i.e., Taylor United mussel farm) and for each station after 61-d exposure are provided in Table 10. The average concentration in mussel tissues at the start of the test was 91 ug/kg-dw. The 61-d TPAH_{0-ND} concentrations by station ranged from 0.00 to 526 ug/kg-dw.

Table 9. TPAH and Lipid-normalized TPAH by Site

| | Non-detects at ½ Detection Limit | | | | | "0" for Non-detects | | | | |
|---|----------------------------------|------|-------|-------|----------------|---------------------|------|------|------|----------------|
| | PW | CP | GR | IT | I ₀ | PW | CP | GR | IT | I ₀ |
| TPAH (ug/kg-dw) | | | | | | | | | | |
| Mean | 399 | 425 | 516 | 418 | 400 | 156 | 169 | 276 | 168 | 91 |
| StDev | 52 | 78 | 120 | 97 | 124 | 80 | 119 | 161 | 125 | 91 |
| N | 7 | 7 | 7 | 18 | 3 | 7 | 7 | 7 | 18 | 3 |
| Lipid-normalized TPAH (ug TPAH/g lipid-dw) | | | | | | | | | | |
| Mean | 8795 | 7892 | 12545 | 11759 | 7254 | 3641 | 3025 | 6642 | 4644 | 1585 |
| StDev | 3565 | 1926 | 5269 | 5744 | 1601 | 2638 | 2279 | 5409 | 4732 | 1201 |
| N | 7 | 7 | 7 | 18 | 3 | 7 | 7 | 7 | 18 | 3 |

Prior to deployment, mussel tissues had an average TPAH_{0-ND} concentration of 91ug/kg. After the 61-d exposure, 30 of 39 (77%) mussel tissue samples had a higher TPAH_{0-ND} concentration than mussels collected from the Taylor United Mussel Farm (Table 10, Figure 14C), and the average by site was higher in each case than the beginning-of-test concentration (Table 9, Figure 14C). The highest TPAH_{0-ND} concentration, 525.80 ug/kg-dw, after the 61-d exposure period was measured in mussels from GR-01 (Figure 14C). The highest TPAH_{0-ND} concentrations were measured in mussels at Gulf Road, on an average basis for each site. The accumulated TPAH_{0-ND} concentrations were approximately 68% higher than those measured at the other three sites. The concentrations of TPAH_{0-ND} in tissues at Point Whitehorn, Cherry Point, and Intalco-Tosco were very similar, ranging from 156 to 169 ug/kg-dw. Based on the ANOVA results, there were no significant differences between BOT and EOT TPAH_{0-ND} concentrations for any site (p=0.1998) or when comparing EOT concentrations among sites (p=0.2251).

TPAH content (ug TPAH/mussel) by station and site is summarized in Table 11. On a site basis, the content data support the trends shown by the TPAH_{0-ND} data, with the content increasing at all sites during the 61-day exposure period and an increasing content from north to south with the highest at Gulf Road (Figure 15). The EOT content at Gulf Road of 0.17 ug TPAH/animal is about a factor of 5.6 higher than the T₀ content of 0.03 ug TPAH/animal. There was no significant difference in end-of-test TPAH content across sites (p=0.1824). Based on the ANOVA results, there were no significant differences between BOT and EOT TPAH_{0-ND} contents for any site (p=0.1824) or when comparing EOT concentrations among sites (p=0.0980). However, the content for Gulf Road was marginally insignificant when compared to BOT.

The relative concentrations of five selected PAH parent compounds (naphthalene, fluorene, dibenzothiophene, phenanthrene, and chrysene) and their respective alkylated homologs are shown in Table 12. Sums were calculated based on the TPAH_{0-ND} concentrations. The respective ratios are shown by site in Figure 16 and compared with beginning of test. The data show considerable differences in the chemical composition of TPAHs at each site and when compared to BOT. Most notable among these was the composition of the BOT samples from the Taylor United Mussel Farm where the only parent compound detected was chrysene. None of the other PAH compounds, including the alkylated homologs, were detected in this sample. Although similar concentrations of chrysene were measured in tissue samples from the four test sites, the relative contribution of the alkylated homologs ranged from 1.90 to 3.48.

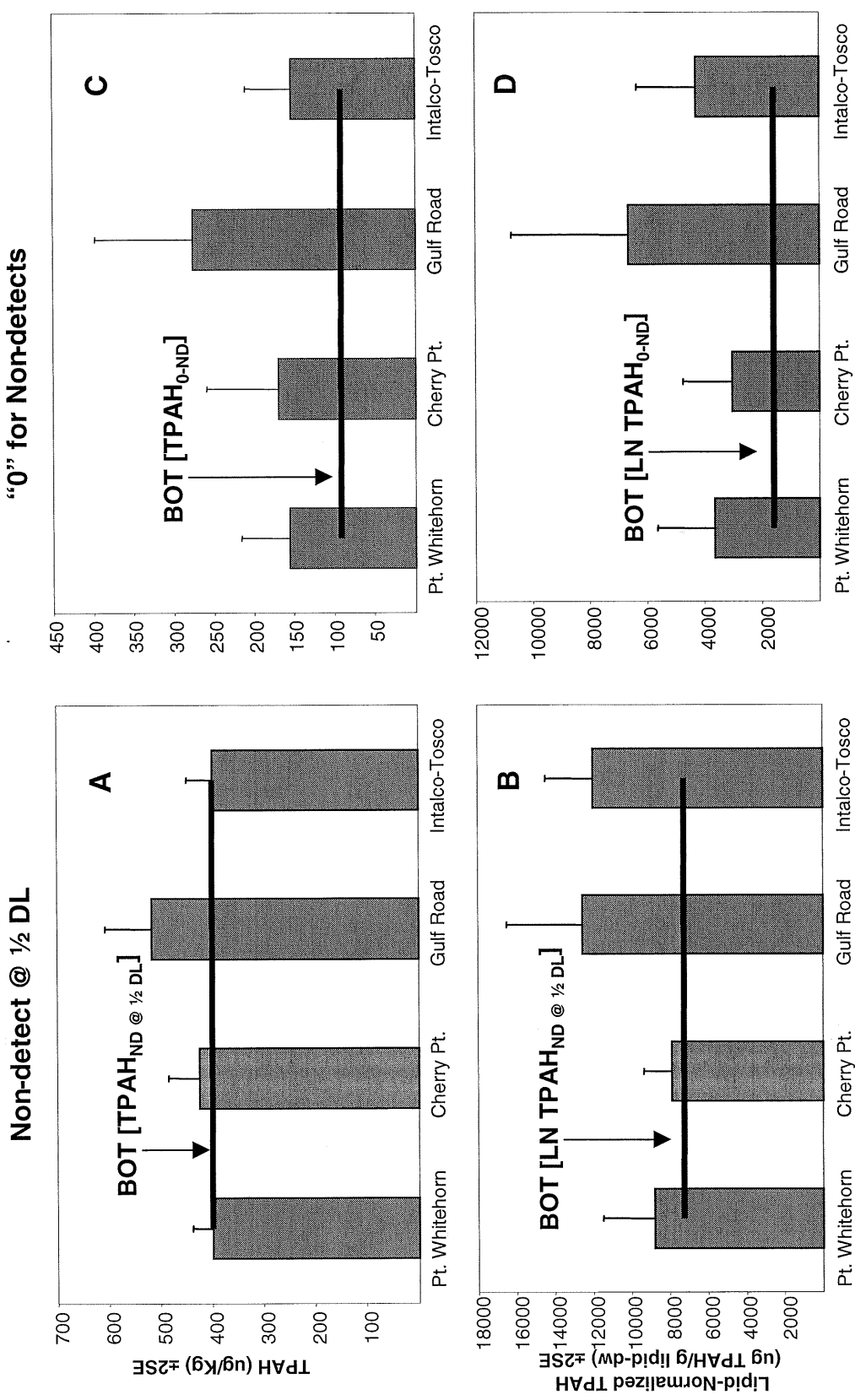
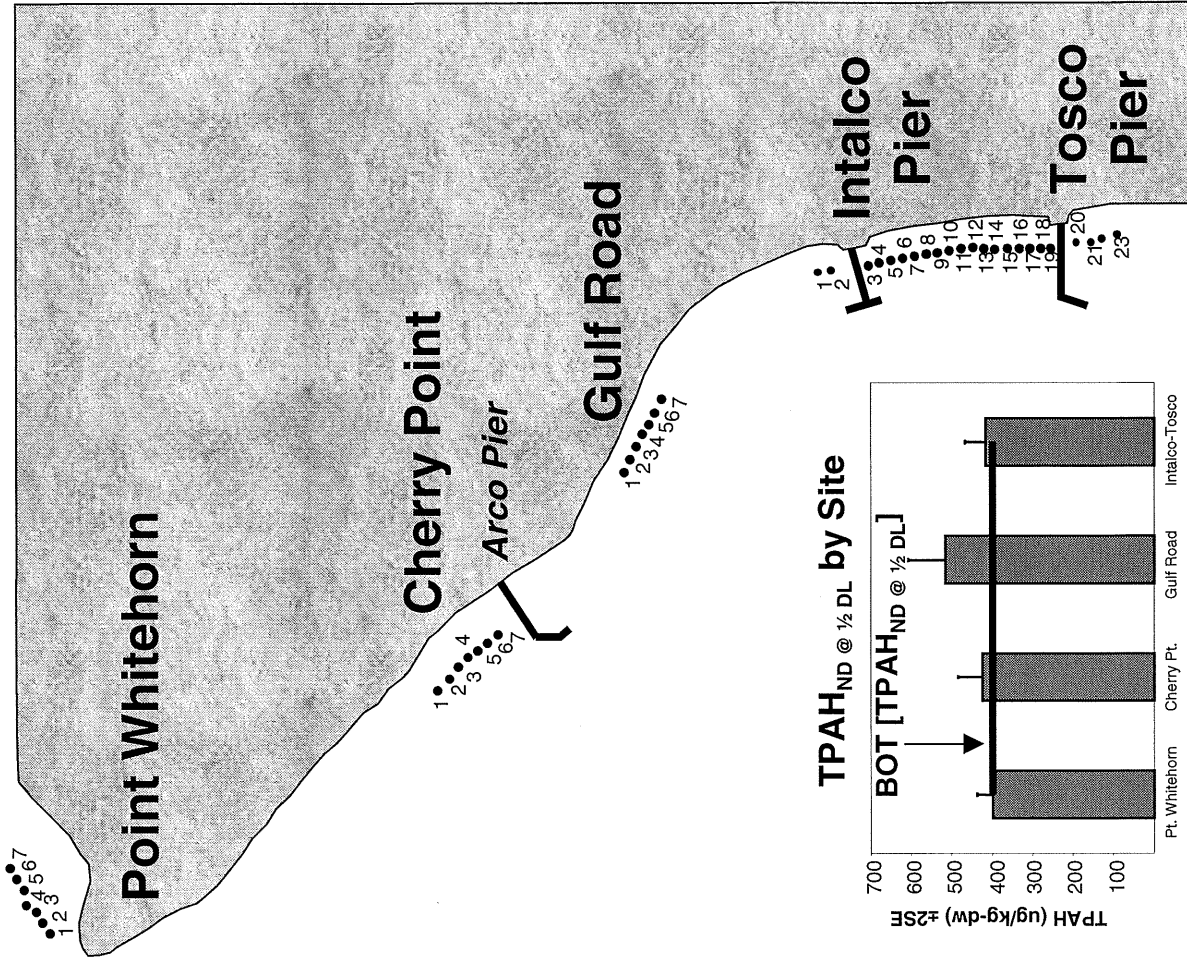
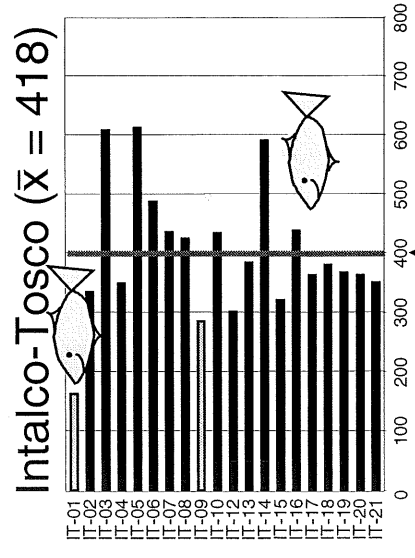
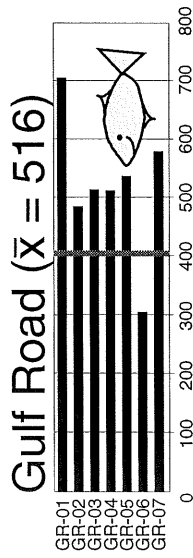
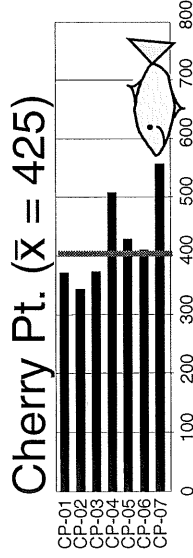
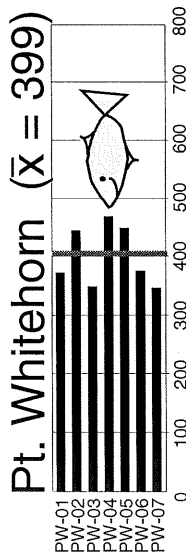


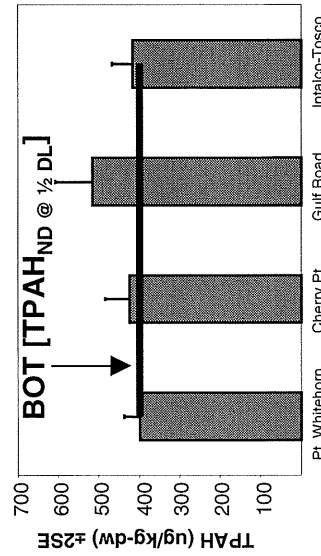
Figure 13. Comparison of various ways to report TPAH: A = dry weight, non-detects included in total at 1/2 detection limit; B = lipid-normalized dry weight, non-detects included in total at 1/2 detection limit; C = dry weight, "0" for non-detects in total; D = lipid-normalized dry weight, "0" for non-detects in total.



TPAH_{ND} @ ½ DL by Station (ug/kg dry wt)

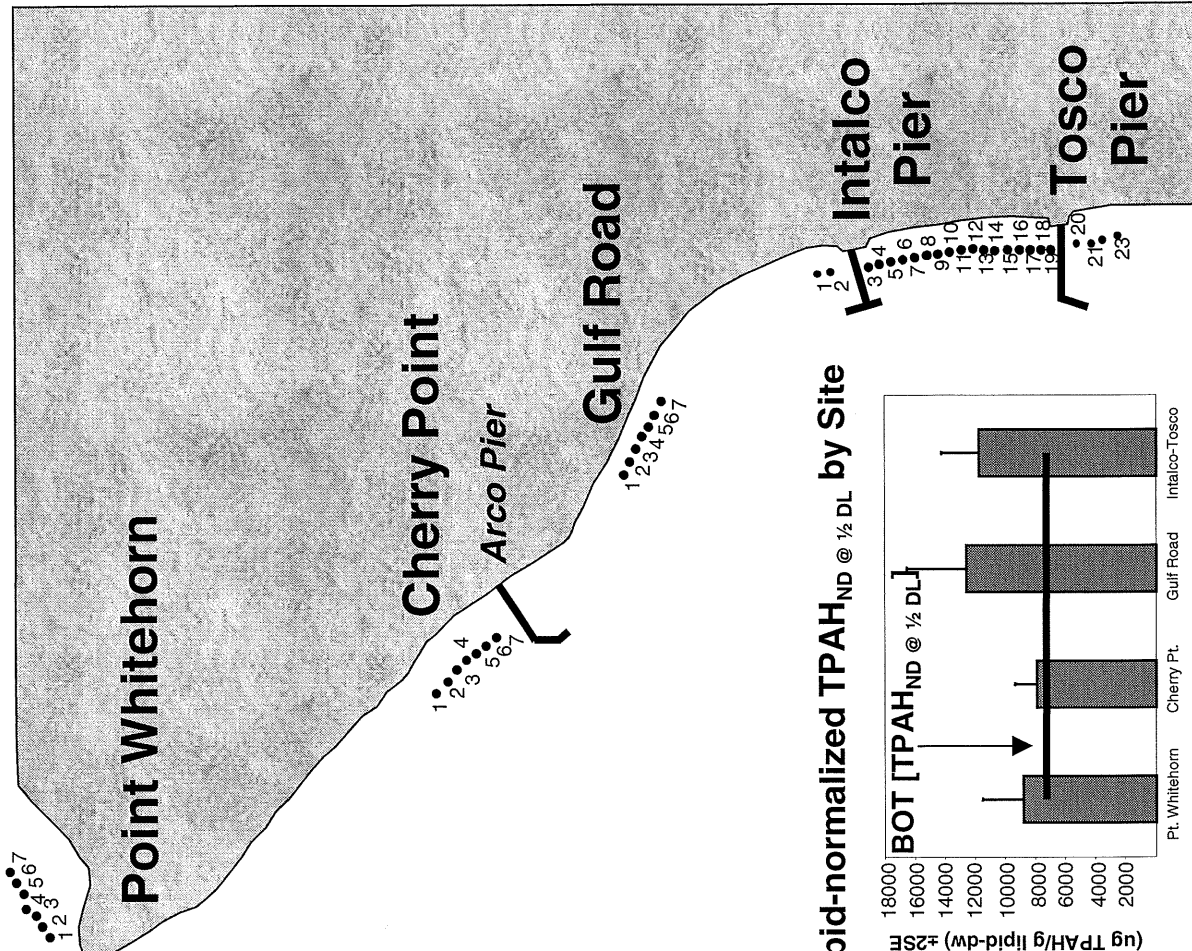


TPAH_{ND} @ ½ DL by Site



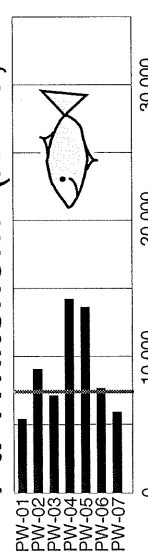
BOT [TPAH_{ND} @ ½ DL]

Figure 14A. TPAH concentration (ug/kg-dw; Non-detects included at ½ DL) in mussel tissues by station and site. * = herring egg deployment site. * = stations not included in average.

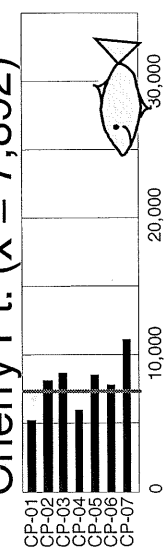


Lipid-normalized TPAH_{ND} @ ½ DL by Station (ug TPAH/g lipid-dw)

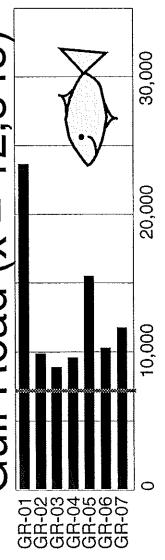
Pt. Whitehorn ($\bar{x} = 8,795$)



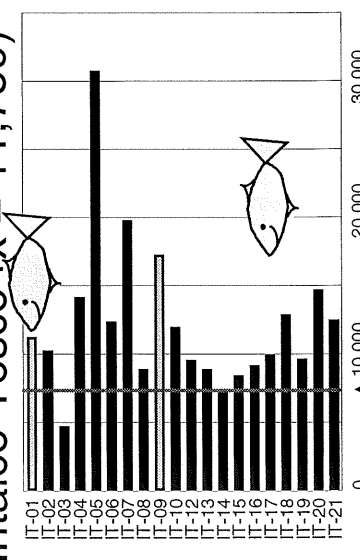
Cherry Pt. ($\bar{x} = 7,892$)



Gulf Road ($\bar{x} = 12,545$)



Intalco-Tosco ($\bar{x} = 11,759$)



Lipid-normalized TPAH_{ND} @ ½ DL by Site

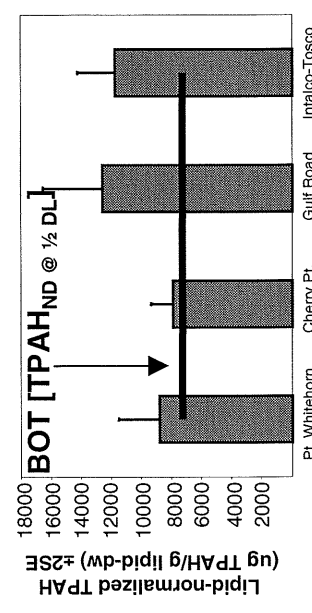


Figure 14B. Lipid-normalized TPAH concentration (ug TPAH/g lipid-dw; Non-detects included at ½ DL) in mussel tissues by station and site. = herring egg deployment site. * = stations not included in average.

TPAH₀ for NDs by Station (ug/kg dry wt)

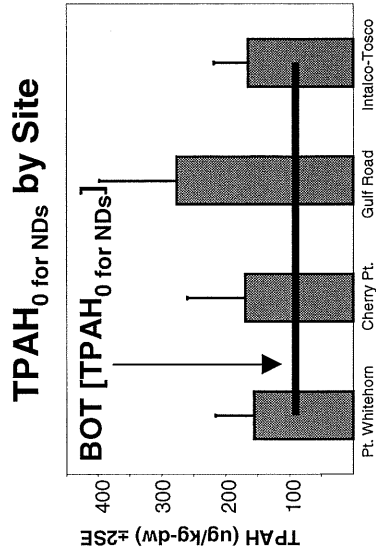
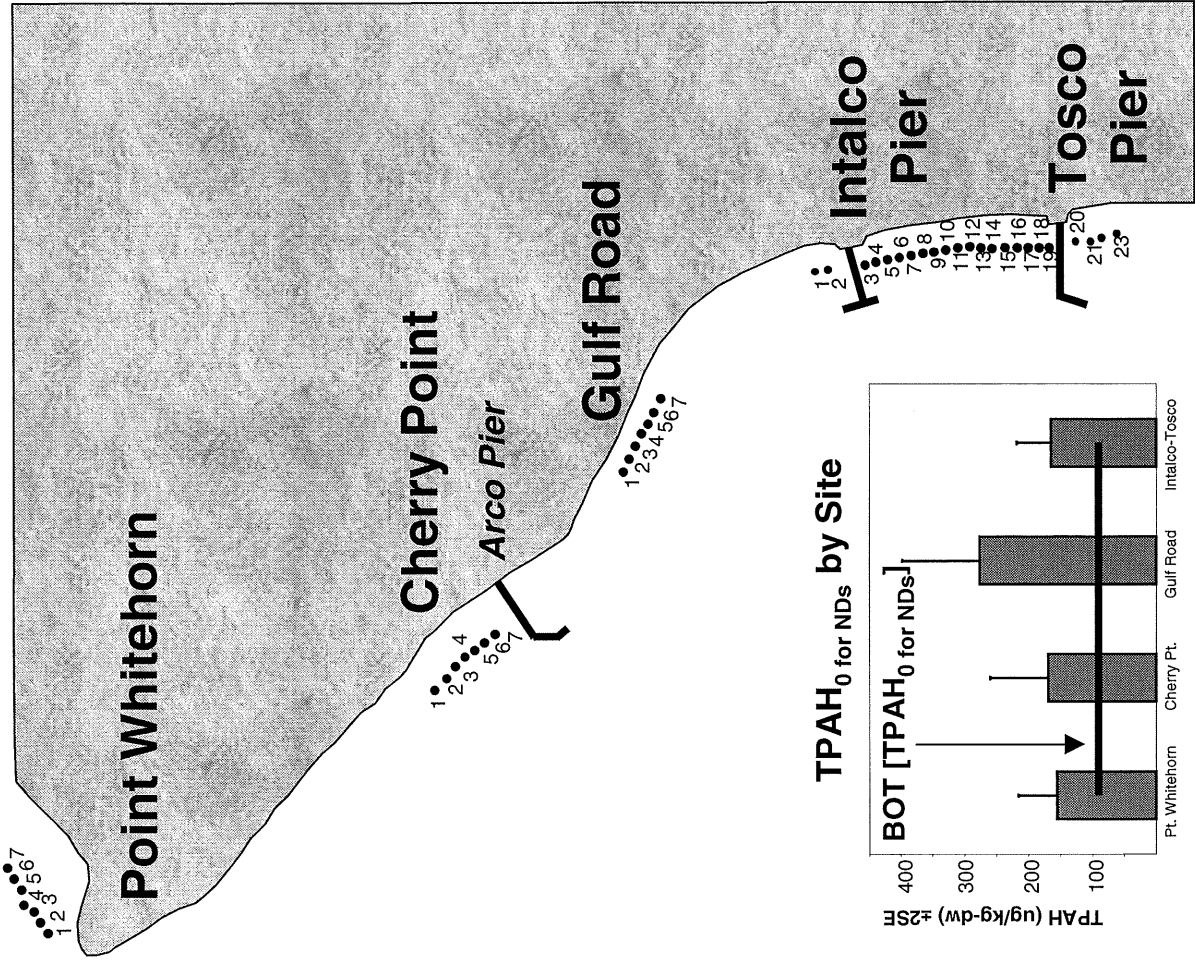
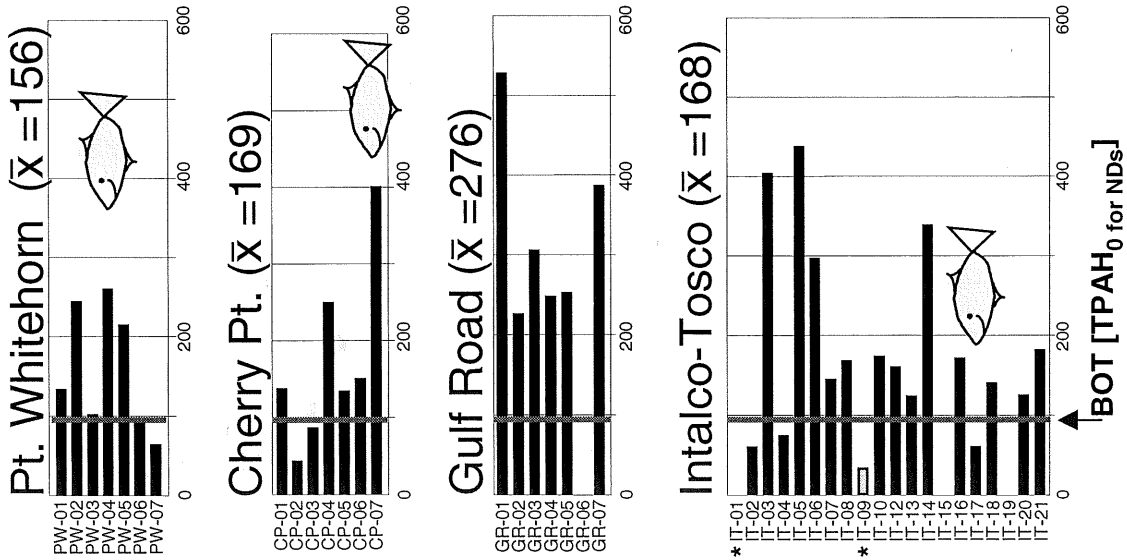
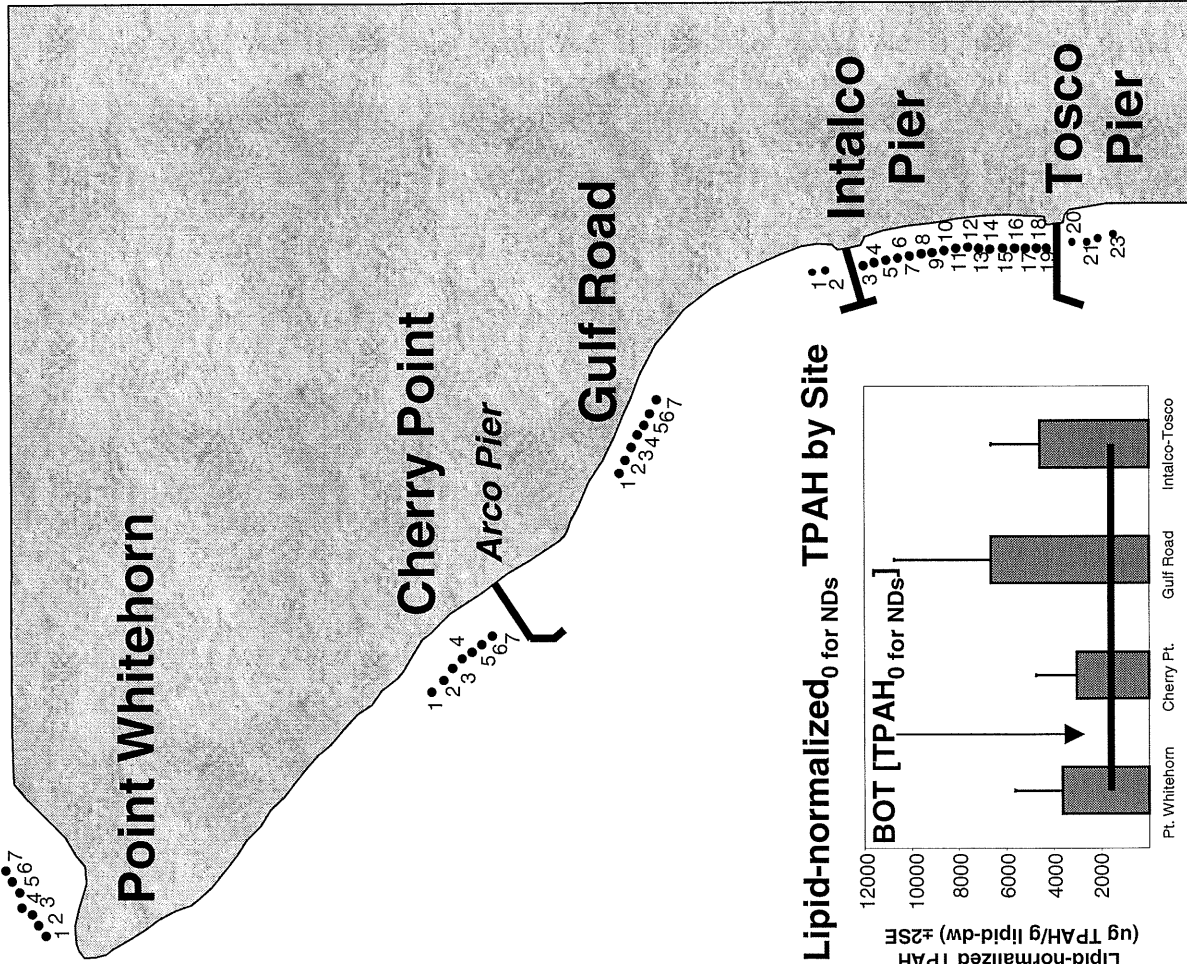
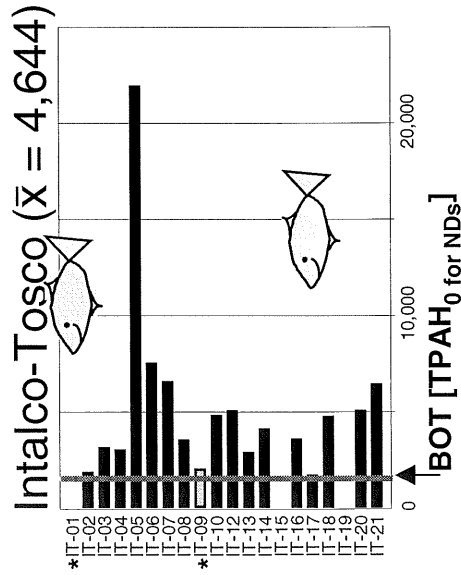
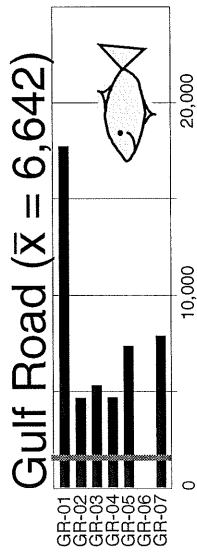
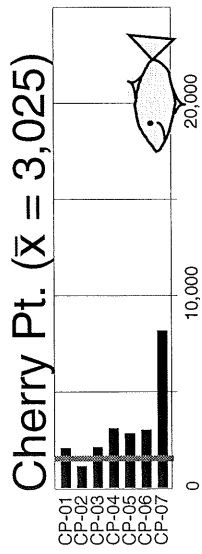
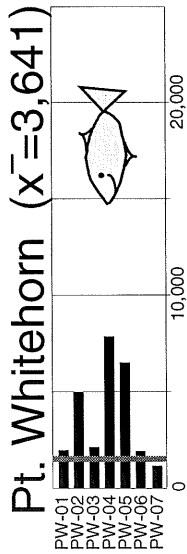


Figure 14C. TPAH concentration (ug/kg-dw; "0" for non-detects in total) in mussel tissues by station and site. = herring egg deployment site. * = stations not included in average.

Lipid-normalized TPAH₀ for NDs by Station
(ug TPAH/g lipid-dw)



Lipid-normalized₀ for NDs TPAH by Site

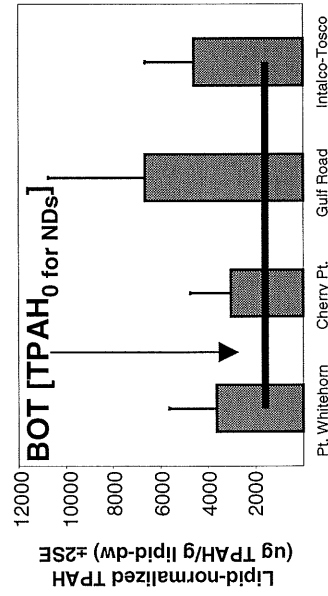


Figure 14D. Lipid-normalized TPAH concentration (ug TPAH/g lipid-dw; "0" for non-detects in total) in mussel tissues by station and site. * = herring egg deployment site. * = stations not included in average.

**Table 10. PAH compounds (ug/kg-dw) measured in mussel tissues at the start of the test
and after 61 days exposure period**

| Chemical | T0-01 | T0-02 | T0-03 | PW-01 | PW-02 | PW-03 | PW-04 | PW-05 | PW-06 | PW-07 |
|--|---------|---------|----------|----------|----------|----------|----------|----------|----------|----------|
| C1-Naphthalenes | 41.11 U | 8.89 U | 20.56 U | 28.57 NJ | 18.18 UJ | 18.64 NJ | 27.14 NJ | 61.90 NJ | 28.00 NJ | 34.00 NJ |
| C2 -Naphthalenes | 41.11 U | 12.78 U | 38.89 U | 20.48 NJ | 20.91 UJ | 17.27 U | 21.43 NJ | 26.19 NJ | 25.00 U | 19.00 U |
| C3 -Naphthalenes | 20.56 U | 12.22 U | 38.89 U | 19.05 U | 14.09 UJ | 20.91 NJ | 27.62 NJ | 21.90 U | 21.50 NJ | 19.50 U |
| C4 -Naphthalenes | 20.56 U | 17.22 U | 19.44 U | 19.05 U | 16.82 U | 17.27 U | 16.67 U | 18.57 U | 20.00 U | 19.50 U |
| C1-Fluorenes | 41.11 U | 10.56 U | 38.89 U | 19.05 U | 32.73 UJ | 24.55 U | 47.14 NJ | 14.76 U | 11.50 U | 19.50 U |
| C2-Fluorenes | 20.56 U | 17.22 U | 19.44 U | 19.05 U | 16.82 U | 17.27 U | 16.67 U | 18.57 U | 20.00 U | 19.50 U |
| C3-Fluorenes | 20.56 U | 17.22 U | 19.44 U | 19.05 U | 16.82 U | 17.27 U | 16.67 U | 18.57 U | 20.00 U | 19.50 U |
| C1-Dibenzothiophenes | 20.56 U | 17.22 U | 19.44 U | 19.05 U | 16.82 U | 17.27 U | 16.67 U | 18.57 U | 20.00 U | 19.50 U |
| C2-Dibenzothiophenes | 20.56 U | 4.33 U | 19.44 U | 19.05 U | 5.45 U | 17.27 U | 8.57 U | 18.57 U | 5.50 U | 19.50 U |
| C3-Dibenzothiophenes | 20.56 U | 17.22 U | 19.44 U | 19.05 U | 16.82 U | 17.27 U | 16.67 U | 18.57 U | 20.00 U | 19.50 U |
| C1-Phenanthrenes/Anthracenes | 14.44 U | 12.22 U | 38.89 U | 17.62 U | 27.27 NJ | 22.27 U | 34.76 U | 13.33 U | 28.00 U | 12.00 U |
| C2-Phenanthrenes/Anthracenes | 20.56 U | 17.22 U | 19.44 U | 19.05 U | 17.73 U | 17.27 U | 42.86 U | 18.57 U | 17.00 U | 19.50 U |
| C3-Phenanthrenes/Anthracenes | 20.56 U | 17.22 U | 19.44 U | 19.05 U | 16.82 U | 17.27 U | 16.67 U | 18.57 U | 20.00 U | 19.50 U |
| C4-Phenanthrenes/Anthracenes | 20.56 U | 17.22 U | 19.44 U | 19.05 U | 9.55 U | 17.27 U | 15.24 U | 18.57 U | 20.00 U | 19.50 U |
| C1-Fluoranthene/Pyrene | 28.89 U | 17.22 U | 66.67 NJ | 19.05 U | 16.82 U | 17.27 U | 16.67 U | 18.57 U | 20.00 U | 19.50 U |
| C1-Chrysenes | 20.56 U | 17.22 U | 19.44 U | 19.05 U | 16.82 U | 17.27 U | 16.67 U | 18.57 U | 20.00 U | 19.50 U |
| C2-Chrysenes | 20.56 U | 17.22 U | 19.44 U | 19.05 U | 16.82 U | 17.27 U | 16.67 U | 18.57 U | 20.00 U | 19.50 U |
| C3-Chrysenes | 20.56 U | 17.22 U | 19.44 U | 19.05 U | 16.82 U | 17.27 U | 16.67 U | 18.57 U | 20.00 U | 19.50 U |
| C4-Chrysenes | 5.11 U | 4.33 U | 4.89 U | 4.71 U | 4.18 U | 4.36 U | 4.14 U | 4.67 U | 5.00 U | 4.85 U |
| Anthracene | 5.11 U | 4.33 U | 4.89 U | 4.71 U | 4.18 U | 4.36 U | 4.14 U | 4.67 U | 5.00 U | 4.85 U |
| 4,6-Dimethyldibenzothiophene | 5.11 U | 16.11 U | 4.89 U | 4.71 U | 15.91 U | 4.36 U | 15.71 U | 4.67 U | 19.00 U | 4.85 U |
| Pyrene | 10.00 U | 7.22 | 17.78 | 5.71 U | 5.45 U | 5.45 U | 6.19 U | 4.76 U | 7.50 U | 4.85 U |
| Dibenzofuran | 14.44 U | 11.11 U | 15.56 U | 15.71 | 17.27 UJ | 14.55 U | 17.14 | 14.76 U | 15.50 U | 15.00 U |
| Dibenzothiophene | 5.11 U | 4.33 U | 4.89 U | 5.24 U | 5.45 U | 4.36 U | 4.14 U | 18.57 U | 5.00 U | 4.85 U |
| Phenanthrene, 3,6-dimethyl- | 5.11 U | 4.33 U | 4.89 U | 4.71 U | 5.45 UJ | 4.36 U | 8.57 U | 4.67 U | 5.00 U | 4.85 U |
| 9H-Fluorene, 1-methyl | 10.00 U | 6.67 U | 10.00 U | 10.48 U | 20.91 UJ | 15.45 U | 19.05 | 4.67 U | 11.50 U | 4.85 U |
| Benzo(ghi)perylene | 5.11 U | 4.33 U | 4.89 U | 4.71 U | 4.18 U | 4.36 U | 4.14 U | 4.67 U | 5.00 U | 4.85 U |
| Benzo[e]pyrene | 7.78 U | 5.44 | 13.89 | 4.71 U | 4.18 U | 4.36 U | 4.14 U | 4.67 U | 5.00 U | 4.85 U |
| Indeno(1,2,3-cd)pyrene | 5.11 U | 4.33 U | 4.89 U | 4.71 U | 4.18 U | 4.36 U | 4.14 U | 4.67 U | 5.00 U | 4.85 U |
| Perylene | 5.11 U | 4.33 U | 4.89 U | 4.71 U | 4.18 U | 4.36 U | 4.14 U | 4.67 U | 5.00 U | 4.85 U |
| Benzo(b)fluoranthene | 6.67 U | 4.72 | 12.22 | 4.71 U | 4.18 U | 4.36 U | 4.14 U | 4.67 U | 5.00 U | 4.85 U |
| Fluoranthene | 17.78 U | 13.33 | 31.67 | 8.57 U | 7.73 U | 8.18 U | 8.57 U | 7.62 U | 10.00 U | 8.00 U |
| Benzo(k)fluoranthene | 5.11 U | 12.22 | 20.56 | 4.71 U | 4.18 U | 4.36 U | 4.14 U | 4.67 U | 5.00 U | 4.85 U |
| Acenaphthylene | 5.11 U | 4.33 U | 4.89 U | 4.71 U | 4.18 U | 4.36 U | 4.14 U | 4.67 U | 5.00 U | 4.85 U |
| Chrysene | 20.56 | 15.56 | 31.67 | 13.81 U | 12.27 U | 4.36 U | 4.14 U | 4.67 U | 16.50 U | 14.50 U |
| 1,6,7-Trimethylnaphthalene | 5.11 U | 10.56 U | 4.89 U | 4.71 U | 24.55 UJ | 4.36 U | 13.33 | 4.67 U | 5.00 U | 4.85 U |
| 2-Methylphenanthrene | 6.11 U | 5.56 U | 10.00 U | 6.67 U | 9.55 | 9.09 U | 7.62 U | 6.19 U | 9.50 U | 4.95 U |
| 2-Methylfluoranthene | 5.11 U | 4.33 U | 4.89 U | 4.71 U | 4.18 U | 4.36 U | 4.14 U | 4.67 U | 5.00 U | 4.85 U |
| Chrysene, 5-methyl- | 5.11 U | 4.33 U | 4.89 U | 4.71 U | 4.18 U | 4.36 U | 4.14 U | 4.67 U | 5.00 U | 4.85 U |
| Retene | 5.11 U | 4.33 U | 4.89 U | 4.71 U | 10.00 U | 4.36 U | 11.90 U | 4.67 U | 5.00 U | 4.85 U |
| Benzo(a)pyrene | 5.11 U | 4.33 U | 4.89 U | 4.71 U | 4.18 U | 4.36 U | 4.14 U | 4.67 U | 5.00 U | 4.85 U |
| Dibenzo(a,h)anthracene | 5.11 U | 4.33 U | 4.89 U | 4.71 U | 4.18 U | 4.36 U | 4.14 U | 4.67 U | 5.00 U | 4.85 U |
| Benzo(a)anthracene | 5.11 U | 4.33 U | 11.67 U | 4.71 U | 4.18 U | 4.36 U | 4.14 U | 4.67 U | 5.00 U | 4.85 U |
| 2,6-Dimethylnaphthalene | 20.56 U | 17.22 U | 38.89 U | 11.43 J | 14.09 U | 17.27 J | 11.90 J | 13.81 J | 12.00 U | 12.00 U |
| 1-Methylphenanthrene | 8.33 U | 6.67 U | 10.00 U | 8.10 U | 8.64 U | 9.09 U | 10.48 U | 7.62 U | 13.00 U | 8.00 U |
| Acenaphthene | 5.11 U | 9.44 U | 14.44 U | 12.38 | 14.09 U | 4.36 U | 8.10 U | 13.33 | 15.50 U | 13.50 U |
| Phenanthrene | 18.89 U | 14.44 U | 25.56 U | 22.38 U | 28.64 UJ | 27.73 | 27.14 | 16.67 U | 23.00 U | 16.50 U |
| Fluorene | 5.11 U | 4.33 U | 4.89 U | 4.71 U | 14.09 U | 0.86 J | 3.19 J | 4.67 U | 5.00 U | 4.85 U |
| Carbazol | | | | | 4.18 U | 4.36 U | 4.14 U | | 5.00 U | |
| 1-Methylnaphthalene | 5.11 U | 4.33 U | 10.00 U | 4.48 J | 14.09 U | 17.27 U | 4.62 | 13.33 | 3.25 J | 5.50 |
| Naphthalene | 10.00 U | 4.33 U | 10.00 U | 14.76 | 9.09 UJ | 8.18 U | 12.38 | 35.24 | 17.00 | 18.00 U |
| 2-Methylnaphthalene | 10.00 U | 6.67 U | 15.00 U | 20.00 | 12.73 UJ | 13.64 | 19.05 | 44.29 | 18.50 | 22.00 |
| 2-Chloronaphthalene | 5.11 U | 4.33 U | 4.89 U | 4.71 U | 4.18 U | 4.36 U | 4.14 U | 4.67 U | 5.00 U | 4.85 U |
| 1,1'-Biphenyl | 20.56 U | 17.22 U | 19.44 U | 2.33 J | 14.09 U | 17.27 U | 5.71 J | 3.86 J | 5.00 U | 19.50 U |
| Total PAHs* | 20.56 | 58.50 | 194.44 | 130.14 | 241.36 | 99.05 | 256.86 | 211.95 | 88.25 | 61.50 |
| Lipid Normalized TPAH as ug | 308 | 1755 | 2692 | 1822 | 4827 | 1981 | 7706 | 6359 | 1765 | 1025 |
| PAH/g lipid on a dry basis | | | | | | | | | | |
| *TPAH calculated as the sum of all PAHs, including homologs. "0" for Non-detects | | | | | | | | | | |
| Lipid-normalized TPAH = [Tissue TPAH ug/kg-dw]/%lipid as decimal fraction | | | | | | | | | | |

| (Table 10 Cont) Chemical | CP-01 | CP-02 | CP-03 | CP-04 | CP-05 | CP-06 | CP-07 |
|--|----------|----------|----------|----------|----------|----------|----------|
| C1-Naphthalenes | 40.42 NJ | 22.63 NJ | 46.19 NJ | 48.00 NJ | 30.00 NJ | 30.00 NJ | 50.00 NJ |
| C2 -Naphthalenes | 25.83 NJ | 15.26 U | 22.86 U | 27.00 NJ | 19.00 U | 19.05 U | 28.18 NJ |
| C3 -Naphthalenes | 21.67 U | 21.05 U | 22.86 U | 24.50 NJ | 16.00 U | 18.57 U | 26.82 NJ |
| C4 -Naphthalenes | 16.25 U | 21.05 U | 18.57 U | 20.00 U | 19.50 U | 18.57 U | 16.82 U |
| C1-Fluorenes | 16.25 U | 21.05 U | 18.57 U | 21.00 U | 16.00 U | 24.76 U | 32.27 NJ |
| C2-Fluorenes | 16.25 U | 21.05 U | 18.57 U | 20.00 U | 19.50 U | 18.57 U | 16.82 U |
| C3-Fluorenes | 16.25 U | 21.05 U | 18.57 U | 20.00 U | 19.50 U | 18.57 U | 16.82 U |
| C1-Dibenzothiophenes | 16.25 U | 21.05 U | 18.57 U | 20.00 U | 19.50 U | 18.57 U | 16.82 U |
| C2-Dibenzothiophenes | 16.25 U | 21.05 U | 18.57 U | 20.00 U | 19.50 U | 17.62 U | 5.91 U |
| C3-Dibenzothiophenes | 16.25 U | 21.05 U | 18.57 U | 20.00 U | 19.50 U | 18.57 U | 16.82 U |
| C1-Phenanthrenes/Anthracenes | 16.67 U | 13.16 U | 21.90 U | 32.00 U | 19.50 U | 29.52 U | 28.64 NJ |
| C2-Phenanthrenes/Anthracenes | 16.25 U | 21.05 U | 18.57 U | 20.00 U | 15.00 U | 17.62 U | 16.82 U |
| C3-Phenanthrenes/Anthracenes | 16.25 U | 21.05 U | 18.57 U | 20.00 U | 19.50 U | 18.57 U | 16.82 U |
| C4-Phenanthrenes/Anthracenes | 16.25 U | 21.05 U | 18.57 U | 20.00 U | 10.00 U | 12.38 U | 14.55 U |
| C1-Fluoranthene/Pyrene | 16.25 U | 21.05 U | 18.57 U | 20.00 U | 19.50 U | 18.57 U | 16.82 U |
| C1-Chrysenes | 16.25 U | 21.05 U | 18.57 U | 20.00 U | 19.50 U | 18.57 U | 16.82 U |
| C2-Chrysenes | 16.25 U | 21.05 U | 18.57 U | 20.00 U | 19.50 U | 18.57 U | 16.82 U |
| C3-Chrysenes | 16.25 U | 21.05 U | 18.57 U | 20.00 U | 19.50 U | 18.57 U | 16.82 U |
| C4-Chrysenes | 4.04 U | 5.26 U | 4.67 U | 5.00 U | 4.90 U | 4.71 U | 4.23 U |
| Anthracene | 4.04 U | 5.26 U | 4.67 U | 5.00 U | 4.90 U | 4.71 U | 4.23 U |
| 4,6-Dimethyldibenzothiophene | 15.42 U | 5.26 U | 4.67 U | 5.00 U | 18.50 U | 17.62 U | 15.91 U |
| Pyrene | 6.67 U | 6.32 U | 7.14 U | 8.50 | 7.50 U | 8.57 U | 7.73 |
| Dibenzofuran | 13.75 U | 15.26 U | 14.76 U | 16.50 U | 15.00 | 14.76 | 15.91 |
| Dibenzothiophene | 4.04 U | 5.26 U | 4.67 U | 6.00 U | 4.90 U | 4.76 U | 5.91 |
| Phenanthrene, 3,6-dimethyl- | 4.04 U | 5.26 U | 4.67 U | 5.00 U | 19.50 U | 17.62 U | 4.23 U |
| 9H-Fluorene, 1-methyl- | 4.04 U | 5.26 U | 4.67 U | 15.00 U | 10.00 U | 11.90 U | 18.18 |
| Benzo(ghi)perylene | 4.04 U | 5.26 U | 4.67 U | 5.00 U | 9.50 U | 4.71 U | 4.23 U |
| Benzo[e]pyrene | 4.04 U | 5.26 U | 4.67 U | 5.00 U | 6.50 U | 4.76 U | 4.23 U |
| Indeno(1,2,3-cd)pyrene | 4.04 U | 5.26 U | 4.67 U | 5.00 U | 4.90 U | 4.71 U | 4.23 U |
| Perylene | 4.04 U | 5.26 U | 4.67 U | 5.00 U | 10.00 U | 4.71 U | 4.23 U |
| Benzo(b)fluoranthene | 4.04 U | 5.26 U | 4.67 U | 5.00 U | 7.00 U | 4.71 U | 4.23 U |
| Fluoranthene | 9.58 U | 9.47 U | 9.52 U | 16.50 | 11.00 U | 13.33 | 13.64 |
| Benzo(k)fluoranthene | 4.04 U | 5.26 U | 4.67 U | 5.00 U | 16.00 | 4.71 U | 4.23 U |
| Acenaphthylene | 4.04 U | 5.26 U | 4.67 U | 5.00 U | 6.00 U | 5.71 | 4.23 U |
| Chrysene | 13.33 U | 16.84 U | 15.24 U | 5.00 U | 16.00 | 14.29 U | 4.23 U |
| 1,6,7-Trimethylnaphthalene | 11.25 U | 5.26 U | 4.67 U | 5.00 U | 12.50 U | 4.71 U | 11.82 |
| 2-Methylphenanthrene | 6.67 U | 5.26 U | 7.62 U | 10.50 U | 7.50 U | 10.48 U | 10.91 |
| 2-Methylfluoranthene | 4.04 U | 5.26 U | 4.67 U | 5.00 U | 4.90 U | 4.71 U | 4.23 U |
| Chrysene, 5-methyl- | 4.04 U | 5.26 U | 4.67 U | 5.00 U | 4.90 U | 4.71 U | 4.23 U |
| Retene | 4.04 U | 5.26 U | 4.67 U | 5.00 U | 9.50 U | 10.95 U | 14.55 U |
| Benzo(a)pyrene | 4.04 U | 5.26 U | 4.67 U | 5.00 U | 8.00 U | 4.71 U | 4.23 U |
| Dibenzo(a,h)anthracene | 4.04 U | 5.26 U | 4.67 U | 5.00 U | 25.00 U | 4.71 U | 4.23 U |
| Benzo(a)anthracene | 7.92 U | 9.47 U | 4.67 U | 5.00 U | 9.50 U | 4.71 U | 4.23 U |
| 2,6-Dimethylnaphthalene | 12.50 J | 11.05 U | 12.38 U | 14.00 J | 11.50 U | 11.90 U | 12.73 J |
| 1-Methylphenanthrene | 7.50 U | 5.26 U | 8.10 U | 11.00 U | 9.00 U | 10.00 U | 9.55 |
| Acenaphthene | 11.25 U | 15.26 U | 4.67 U | 5.00 U | 13.50 | 12.86 | 13.64 |
| Phenanthrene | 18.75 U | 17.89 U | 20.00 U | 40.00 | 21.00 U | 27.14 | 32.73 |
| Fluorene | 1.04 J | 5.26 U | 4.67 U | 15.00 U | 4.90 U | 4.71 U | 2.18 J |
| Carbazol | | | | 5.00 U | | | 4.23 U |
| 1-Methylnaphthalene | 8.33 | 2.11 J | 8.57 | 11.50 | 4.15 J | 5.24 | 12.27 |
| Naphthalene | 20.83 | 11.58 U | 24.29 U | 25.00 | 15.50 | 14.29 | 27.27 |
| 2-Methylnaphthalene | 26.25 | 16.32 | 29.05 | 32.00 | 22.00 | 21.90 | 33.18 |
| 2-Chloronaphthalene | 4.04 U | 5.26 U | 4.67 U | 5.00 U | 4.90 U | 4.71 U | 4.23 U |
| 1,1'-Biphenyl | 4.04 U | 21.05 U | 18.57 U | 20.00 U | 19.50 U | 2.86 J | 4.55 J |
| Total PAHs* | 135.21 | 41.05 | 83.81 | 247.00 | 132.15 | 148.10 | 398.09 |
| Lipid Normalized TPAH as ug PAH/g lipid on a dry basis | 1909 | 975 | 1956 | 2906 | 2643 | 2827 | 7962 |
| *TPAH calculated as the sum of all PAHs, including homologs, "0" for Non-detects | | | | | | | |
| Lipid-normalized TPAH = [Tissue TPAH ug/kg-dw]/%lipid as decimal fraction | | | | | | | |

| (Table 10 Cont) Chemical | GR-01 | GR-02 | GR-03 | GR-04 | GR-05 | GR-06 | GR-07 |
|--|----------|----------|----------|----------|----------|---------|----------|
| C1-Naphthalenes | 29.50 NJ | 36.82 NJ | 32.27 NJ | 63.64 NJ | 60.00 NJ | 28.50 U | 34.00 NJ |
| C2 -Naphthalenes | 23.00 NJ | 24.09 NJ | 18.18 NJ | 30.00 NJ | 27.00 NJ | 18.50 U | 18.00 NJ |
| C3 -Naphthalenes | 35.00 NJ | 16.36 U | 17.73 U | 35.91 NJ | 19.00 U | 22.50 U | 25.00 NJ |
| C4 -Naphthalenes | 19.00 U | 17.73 U | 17.73 U | 18.18 U | 19.00 U | 19.00 U | 19.00 U |
| C1-Fluorenes | 43.00 NJ | 32.73 U | 41.82 NJ | 22.73 U | 23.00 U | 14.00 U | 28.00 NJ |
| C2-Fluorenes | 19.00 U | 17.73 U | 17.73 U | 18.18 U | 19.00 U | 19.00 U | 19.00 U |
| C3-Fluorenes | 19.00 U | 17.73 U | 17.73 U | 18.18 U | 19.00 U | 19.00 U | 19.00 U |
| C1-Dibenzothiophenes | 19.00 U | 17.73 U | 17.73 U | 18.18 U | 19.00 U | 19.00 U | 19.00 U |
| C2-Dibenzothiophenes | 16.00 U | 16.82 U | 10.91 U | 18.18 U | 8.50 U | 19.00 U | 19.00 U |
| C3-Dibenzothiophenes | 19.00 U | 17.73 U | 17.73 U | 18.18 U | 19.00 U | 19.00 U | 19.00 U |
| C1-Phenanthrenes/Anthracenes | 50.00 NJ | 35.45 U | 29.09 U | 34.55 U | 39.50 U | 19.00 U | 40.50 NJ |
| C2-Phenanthrenes/Anthracenes | 50.00 NJ | 22.27 U | 21.82 U | 30.45 U | 37.50 U | 19.00 U | 32.50 NJ |
| C3-Phenanthrenes/Anthracenes | 19.00 U | 17.73 U | 17.73 U | 18.18 U | 19.00 U | 19.00 U | 19.00 U |
| C4-Phenanthrenes/Anthracenes | 27.00 NJ | 14.55 U | 17.73 U | 10.00 U | 15.00 U | 19.00 U | 18.50 U |
| C1-Fluoranthene/Pyrene | 19.00 U | 17.73 U | 17.73 U | 18.18 U | 19.00 U | 19.00 U | 19.00 U |
| C1-Chrysenes | 19.00 U | 17.73 U | 17.73 U | 18.18 U | 19.00 U | 19.00 U | 19.00 U |
| C2-Chrysenes | 19.00 U | 17.73 U | 17.73 U | 18.18 U | 19.00 U | 19.00 U | 19.00 U |
| C3-Chrysenes | 19.00 U | 17.73 U | 17.73 U | 18.18 U | 19.00 U | 19.00 U | 19.00 U |
| C4-Chrysenes | 4.80 U | 4.41 U | 17.73 U | 4.50 U | 4.80 U | 4.70 U | 4.70 U |
| Anthracene | 4.80 U | 4.41 U | 4.45 U | 4.50 U | 4.80 U | 4.70 U | 4.70 U |
| 4,6-Dimethyldibenzothiophene | 18.50 U | 16.82 U | 17.27 U | 4.50 U | 18.50 U | 4.70 U | 4.70 U |
| Pyrene | 10.50 | 9.55 U | 11.82 | 8.64 U | 10.50 U | 6.00 U | 14.50 |
| Dibenzofuran | 18.50 | 15.00 | 14.55 | 13.64 U | 15.00 U | 13.50 U | 15.50 U |
| Dibenzothiophene | 7.00 | 5.45 U | 5.45 | 5.45 U | 5.50 U | 4.70 U | 6.00 U |
| Phenanthrene, 3,6-dimethyl- | 16.00 U | 16.82 U | 17.27 U | 4.50 U | 4.80 U | 4.70 U | 1.50 J |
| 9H-Fluorene, 1-methyl- | 30.50 | 19.09 U | 19.09 | 13.64 U | 15.50 U | 10.00 U | 16.50 U |
| Benzo(ghi)perylene | 4.80 U | 7.73 U | 4.45 U | 4.50 U | 7.50 U | 4.70 U | 4.70 U |
| Benzo[e]pyrene | 6.50 U | 6.82 | 5.45 | 5.45 U | 7.00 U | 4.70 U | 4.70 U |
| Indeno(1,2,3-cd)pyrene | 4.80 U | 4.41 U | 4.45 U | 4.50 U | 4.80 U | 4.70 U | 4.70 U |
| Perylene | 4.80 U | 4.41 U | 4.45 U | 4.50 U | 4.80 U | 4.70 U | 4.70 U |
| Benzo(b)fluoranthene | 4.80 U | 4.41 U | 4.45 U | 4.50 U | 6.00 U | 4.70 U | 4.70 U |
| Fluoranthene | 17.50 | 13.64 | 17.27 | 12.73 U | 14.00 U | 9.00 U | 24.00 |
| Benzo(k)fluoranthene | 4.80 U | 4.41 U | 4.45 U | 4.50 U | 14.50 U | 4.70 U | 4.70 U |
| Acenaphthylene | 4.80 U | 4.41 U | 7.27 | 4.50 U | 4.80 U | 4.70 U | 4.70 U |
| Chrysene | 16.50 | 15.45 | 15.00 | 14.55 U | 17.00 | 4.70 U | 17.00 |
| 1,6,7-Trimethylnaphthalene | 14.50 U | 11.82 U | 4.45 U | 13.18 | 4.80 U | 4.70 U | 13.00 |
| 2-Methylphenanthrene | 16.00 | 11.36 U | 11.36 U | 11.82 U | 13.50 U | 6.00 U | 14.00 |
| 2-Methylfluoranthene | 4.80 U | 4.41 U | 4.45 U | 4.50 U | 4.80 U | 4.70 U | 4.70 U |
| Chrysene, 5-methyl- | 4.80 U | 4.41 U | 4.45 U | 4.50 U | 4.80 U | 4.70 U | 4.70 U |
| Retene | 27.50 | 13.64 U | 13.18 U | 14.09 U | 15.00 U | 4.70 U | 18.50 U |
| Benzo(a)pyrene | 4.80 U | 4.41 U | 4.45 U | 4.50 U | 4.80 U | 4.70 U | 4.70 U |
| Dibenzo(a,h)anthracene | 4.80 U | 21.82 U | 4.45 U | 4.50 U | 4.80 U | 4.70 U | 4.70 U |
| Benzo(a)anthracene | 4.80 U | 7.73 U | 8.18 | 4.50 U | 9.00 U | 4.70 U | 10.00 |
| 2,6-Dimethylnaphthalene | 12.00 J | 11.82 J | 10.91 J | 15.45 J | 15.00 J | 12.50 U | 11.50 J |
| 1-Methylphenanthrene | 12.50 | 10.00 U | 9.55 U | 11.36 U | 11.00 U | 4.70 U | 12.00 |
| Acenaphthene | 15.00 | 12.27 | 14.09 | 11.36 U | 13.50 U | 4.70 U | 4.70 U |
| Phenanthrene | 48.00 | 32.27 | 38.18 | 30.45 U | 36.00 | 18.00 U | 46.00 |
| Fluorene | 2.55 J | 0.73 J | 1.09 J | 0.30 J | 1.90 J | 4.70 U | 1.70 J |
| Carbazol | 4.80 U | | | 4.50 U | | 4.70 U | 4.70 U |
| 1-Methylnaphthalene | 3.75 J | 5.91 | 5.00 | 13.18 | 14.50 | 4.90 U | 4.90 |
| Naphthalene | 12.00 J | 18.64 | 14.55 | 28.64 | 32.00 | 14.00 U | 15.50 |
| 2-Methylnaphthalene | 18.50 J | 27.27 | 20.91 | 41.36 | 43.50 | 22.00 U | 21.00 |
| 2-Chloronaphthalene | 4.80 U | 4.41 U | 4.45 U | 4.50 U | 4.80 U | 4.70 U | 4.70 U |
| 1,1'-Biphenyl | 19.00 U | 2.77 J | 2.41 J | 3.95 J | 3.85 J | 19.00 U | 19.00 U |
| Total PAHs* | 525.80 | 223.50 | 303.50 | 245.61 | 250.75 | 0.00 | 384.60 |
| Lipid Normalized TPAH as ug PAH/g lipid on a dry basis | 17527 | 4470 | 5136 | 4503 | 7164 | 0 | 7692 |

*TPAH calculated as the sum of all PAHs, including homologs, "0" for Non-detects

Lipid-normalized TPAH = [Tissue TPAH ug/kg-dw]/%lipid as decimal fraction

| (Table 10 Cont) Chemical | IT-01 | IT-02 | IT-03 | IT-04 | IT-05 | IT-06 | IT-07 | IT-08 | IT-09 | IT-10 |
|------------------------------|---------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| C1-Naphthalenes | 7.80 U | 30.95 NJ | 45.00 NJ | 30.50 NJ | 46.00 NJ | 45.00 NJ | 59.26 NJ | 33.68 NJ | 13.67 U | 36.32 NJ |
| C2 -Naphthalenes | 8.29 U | 16.19 U | 27.50 NJ | 19.00 U | 26.00 NJ | 28.00 NJ | 25.93 U | 23.68 NJ | 14.67 NJ | 22.11 NJ |
| C3 -Naphthalenes | 9.76 U | 11.43 U | 29.00 NJ | 19.50 U | 27.50 NJ | 21.50 NJ | 27.78 NJ | 25.79 NJ | 18.00 U | 26.84 NJ |
| C4 -Naphthalenes | 9.76 U | 18.10 U | 19.50 U | 19.50 U | 18.50 U | 18.00 U | 14.81 U | 20.00 U | 18.00 U | 20.53 U |
| C1-Fluorenes | 8.54 U | 10.48 U | 27.50 NJ | 19.50 U | 41.00 NJ | 18.50 U | 44.44 U | 15.26 U | 14.67 U | 13.68 U |
| C2-Fluorenes | 9.76 U | 18.10 U | 19.50 U | 19.50 U | 18.50 U | 18.00 U | 14.81 U | 20.00 U | 18.00 U | 20.53 U |
| C3-Fluorenes | 9.76 U | 18.10 U | 19.50 U | 19.50 U | 18.50 U | 18.00 U | 14.81 U | 20.00 U | 18.00 U | 20.53 U |
| C1-Dibenzothiophenes | 9.76 U | 18.10 U | 19.50 U | 19.50 U | 18.50 U | 18.00 U | 14.81 U | 20.00 U | 18.00 U | 20.53 U |
| C2-Dibenzothiophenes | 2.68 U | 18.10 U | 19.50 U | 19.50 U | 6.00 NJ | 4.50 U | 14.07 U | 20.00 U | 18.00 U | 20.53 U |
| C3-Dibenzothiophenes | 9.76 U | 18.10 U | 19.50 U | 19.50 U | 18.50 U | 18.00 U | 14.81 U | 20.00 U | 18.00 U | 20.53 U |
| C1-Phenanthrenes/Anthracenes | 12.68 U | 15.71 U | 45.00 NJ | 11.50 U | 38.00 NJ | 25.50 U | 31.11 U | 16.84 U | 17.33 U | 27.37 U |
| C2-Phenanthrenes/Anthracenes | 8.29 U | 18.10 U | 19.50 U | 19.50 U | 18.00 U | 17.50 U | 27.04 U | 20.00 U | 18.00 U | 17.89 U |
| C3-Phenanthrenes/Anthracenes | 9.76 U | 18.10 U | 19.50 U | 19.50 U | 18.50 U | 18.00 U | 14.81 U | 20.00 U | 18.00 U | 20.53 U |
| C4-Phenanthrenes/Anthracenes | 9.76 U | 18.10 U | 19.50 U | 19.50 U | 14.00 U | 11.00 NJ | 15.93 U | 20.00 U | 18.00 U | 12.11 NJ |
| C1-Fluoranthene/Pyrene | 9.76 U | 18.10 U | 19.50 U | 19.50 U | 18.50 U | 18.00 U | 14.81 U | 20.00 U | 18.00 U | 20.53 U |
| C1-Chrysenes | 9.76 U | 18.10 U | 19.50 U | 19.50 U | 18.50 U | 18.00 U | 14.81 U | 20.00 U | 18.00 U | 20.53 U |
| C2-Chrysenes | 9.76 U | 18.10 U | 19.50 U | 19.50 U | 18.50 U | 18.00 U | 14.81 U | 20.00 U | 18.00 U | 20.53 U |
| C3-Chrysenes | 9.76 U | 18.10 U | 19.50 U | 19.50 U | 18.50 U | 18.00 U | 14.81 U | 20.00 U | 18.00 U | 20.53 U |
| C4-Chrysenes | 2.41 U | 4.48 U | 4.90 U | 4.95 U | 4.65 U | 4.50 U | 3.70 U | 5.00 U | 4.33 U | 5.11 U |
| Anthracene | 2.41 U | 4.48 U | 4.90 U | 4.95 U | 4.65 U | 4.50 U | 3.70 U | 5.00 U | 4.33 U | 5.11 U |
| 4,6-Dimethyldibenzothiophene | 9.02 U | 4.48 U | 4.90 U | 4.95 U | 18.00 U | 17.00 U | 14.07 U | 5.00 U | 4.33 U | 5.11 U |
| Pyrene | 4.39 U | 11.43 U | 10.50 U | 6.50 U | 9.00 | 10.00 | 9.26 U | 6.32 U | 5.00 U | 8.95 U |
| Dibenzofuran | 7.32 U | 13.81 U | 16.00 | 14.00 U | 17.50 | 13.50 | 14.81 U | 14.21 U | 4.33 U | 14.21 U |
| Dibenzothiophene | 2.41 U | 4.48 U | 6.50 U | 4.95 U | 7.00 | 4.75 U | 4.81 U | 5.00 U | 4.33 U | 5.26 U |
| Phenanthrene, 3,6-dimethyl- | 2.41 U | 4.48 U | 4.90 U | 4.95 U | 11.00 U | 5.00 U | 2.00 U | 5.00 U | 4.33 U | 5.11 U |
| 9H-Fluorene, 1-methyl- | 5.85 U | 8.57 U | 16.50 | 4.95 U | 23.00 | 11.50 U | 18.15 U | 11.05 U | 10.67 U | 11.05 U |
| Benzo(ghi)perylene | 2.41 U | 4.48 U | 4.90 U | 4.95 U | 4.65 U | 7.00 U | 3.70 U | 5.00 U | 4.33 U | 5.11 U |
| Benzo[e]pyrene | 2.68 U | 6.67 U | 4.90 U | 5.50 U | 6.00 U | 5.50 U | 3.70 U | 5.26 U | 4.33 U | 5.79 U |
| Indeno(1,2,3-cd)pyrene | 2.41 U | 4.48 U | 4.90 U | 4.95 U | 4.65 U | 4.50 U | 3.70 U | 5.00 U | 4.33 U | 5.11 U |
| Perylene | 2.41 U | 4.48 U | 4.90 U | 4.95 U | 4.65 U | 4.50 U | 3.70 U | 5.00 U | 4.33 U | 5.11 U |
| Benzo(b)fluoranthene | 2.41 U | 4.48 U | 4.90 U | 4.95 U | 4.65 U | 4.50 U | 5.19 U | 5.00 U | 4.33 U | 5.11 U |
| Fluoranthene | 5.37 U | 11.90 U | 22.00 | 9.50 U | 13.00 | 13.50 | 12.96 U | 9.47 U | 7.00 U | 13.16 U |
| Benzo(k)fluoranthene | 2.41 U | 4.48 U | 4.90 U | 4.95 U | 4.65 U | 4.50 U | 3.70 U | 5.00 U | 4.33 U | 5.11 U |
| Acenaphthylene | 2.41 U | 4.48 U | 4.90 U | 4.95 U | 4.65 U | 4.50 U | 3.70 U | 5.00 U | 4.33 U | 5.11 U |
| Chrysene | 7.32 U | 16.19 U | 16.00 U | 16.00 U | 15.00 U | 14.50 | 12.59 U | 14.74 U | 4.33 U | 15.79 |
| 1,6,7-Trimethylnaphthalene | 2.41 U | 23.33 U | 14.50 | 4.95 U | 26.00 | 11.50 | 3.70 U | 13.16 | 4.33 U | 13.16 |
| 2-Methylphenanthrene | 4.63 U | 6.19 U | 17.00 | 5.50 U | 13.50 | 9.50 U | 10.37 U | 6.84 U | 6.00 U | 8.95 U |
| 2-Methylfluoranthene | 2.41 U | 4.48 U | 4.90 U | 4.95 U | 4.65 U | 4.50 U | 3.70 U | 5.00 U | 4.33 U | 5.11 U |
| Chrysene, 5-methyl- | 2.41 U | 4.48 U | 4.90 U | 4.95 U | 4.65 U | 4.50 U | 3.70 U | 5.00 U | 4.33 U | 5.11 U |
| Retene | 2.41 U | 4.48 U | 4.90 U | 4.95 U | 14.00 U | 11.00 | 15.93 U | 8.42 U | 4.33 U | 12.11 U |
| Benzo(a)pyrene | 2.41 U | 4.48 U | 4.90 U | 4.95 U | 4.65 U | 4.50 U | 3.70 U | 5.00 U | 4.33 U | 5.11 U |
| Dibenzo(a,h)anthracene | 2.41 U | 4.48 U | 4.90 U | 4.95 U | 4.65 U | 4.50 U | 3.70 U | 5.00 U | 4.33 U | 5.11 U |
| Benzo(a)anthracene | 2.41 U | 9.05 U | 4.90 U | 4.95 U | 4.65 U | 4.50 U | 3.70 U | 8.42 U | 4.33 U | 8.42 U |
| 2,6-Dimethylnaphthalene | 9.76 U | 11.90 U | 13.00 J | 12.50 U | 14.00 J | 13.50 J | 13.33 J | 13.16 J | 18.00 U | 13.68 J |
| 1-Methylphenanthrene | 5.37 U | 7.62 U | 11.00 U | 4.95 U | 11.00 | 8.50 U | 8.89 U | 8.42 U | 4.33 U | 11.05 U |
| Acenaphthene | 6.59 U | 11.90 U | 14.50 | 12.50 U | 13.50 | 12.50 | 10.74 U | 14.21 | 4.33 U | 12.63 U |
| Phenanthrene | 14.15 U | 20.48 U | 44.50 | 13.50 U | 37.00 | 28.00 | 36.30 U | 20.53 U | 17.00 | 23.16 U |
| Fluorene | 2.41 U | 4.48 U | 2.25 J | 4.95 U | 2.40 J | 4.50 U | 4.07 U | 5.00 U | 4.33 U | 5.11 U |
| Carbazol | 2.41 U | 4.48 U | 4.90 U | 4.95 U | 4.65 U | 4.50 U | 3.70 U | 5.00 U | 4.33 U | 5.11 U |
| 1-Methylnaphthalene | 4.39 U | 3.95 J | 10.50 | 4.85 J | 9.00 | 8.00 | 13.70 U | 5.26 | 13.67 U | 8.42 |
| Naphthalene | 4.39 U | 13.81 U | 23.00 | 15.00 | 19.50 | 19.50 | 24.81 U | 13.16 | 5.67 U | 10.00 U |
| 2-Methylnaphthalene | 7.07 U | 22.86 | 34.00 | 21.50 | 30.00 | 30.00 | 35.93 | 23.68 | 10.00 U | 23.16 |
| 2-Chloronaphthalene | 2.41 U | 4.48 U | 4.90 U | 4.95 U | 4.65 U | 4.50 U | 3.70 U | 5.00 U | 4.33 U | 5.11 U |
| 1,1'-Biphenyl | 9.76 U | 4.48 U | 19.50 U | 19.50 U | 5.50 J | 3.55 J | 5.93 J | 20.00 U | 18.00 U | 5.11 U |
| Total PAHs* | 0.00 | 57.76 | 401.75 | 71.85 | 435.40 | 294.55 | 142.22 | 165.79 | 31.67 | 171.58 |
| Lipid Normalized TPAH as ug | 0 | 1733 | 2976 | 2874 | 21770 | 7364 | 6400 | 3387 | 1896 | 4657 |
| PAH/g lipid on a dry basis | | | | | | | | | | |

*TPAH calculated as the sum of all PAHs, including homologs, "0" for Non-detects

Lipid-normalized TPAH = ITissue TPAH ug/kg-dw/%lipid as decimal fraction

| (Table 10 Cont) Chemical | IT-12 | IT-13 | IT-14 | IT-15 | IT-16 | IT-17 | IT-18 | IT-19 | IT-20 | IT-21 |
|---|----------|----------|----------|---------|----------|-------|----------|---------|----------|----------|
| C1-Naphthalenes | 31.07 NJ | 47.14 NJ | 21.50 NJ | 21.11 U | 44.21 NJ | 31.05 | 33.00 NJ | 44.44 U | 34.50 NJ | 33.81 NJ |
| C2 -Naphthalenes | 16.43 NJ | 24.29 U | 23.50 NJ | 15.56 U | 20.00 U | 18.95 | 18.00 NJ | 22.22 U | 18.00 NJ | 16.67 U |
| C3 -Naphthalenes | 16.07 NJ | 23.33 NJ | 29.00 NJ | 21.11 U | 20.00 U | 18.42 | 21.00 NJ | 22.22 U | 15.00 NJ | 15.71 NJ |
| C4 -Naphthalenes | 12.50 U | 18.10 U | 20.00 U | 21.11 U | 18.95 U | 18.42 | 19.00 U | 22.22 U | 18.00 U | 15.71 U |
| C1-Fluorenes | 13.93 U | 17.62 U | 20.00 U | 21.11 U | 18.95 U | 18.42 | 19.00 U | 22.22 U | 19.00 U | 14.29 U |
| C2-Fluorenes | 12.50 U | 18.10 U | 20.00 U | 21.11 U | 18.95 U | 18.42 | 19.00 U | 22.22 U | 18.00 U | 15.71 U |
| C3-Fluorenes | 12.50 U | 18.10 U | 20.00 U | 21.11 U | 18.95 U | 18.42 | 19.00 U | 22.22 U | 18.00 U | 15.71 U |
| C1-Dibenzothiophenes | 12.50 U | 18.10 U | 20.00 U | 21.11 U | 18.95 U | 18.42 | 19.00 U | 22.22 U | 18.00 U | 15.71 U |
| C2-Dibenzothiophenes | 3.11 U | 18.10 U | 20.00 U | 21.11 U | 18.95 U | 18.42 | 19.00 U | 22.22 U | 18.00 U | 15.71 U |
| C3-Dibenzothiophenes | 12.50 U | 18.10 U | 20.00 U | 21.11 U | 18.95 U | 18.42 | 19.00 U | 22.22 U | 18.00 U | 15.71 U |
| C1-Phenanthrenes/Anthracenes | 15.71 U | 13.33 U | 55.00 NJ | 21.11 U | 18.95 U | 36.84 | 14.50 U | 44.44 U | 19.00 U | 14.76 U |
| C2-Phenanthrenes/Anthracenes | 4.64 U | 18.10 U | 48.00 NJ | 21.11 U | 18.95 U | 18.42 | 19.00 U | 22.22 U | 18.00 U | 4.00 U |
| C3-Phenanthrenes/Anthracenes | 12.50 U | 18.10 U | 20.00 U | 21.11 U | 18.95 U | 18.42 | 19.00 U | 22.22 U | 18.00 U | 15.71 U |
| C4-Phenanthrenes/Anthracenes | 6.43 U | 18.10 U | 27.00 U | 21.11 U | 18.95 U | 36.84 | 19.00 U | 22.22 U | 18.00 U | 5.71 U |
| C1-Fluoranthene/Pyrene | 12.50 U | 18.10 U | 20.00 U | 21.11 U | 18.95 U | 18.42 | 19.00 U | 22.22 U | 18.00 U | 15.71 U |
| C1-Chrysenes | 12.50 U | 18.10 U | 20.00 U | 21.11 U | 18.95 U | 18.42 | 19.00 U | 22.22 U | 18.00 U | 15.71 U |
| C2-Chrysenes | 12.50 U | 18.10 U | 20.00 U | 21.11 U | 18.95 U | 18.42 | 19.00 U | 22.22 U | 18.00 U | 15.71 U |
| C3-Chrysenes | 12.50 U | 18.10 U | 20.00 U | 21.11 U | 18.95 U | 18.42 | 19.00 U | 22.22 U | 18.00 U | 15.71 U |
| C4-Chrysenes | 3.11 U | 4.57 U | 5.00 U | 5.28 U | 4.74 U | 4.63 | 4.70 U | 5.56 U | 4.50 U | 3.95 U |
| Anthracene | 3.11 U | 4.57 U | 5.00 U | 5.28 U | 4.74 U | 4.63 | 4.70 U | 5.56 U | 4.50 U | 3.95 U |
| 4,6-Dimethyldibenzothiophene | 11.79 U | 4.57 U | 5.00 U | 5.28 U | 4.74 U | 4.63 | 4.70 U | 5.56 U | 4.50 U | 3.95 U |
| Pyrene | 6.07 | 8.10 U | 16.50 | 5.39 U | 9.47 U | 9.47 | 6.50 U | 11.11 U | 7.00 U | 6.67 |
| Dibenzofuran | 10.00 | 13.33 U | 17.00 U | 15.00 U | 4.74 U | 13.68 | 13.50 U | 5.56 U | 12.50 U | 11.43 |
| Dibenzothiophene | 3.36 U | 4.57 U | 7.00 | 5.28 U | 4.74 U | 4.63 | 4.70 U | 5.56 U | 4.50 U | 4.05 U |
| Phenanthrene, 3,6-dimethyl- | 3.11 U | 4.57 U | 14.00 U | 5.28 U | 4.74 U | 4.63 | 4.70 U | 5.56 U | 4.50 U | 3.95 U |
| 9H-Fluorene, 1-methyl- | 8.21 U | 9.52 U | 5.00 U | 8.33 U | 4.74 U | 4.63 | 10.00 U | 5.56 U | 9.00 U | 8.10 U |
| Benzo(ghi)perylene | 3.11 U | 4.57 U | 5.00 U | 5.28 U | 31.58 | 4.63 | 4.70 U | 5.56 U | 4.50 U | 3.95 U |
| Benzo[e]pyrene | 4.29 | 5.71 U | 7.00 U | 5.28 U | 6.84 U | 4.63 | 5.00 U | 5.56 U | 4.50 U | 4.57 U |
| Indeno(1,2,3-cd)pyrene | 3.11 U | 4.57 U | 5.00 U | 5.28 U | 11.58 | 4.63 | 4.70 U | 5.56 U | 4.50 U | 3.95 U |
| Perylene | 3.11 U | 4.57 U | 5.00 U | 5.28 U | 13.16 U | 4.63 | 4.70 U | 5.56 U | 4.50 U | 3.95 U |
| Benzo(b)fluoranthene | 3.11 U | 4.57 U | 5.00 U | 5.44 U | 4.74 U | 4.63 | 4.70 U | 5.56 U | 4.50 U | 3.95 U |
| Fluoranthene | 7.86 | 9.52 U | 29.50 | 8.33 U | 9.47 U | 8.95 | 9.00 U | 12.78 U | 9.00 U | 9.05 |
| Benzo(k)fluoranthene | 3.11 U | 4.57 U | 17.00 U | 5.28 U | 4.74 U | 4.63 | 4.70 U | 5.56 U | 12.50 U | 10.48 U |
| Acenaphthylene | 3.11 U | 4.57 U | 5.00 U | 5.28 U | 4.74 U | 4.63 | 4.70 U | 5.56 U | 4.50 U | 3.95 U |
| Chrysene | 9.64 | 14.29 U | 19.50 U | 15.56 U | 4.74 U | 13.16 | 13.00 U | 16.67 U | 12.50 U | 11.90 |
| 1,6,7-Trimethylnaphthalene | 7.86 | 12.86 U | 5.00 U | 5.28 U | 4.74 U | 4.63 | 12.00 | 5.56 U | 10.50 U | 10.00 |
| 2-Methylphenanthrene | 5.71 U | 4.76 U | 15.00 | 5.28 U | 4.74 U | 7.37 | 6.00 U | 6.67 U | 5.00 U | 5.71 U |
| 2-Methylfluoranthene | 3.11 U | 4.57 U | 5.00 U | 5.28 U | 4.74 U | 4.63 | 4.70 U | 5.56 U | 4.50 U | 3.95 U |
| Chrysene, 5-methyl- | 3.11 U | 4.57 U | 5.00 U | 5.28 U | 4.74 U | 4.63 | 4.70 U | 5.56 U | 4.50 U | 3.95 U |
| Retene | 6.43 U | 4.57 U | 25.00 U | 5.28 U | 4.74 U | 10.00 | 7.50 U | 5.56 U | 4.50 U | 5.71 U |
| Benzo(a)pyrene | 3.11 U | 4.57 U | 5.00 U | 5.28 U | 8.95 U | 4.63 | 4.70 U | 5.56 U | 4.50 U | 3.95 U |
| Dibenzo(a,h)anthracene | 3.11 U | 4.57 U | 5.00 U | 5.28 U | 44.21 | 4.63 | 4.70 U | 5.56 U | 4.50 U | 3.95 U |
| Benzo(a)anthracene | 3.11 U | 8.57 U | 9.50 U | 5.28 U | 4.74 U | 4.63 | 7.50 U | 5.56 U | 8.00 U | 6.67 U |
| 2,6-Dimethylnaphthalene | 8.93 J | 12.86 J | 12.00 J | 11.11 U | 12.63 U | 36.84 | 12.00 J | 22.22 U | 12.00 J | 10.48 J |
| 1-Methylphenanthrene | 5.71 U | 7.62 U | 14.00 | 7.78 U | 4.74 U | 8.42 | 7.50 U | 5.56 U | 7.00 U | 7.62 U |
| Acenaphthene | 8.21 U | 11.43 U | 5.00 U | 12.78 U | 4.74 U | 4.63 | 12.00 U | 5.56 U | 11.50 U | 10.48 |
| Phenanthrene | 16.07 U | 16.67 U | 49.00 | 21.11 U | 13.16 U | 13.68 | 20.00 U | 20.56 U | 16.50 U | 20.00 |
| Fluorene | 0.46 J | 4.57 U | 1.45 J | 5.28 U | 4.74 U | 4.63 | 4.70 U | 5.56 U | 4.50 U | 3.95 U |
| Carbazol | 3.11 U | 4.57 U | 5.00 U | 0.00 | | | | | | |
| 1-Methylnaphthalene | 5.71 | 9.05 | 15.00 U | 0.42 U | 7.37 J | 4.58 | 5.00 | 11.11 U | 5.50 | 5.24 |
| Naphthalene | 11.43 | 18.57 U | 10.50 U | 8.33 U | 20.00 U | 14.21 | 14.00 | 11.11 U | 14.50 | 11.90 |
| 2-Methylnaphthalene | 20.00 | 29.05 | 15.00 | 12.78 U | 30.00 | 22.63 | 23.00 | 13.89 U | 23.00 | 20.95 |
| 2-Chloronaphthalene | 3.11 U | 4.57 U | 5.00 U | 5.28 U | 4.74 U | 4.63 | 4.70 U | 5.56 U | 4.50 U | 3.95 U |
| 1,1'-Biphenyl | 2.00 J | 4.57 U | 20.00 U | 21.11 U | 18.95 U | 18.42 | 4.70 U | 22.22 U | 4.50 U | 1.90 J |
| Total PAHs* | 157.82 | 121.43 | 336.45 | 0.00 | 168.95 | 58.26 | 138.00 | 0.00 | 122.50 | 179.52 |
| Lipid Normalized TPAH as ug | 4910 | 2742 | 3958 | 0 | 3452 | 1581 | 4600 | 0.00 | 4900 | 6283 |
| PAH/g lipid on a dry basis | | | | | | | | | | |
| *TPAH calculated as the sum of all PAHs, including homologs. | | | | | | | | | | |
| "0" for Non-detects | | | | | | | | | | |
| Lipid-normalized TPAH = [Tissue TPAH ug/kg-dw]/%lipid as decimal fraction | | | | | | | | | | |

Table 11. TPAH Content (ug/Animal) by Station and Site

| | <u>Pt Whitehorn</u> | <u>Cherry Pt</u> | <u>Gulf Road</u> | <u>IT-TOS</u> | <u>T0</u> |
|-------|---------------------|------------------|------------------|---------------|-----------|
| | 0.08 | 0.10 | 0.31 | 0.00 | 0.01 |
| | 0.16 | 0.02 | 0.13 | 0.03 | 0.02 |
| | 0.07 | 0.05 | 0.20 | 0.21 | 0.06 |
| | 0.17 | 0.14 | 0.17 | 0.04 | |
| | 0.14 | 0.06 | 0.14 | 0.22 | |
| | 0.05 | 0.09 | 0.00 | 0.14 | |
| | 0.04 | 0.31 | 0.21 | 0.11 | |
| | | | | 0.07 | |
| | | | | 0.02 | |
| | | | | 0.09 | |
| | | | | 0.10 | |
| | | | | 0.00 | |
| | | | | 0.19 | |
| | | | | 0.00 | |
| | | | | 0.11 | |
| | | | | 0.03 | |
| | | | | 0.08 | |
| | | | | 0.00 | |
| | | | | 0.08 | |
| | | | | 0.10 | |
| Mean | 0.10 | 0.11 | 0.17 | 0.09 | 0.03 |
| Stdev | 0.054 | 0.097 | 0.096 | 0.069 | 0.026 |
| N | 7 | 7 | 7 | 18 | 3 |
| 2se | 0.041 | 0.073 | 0.072 | 0.032 | 0.030 |

Note: Shaded cells not included in mean calculations; data provided for comparative purposes only

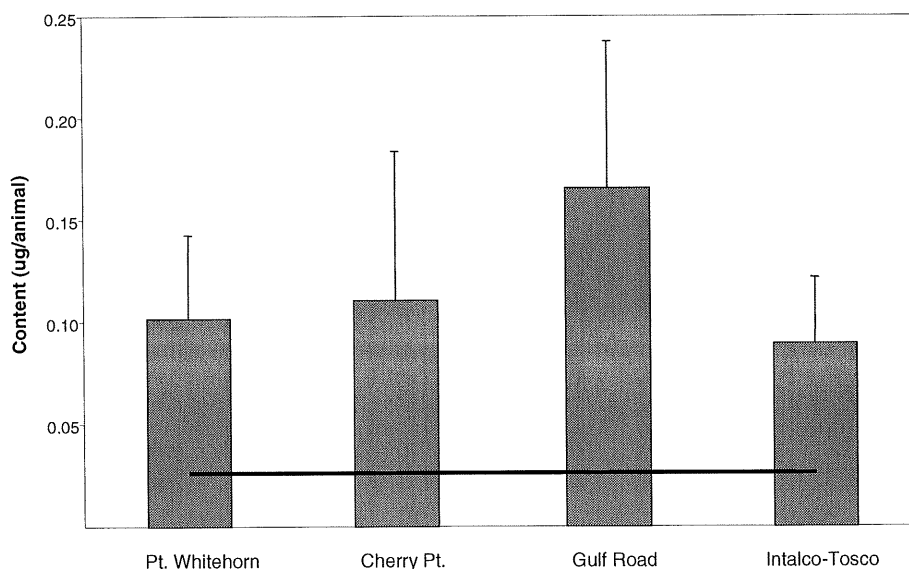


Figure 15. TPAH content (ug/TPAH/animal) in mussel tissues $\pm 2SE$ by site compared with concentrations at the beginning of the test (T_0).

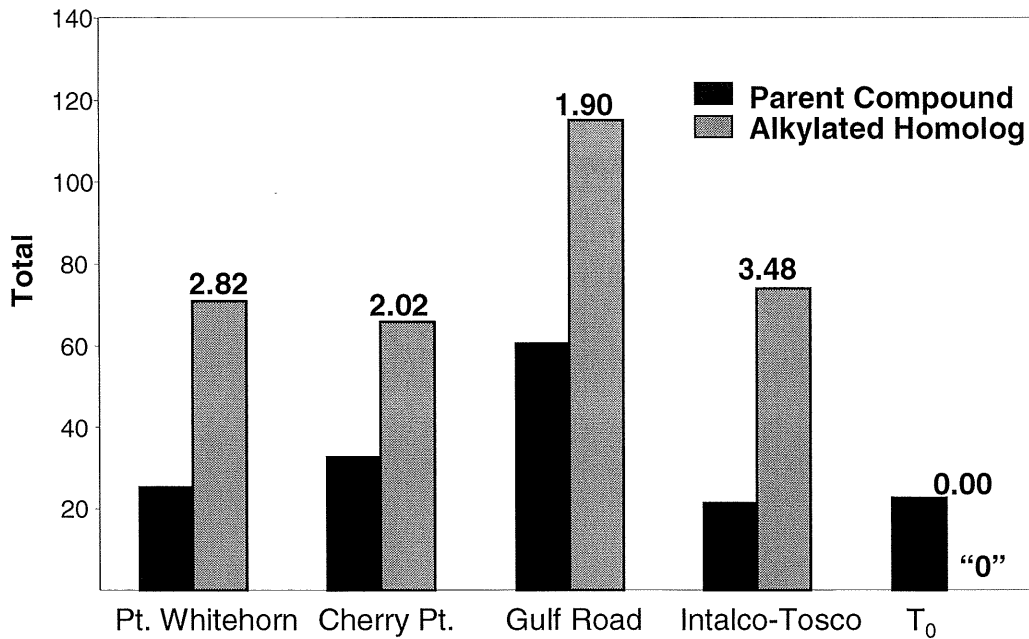


Figure 16A. Relative composition of parent compounds and alkylated homologs for five representative PAHs (naphthalene, fluorene, dibenzothiophene, phenanthrene, and chrysene) for the four test sites and beginning of the test (T₀). The ratio of the substituted compounds versus the parent compounds are given above.

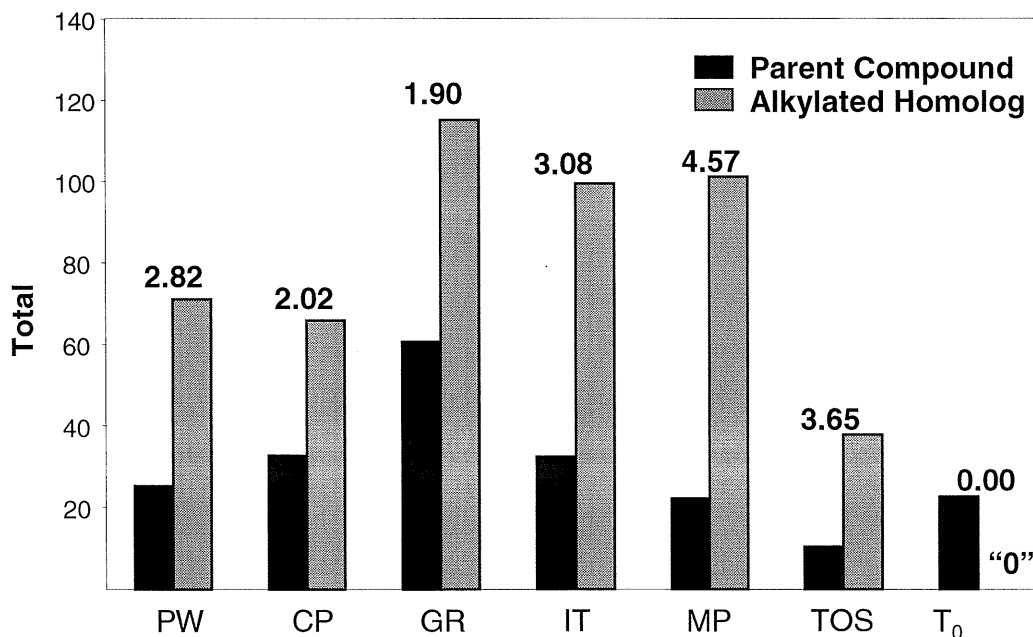


Figure 16B. Relative composition of parent compounds and alkylated homologs for five representative PAHs (naphthalene, fluorene, dibenzothiophene, phenanthrene, and chrysene) for the six regions and beginning of the test (T₀). The ratio of the substituted compounds versus the parent compounds are given above.

Table 12. Relative Composition of Parent Compounds and Alkylated Homologs for Five Representative PAHs (Naphthalene, Fluorene, Dibenzothiophene, Phenanthrene, and Chrysene) & Ratio of the Substituted Compounds versus the Parent Compounds

| | <u>Pt. Whitehorn</u> | <u>Cherry Pt.</u> | <u>Gulf Road</u> | <u>Intalco Tosco</u> | <u>Initial</u> |
|------------------------------|----------------------|-------------------|------------------|----------------------|----------------|
| Naphthalene | 12.64 | 14.70 | 17.33 | 7.10 | 0.00 |
| C1-Naphthalenes | 30.92 | 38.18 | 36.60 | 31.97 | 0.00 |
| C2 -Naphthalenes | 12.71 | 11.57 | 20.04 | 12.00 | 0.00 |
| C3 -Naphthalenes | 12.02 | 7.33 | 13.70 | 15.27 | 0.00 |
| C4 -Naphthalenes | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Fluorene | 0.58 | 0.46 | 1.18 | 0.33 | 0.00 |
| C1-Fluorenes | 11.41 | 4.61 | 16.12 | 3.43 | 0.00 |
| C2-Fluorenes | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| C3-Fluorenes | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Dibenzothiophene | 0.00 | 0.84 | 1.78 | 0.70 | 0.00 |
| C1-Dibenzothiophenes | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| C2-Dibenzothiophenes | 0.00 | 0.00 | 0.00 | 0.30 | 0.00 |
| C3-Dibenzothiophenes | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Phenanthrene | 11.93 | 14.27 | 28.64 | 9.78 | 0.00 |
| C1-Phenanthrenes/Anthracenes | 3.90 | 4.09 | 12.93 | 6.90 | 0.00 |
| C2-Phenanthrenes/Anthracenes | 0.00 | 0.00 | 11.79 | 2.40 | 0.00 |
| C3-Phenanthrenes/Anthracenes | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| C4-Phenanthrenes/Anthracenes | 0.00 | 0.00 | 3.86 | 1.76 | 0.00 |
| Chrysene | 0.00 | 2.29 | 11.56 | 3.38 | 22.59 |
| C1-Chrysenes | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| C2-Chrysenes | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| C3-Chrysenes | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| C4-Chrysenes | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Total of Parent Compounds | 25.15 | 32.56 | 60.49 | 21.28 | 22.59 |
| Total of All Homologs | 70.96 | 65.78 | 115.03 | 74.02 | 0.00 |
| Ratio | 2.82 | 2.02 | 1.90 | 3.48 | 0 |

4.4.2 Trace Metals

The concentration data for each metal shows a decrease in concentration over the course of the exposure period (Table 13; Figure 17) when the end-of-test value is compared to the BOT value. The content data indicate an uptake of arsenic, mercury, cadmium, copper, and zinc during the course of the deployment period (Table 14; Figure 18). Statistical analyses conducted on the concentration data showed no significant differences across sites in end-of-test concentrations for any of the metals (Table 15). The statistical tests comparing end-of-test concentration to BOT concentration showed the EOT concentration for arsenic, mercury, lead, cadmium, and selenium to be significantly lower than BOT concentrations. There was no significant difference in EOT zinc when compared to BOT, and copper was only significantly different for Point Whitehorn when compared to BOT. Metals data for arsenic, lead, cadmium, copper, zinc, and selenium at station IT-07 were excluded because they were all reported as non-detected. Only the mercury data for this station were used.

Because of the significant growth measured for the deployed mussels, it is necessary to evaluate the metals data in terms of content to determine if growth dilution was responsible for the apparent decrease in metal concentration during deployment. The content data indicate that there was an uptake of arsenic, mercury, cadmium, copper, and zinc during the course of the deployment period. There was essentially no change in the amount of lead or selenium accumulated. Statistical analyses of the content data showed several significant differences (1) among sites and the end of the test, and (2) when the EOT value was compared to the T_0 value.

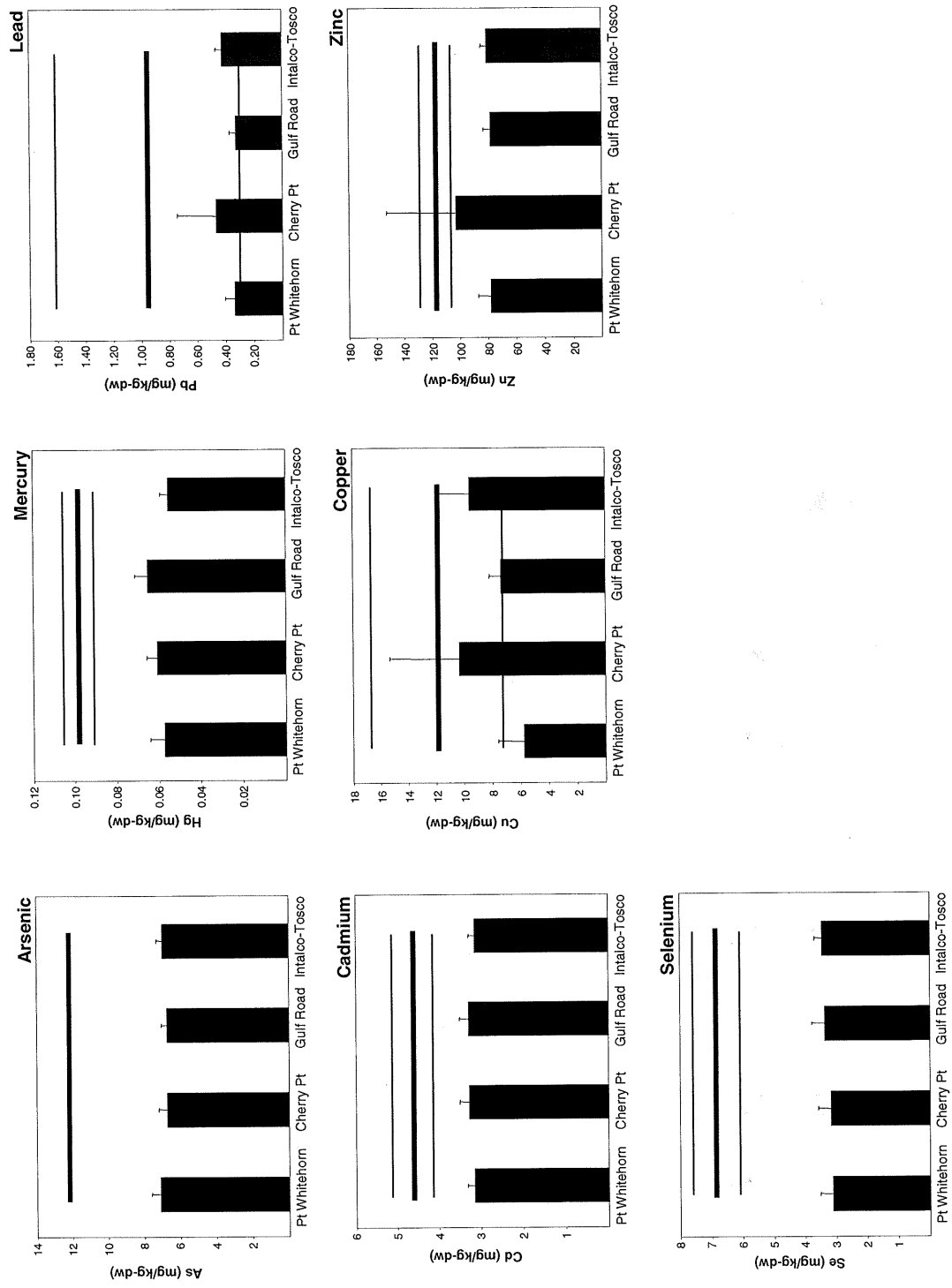


Figure 17. Metal concentration (mg/kg-dry) \pm 2SE in mussel tissues by site compared with concentrations at the beginning of the test (\pm 2SE). BOT concentrations (\pm 2SE) are represented by the solid horizontal lines.

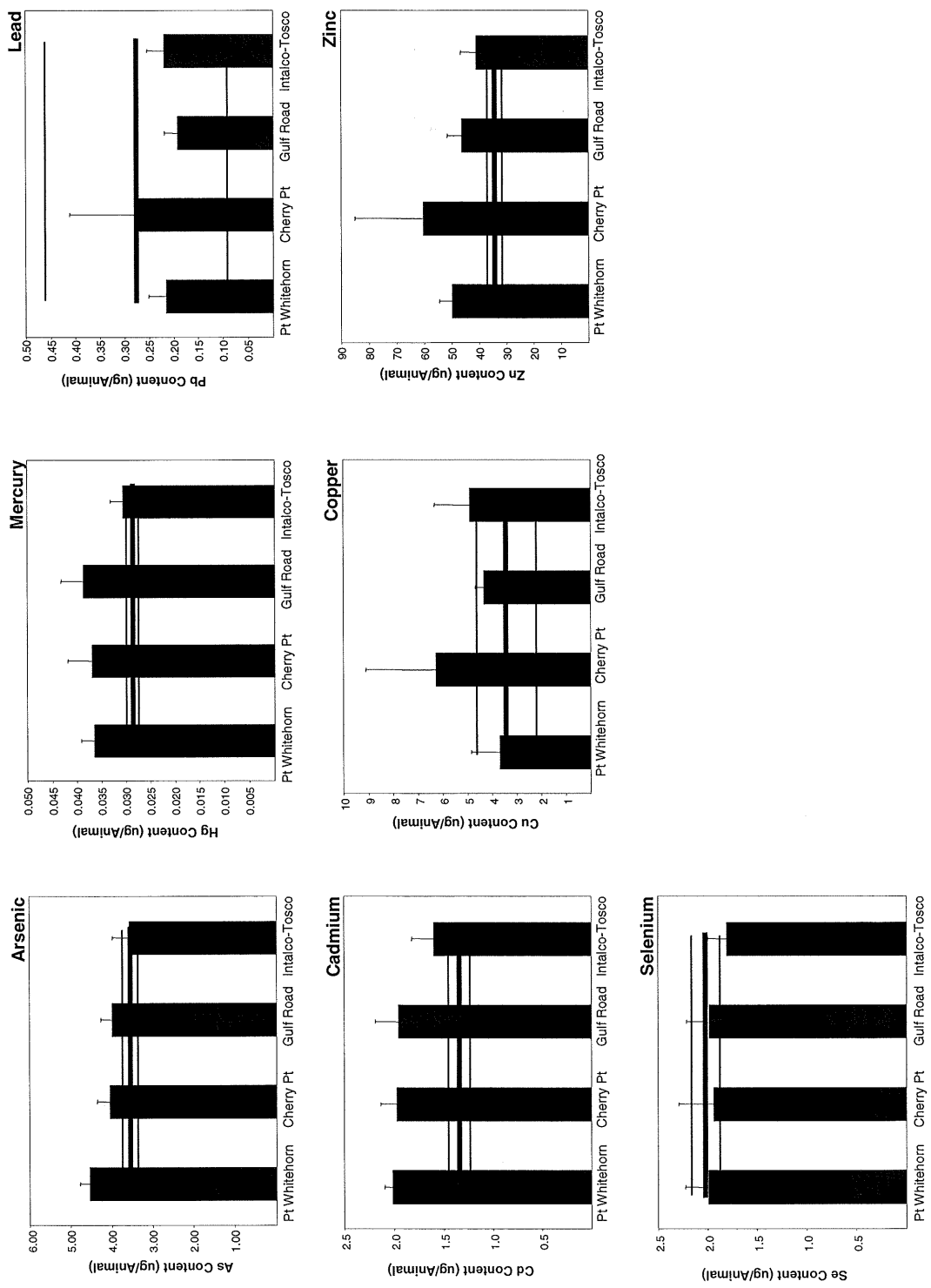


Figure 18. Metal content (ug/Animal) ±2SE in mussel tissues by site compared with content at the beginning of the test (±2SE). BOT contents (±2SE) are represented by the solid horizontal lines.

Table 13. Concentration of metals (mg/kg-dw) in mussel tissues

| | <u>Arsenic</u> | <u>Mercury</u> | <u>Lead</u> | <u>Cadmium</u> | <u>Copper</u> | <u>Zinc</u> | <u>Selenium</u> |
|-------------|----------------|----------------|-------------|----------------|---------------|---------------|-----------------|
| PW-01-04 | 7.14 | 0.057 | 0.44 | 3.29 | 8.95 | 75.24 | 2.95 |
| PW-02-26 | 6.36 | 0.055 | 0.27 | 2.95 | 6.14 | 65.45 | 2.68 |
| PW-03-07 | 6.36 | 0.045 | 0.25 | 2.88 | 6.59 | 66.82 | 2.55 |
| PW-04-42 | 6.67 | 0.062 | 0.26 | 2.96 | 6.57 | 69.52 | 3.71 |
| PW-05-01 | 7.62 | 0.052 | 0.37 | 3.20 | 2.40 | 89.52 | 3.43 |
| PW-06-18 | 8.00 | 0.075 | 0.30 | 3.40 | 6.90 | 84.00 | 3.80 |
| PW-07-29 | 7.50 | 0.055 | 0.47 | 3.36 | 2.60 | 95.50 | 2.70 |
| Mean | 7.09 | 0.057 | 0.34 | 3.15 | 5.74 | 78.01 | 3.12 |
| CP-01-41 | 5.83 | 0.058 | 0.35 | 2.87 | 8.54 | 77.92 | 3.04 |
| CP-02-45 | 6.84 | 0.068 | 1.28 | 3.43 | 5.14 | 251.58 | 3.74 |
| CP-03-15 | 6.67 | 0.057 | 0.33 | 3.16 | 10.10 | 78.10 | 3.14 |
| CP-04-28 | 7.00 | 0.060 | 0.33 | 3.54 | 24.80 | 83.00 | 2.30 |
| CP-05-37 | 7.50 | 0.055 | 0.33 | 3.70 | 7.05 | 84.00 | 2.90 |
| CP-06-36 | 7.14 | 0.071 | 0.40 | 3.24 | 6.71 | 80.00 | 3.90 |
| CP-07-13 | 5.91 | 0.055 | 0.26 | 2.96 | 9.91 | 65.00 | 3.14 |
| Mean | 6.70 | 0.061 | 0.47 | 3.27 | 10.32 | 102.80 | 3.17 |
| GR-01-22 | 6.50 | 0.075 | 0.22 | 3.42 | 7.20 | 84.00 | 4.20 |
| GR-02-21 | 6.36 | 0.059 | 0.40 | 3.36 | 8.14 | 70.45 | 3.55 |
| GR-03-11 | 6.36 | 0.059 | 0.35 | 2.88 | 5.45 | 89.55 | 2.41 |
| GR-04-23 | 6.82 | 0.068 | 0.29 | 3.81 | 7.05 | 71.36 | 3.23 |
| GR-05-20 | 6.50 | 0.055 | 0.38 | 3.08 | 6.85 | 77.50 | 3.20 |
| GR-06-38 | 7.00 | 0.075 | 0.35 | 3.23 | 8.85 | 74.50 | 3.25 |
| GR-07-12 | 7.50 | 0.065 | 0.29 | 3.23 | 7.85 | 78.00 | 3.70 |
| Mean | 6.72 | 0.065 | 0.32 | 3.29 | 7.34 | 77.91 | 3.36 |
| IT-02-27 | 6.67 | 0.048 | 0.29 | 3.03 | 12.48 | 69.52 | 3.38 |
| IT-04-35 | 6.50 | 0.050 | 0.65 | 2.51 | 9.20 | 88.50 | 3.85 |
| IT-05-47 | 7.50 | 0.055 | 0.38 | 2.95 | 7.60 | 85.50 | 3.95 |
| IT-06-32 | 8.00 | 0.050 | 0.49 | 3.61 | 2.70 | 85.00 | 4.00 |
| IT-07-33 | 0.00 | 0.044 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| IT-08-39 | 7.89 | 0.063 | 0.42 | 3.41 | 17.11 | 87.37 | 3.95 |
| IT-10-19 | 6.84 | 0.068 | 0.51 | 3.47 | 9.11 | 91.58 | 3.32 |
| IT-12-25 | 5.71 | 0.054 | 0.39 | 2.71 | 6.21 | 71.79 | 2.32 |
| IT-13-09 | 6.67 | 0.067 | 0.32 | 3.04 | 6.67 | 73.33 | 3.29 |
| IT-15-30 | 7.22 | 0.056 | 0.42 | 3.34 | 9.00 | 92.78 | 3.33 |
| IT-16-17 | 6.32 | 0.048 | 0.37 | 3.24 | 20.16 | 68.42 | 3.63 |
| IT-17-10 | 6.84 | 0.047 | 0.41 | 3.17 | 7.11 | 76.32 | 3.05 |
| IT-18-40 | 7.00 | 0.055 | 0.50 | 3.10 | 10.90 | 74.00 | 3.35 |
| IT-19-46 | 7.22 | 0.052 | 0.37 | 3.19 | 6.24 | 76.67 | 2.83 |
| IT-20-31 | 6.50 | 0.060 | 0.44 | 3.12 | 11.40 | 85.50 | 3.75 |
| IT-21-08 | 7.62 | 0.067 | 0.39 | 3.21 | 7.19 | 81.43 | 3.76 |
| Mean | 6.97 | 0.055 | 0.42 | 3.14 | 9.45 | 80.51 | 3.45 |
| TO-R1-24 | 12.22 | 0.094 | 0.31 | 4.49 | 8.94 | 108.89 | 6.11 |
| TO-R2-03 | 12.22 | 0.094 | 1.13 | 4.33 | 10.44 | 126.67 | 7.22 |
| TO-R3-44 | 12.22 | 0.106 | 1.41 | 5.09 | 16.50 | 118.33 | 7.22 |
| Mean | 12.22 | 0.098 | 0.95 | 4.64 | 11.96 | 117.96 | 6.85 |

Table 14. Content of metals (ug/Animal) in mussel tissues

| | <u>Arsenic</u> | <u>Mercury</u> | <u>Lead</u> | <u>Cadmium</u> | <u>Copper</u> | <u>Zinc</u> | <u>Selenium</u> |
|-------------|----------------|----------------|--------------|----------------|---------------|-------------|-----------------|
| PW-01-04 | 4.385 | 0.035 | 0.269 | 2.02 | 5.50 | 46.2 | 1.81 |
| PW-02-26 | 4.239 | 0.036 | 0.182 | 1.96 | 4.09 | 43.6 | 1.79 |
| PW-03-07 | 4.780 | 0.034 | 0.191 | 2.16 | 4.95 | 50.2 | 1.91 |
| PW-04-42 | 4.307 | 0.040 | 0.169 | 1.91 | 4.25 | 44.9 | 2.40 |
| PW-05-01 | 5.175 | 0.036 | 0.252 | 2.18 | 1.63 | 60.8 | 2.33 |
| PW-06-18 | 4.495 | 0.042 | 0.169 | 1.91 | 3.88 | 47.2 | 2.14 |
| PW-07-29 | 4.361 | 0.032 | 0.273 | 1.95 | 1.51 | 55.5 | 1.57 |
| Mean | 4.535 | 0.036 | 0.215 | 2.01 | 3.69 | 49.8 | 1.99 |
| CP-01-41 | 4.428 | 0.044 | 0.262 | 2.18 | 6.48 | 59.1 | 2.31 |
| CP-02-45 | 3.624 | 0.036 | 0.680 | 1.81 | 2.72 | 133.2 | 1.98 |
| CP-03-15 | 4.111 | 0.035 | 0.203 | 1.95 | 6.23 | 48.2 | 1.94 |
| CP-04-28 | 3.842 | 0.033 | 0.178 | 1.94 | 13.61 | 45.6 | 1.26 |
| CP-05-37 | 3.541 | 0.026 | 0.156 | 1.75 | 3.33 | 39.7 | 1.37 |
| CP-06-36 | 4.101 | 0.041 | 0.227 | 1.86 | 3.85 | 45.9 | 2.24 |
| CP-07-13 | 4.655 | 0.043 | 0.204 | 2.33 | 7.81 | 51.2 | 2.47 |
| Mean | 4.043 | 0.037 | 0.273 | 1.98 | 6.29 | 60.4 | 1.94 |
| GR-01-22 | 3.87 | 0.045 | 0.131 | 2.03 | 4.29 | 50.0 | 2.50 |
| GR-02-21 | 3.63 | 0.034 | 0.226 | 1.92 | 4.65 | 40.2 | 2.03 |
| GR-03-11 | 4.24 | 0.039 | 0.230 | 1.92 | 3.63 | 59.6 | 1.60 |
| GR-04-23 | 4.65 | 0.046 | 0.198 | 2.60 | 4.80 | 48.7 | 2.20 |
| GR-05-20 | 3.55 | 0.030 | 0.208 | 1.68 | 3.74 | 42.4 | 1.75 |
| GR-06-38 | 3.86 | 0.041 | 0.190 | 1.78 | 4.88 | 41.1 | 1.79 |
| GR-07-12 | 4.10 | 0.035 | 0.158 | 1.76 | 4.29 | 42.6 | 2.02 |
| Mean | 3.99 | 0.039 | 0.192 | 1.96 | 4.33 | 46.4 | 1.98 |
| IT-02-27 | 3.50 | 0.025 | 0.152 | 1.59 | 6.54 | 36.5 | 1.77 |
| IT-04-35 | 3.68 | 0.028 | 0.368 | 1.42 | 5.21 | 50.2 | 2.18 |
| IT-05-47 | 3.82 | 0.028 | 0.193 | 1.50 | 3.87 | 43.5 | 2.01 |
| IT-06-32 | 3.92 | 0.025 | 0.238 | 1.77 | 1.32 | 41.7 | 1.96 |
| IT-07-33 | 0.00 | 0.034 | 0.000 | 0.00 | 0.00 | 0.0 | 0.00 |
| IT-08-39 | 3.45 | 0.028 | 0.184 | 1.49 | 7.47 | 38.2 | 1.72 |
| IT-10-19 | 3.65 | 0.037 | 0.270 | 1.85 | 4.86 | 48.9 | 1.77 |
| IT-12-25 | 3.58 | 0.034 | 0.246 | 1.70 | 3.90 | 45.0 | 1.46 |
| IT-13-09 | 3.95 | 0.039 | 0.192 | 1.80 | 3.95 | 43.4 | 1.94 |
| IT-15-30 | 3.52 | 0.027 | 0.203 | 1.63 | 4.38 | 45.2 | 1.62 |
| IT-16-17 | 3.97 | 0.030 | 0.232 | 2.04 | 12.68 | 43.1 | 2.29 |
| IT-17-10 | 4.01 | 0.027 | 0.240 | 1.86 | 4.16 | 44.7 | 1.79 |
| IT-18-40 | 4.02 | 0.032 | 0.287 | 1.78 | 6.25 | 42.4 | 1.92 |
| IT-19-46 | 3.16 | 0.023 | 0.161 | 1.40 | 2.73 | 33.6 | 1.24 |
| IT-20-31 | 4.03 | 0.037 | 0.273 | 1.93 | 7.06 | 53.0 | 2.32 |
| IT-21-08 | 4.17 | 0.037 | 0.211 | 1.76 | 3.94 | 44.6 | 2.06 |
| Mean | 3.53 | 0.031 | 0.216 | 1.59 | 4.90 | 40.9 | 1.75 |
| TO-R1-24 | 3.70 | 0.029 | 0.094 | 1.36 | 2.71 | 32.9 | 1.85 |
| TO-R2-03 | 3.59 | 0.028 | 0.331 | 1.27 | 3.07 | 37.2 | 2.12 |
| TO-R3-44 | 3.47 | 0.030 | 0.400 | 1.45 | 4.68 | 33.6 | 2.05 |
| Mean | 3.58 | 0.029 | 0.275 | 1.36 | 3.48 | 34.6 | 2.01 |

Table 15. Summary of statistics on metal concentration and content

Concentration

| | <u>Differences Among Sites at EOT?</u> | <u>Compared to T₀ Concentration</u> |
|----------|--|---|
| Arsenic | NSD (p = 0.5095) | EOT significantly lower than T ₀ for all stations (p < 0.0001) |
| Mercury | NSD (p = 0.0514) | EOT significantly lower than T ₀ for all stations (p < 0.0001) |
| Lead | NSD (p = 0.0503) | EOT significantly lower than T ₀ for all stations (p < 0.0001) |
| Cadmium | NSD (p = 0.5589) | EOT significantly lower than T ₀ for all stations (p < 0.0001) |
| Copper | NSD (p = 0.0611) | SD (p = 0.0306); PW ≠ T ₀ |
| Zinc | NSD (p = 0.8341) | NSD (p = 0.0902) |
| Selenium | NSD (p = 0.4345) | EOT significantly lower than T ₀ for all stations (p < 0.0001) |

Content

| | <u>Differences Among Sites at EOT?</u> | <u>Compared to T₀ Concentration</u> |
|----------|--|--|
| Arsenic | SD (p = 0.0013); PW ≠ IT | SD (p = 0.0014); PW ≠ T ₀ |
| Mercury | SD (p = 0.0048); GR ≠ IT | SD (p = 0.0048); GR ≠ T ₀ |
| Lead | NSD (p = 0.6408) | NSD (p = 0.7011) |
| Cadmium | SD (p = 0.0021); PW ≠ IT | SD (p = 0.0005); PW, CP, GR ≠ T ₀ |
| Copper | NSD (p = 0.6826) | NSD (p = 0.5597) |
| Zinc | SD (p = 0.0452); not identified | SD (p = 0.0080); PW, CP ≠ T ₀ |
| Selenium | NSD (p = 0.6523) | NSD (p = 0.7672) |

4.5 Water Temperature

Overall, water temperatures during the 1999 study were lower than measured during the 1998 study. In 1999 the differences among stations were small as well as the differences between surface and bottom locations. On one occasion a 4.5°C change in temperature was measured during a 45 minute period. There were frequent excursions of several degrees in a few hours at many stations and several temperature measurements exceeded 17°C. Daily average water temperatures and ranges in daily water temperature are provided in Tables 16 and 17, respectively. Interpretation of the water temperature data will emphasize potential effects on herring eggs since the caged mussel study was intended to support the in-situ herring egg study.

Mussel Deployment Period Temperatures

Water temperature at the surface and bottom locations at all stations displayed similar patterns with daily cycles over the 61-day deployment period (Figures 19 and 20). Water temperature at each monitoring location increased a few degrees during the first month of deployment (i.e., from April 16th to about May 24th). On or about May 25th, there was a sharp increase in water temperature, with the average temperature increasing from about 9.5°C to 11.5°C in less than 24 hours. At all surface monitoring locations, water temperatures approached or exceeded 14°C around 6pm on May 24th (Figure 19). At all bottom monitoring locations, a spike in the water temperature occurred at the same time, with temperatures approaching or exceeding 14°C at some bottom locations (Figure 20). In general, daily average water temperature at all monitoring locations stations increased about 3.5°C during the mussel deployment period, with peak temperatures occurring around May 25th (Table 16). There was a slight decrease in daily average temperature during the last part of May with another increase during the first week of June. Grand means in daily average water temperature calculated across all monitoring locations are shown in Figure 21, which shows that the overall daily average temperatures in 1999 were lower than measured in the 1998 study. It also shows a similar spike in water temperature during May 1999, although this spike occurred about 5 days later than recorded in 1998. The high temperatures recorded during June 1998 were not recorded during June 1999. Results of the one-way ANOVA on daily average temperatures over the entire 61-d exposure period indicated no statistically significant differences among the surface stations (Table 18; $p = 0.8187$), however at the bottom stations, daily average water temperature at PW-07 was significantly higher than CP-07.

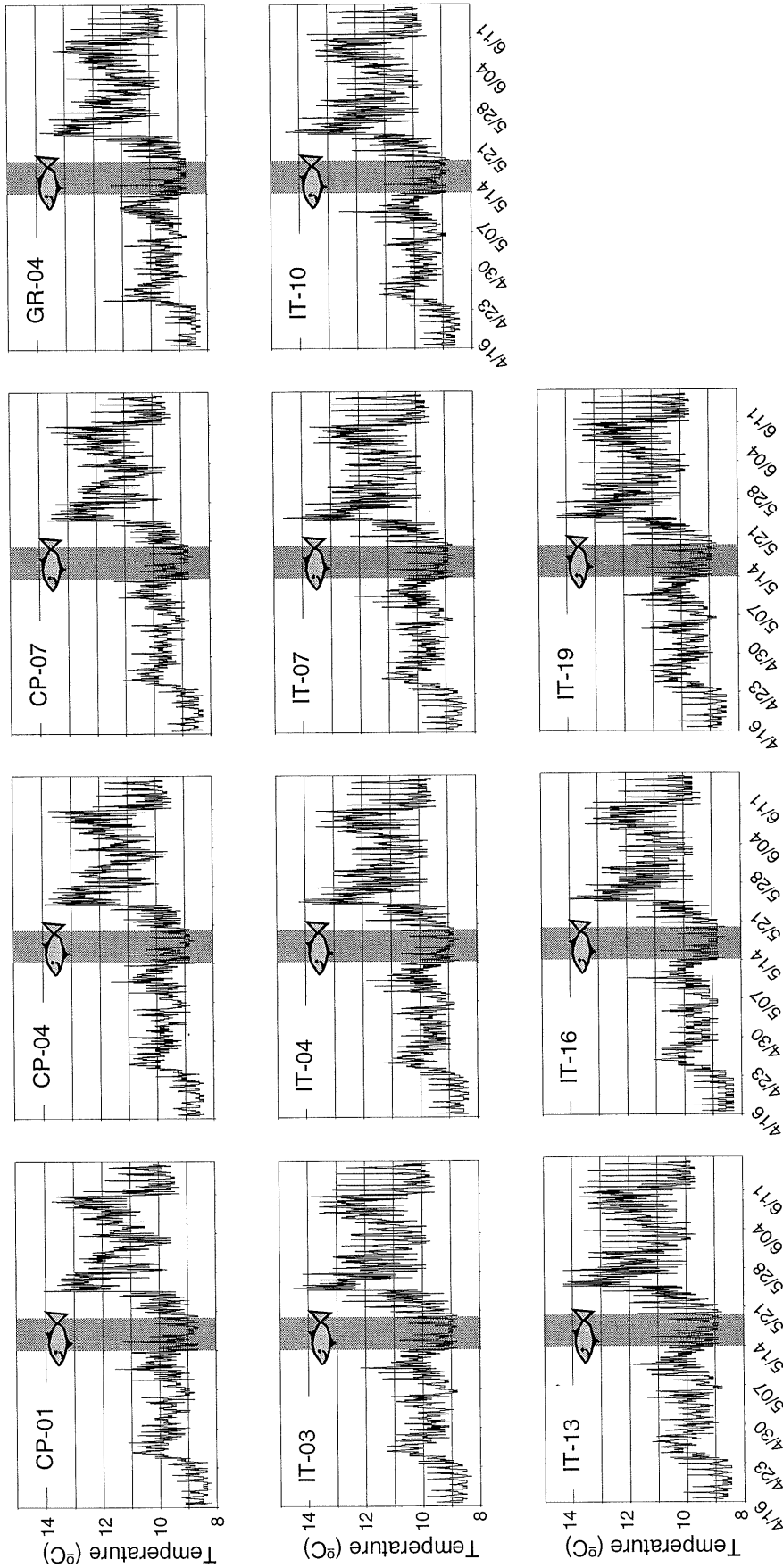
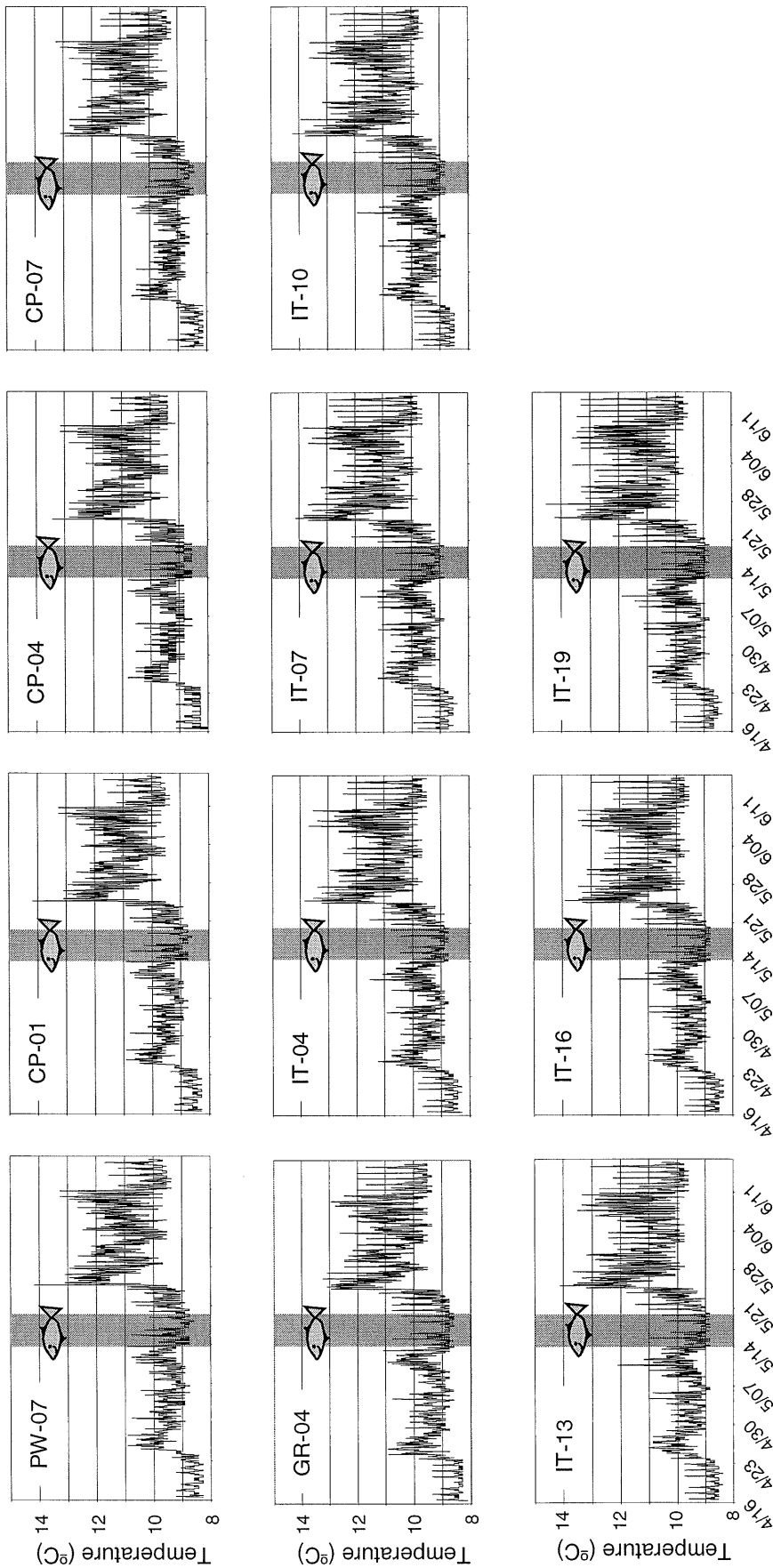


Figure 19. Surface water temperatures during 61-d mussel exposure period.  and gray bar = herring egg deployment site.



| Station | PW-07 | CP-01 | CP-04 | CP-07 | GR-04 | IT-04 | IT-07 | IT-10 | IT-13 | IT-16 | IT-19 |
|---------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Mean | 10.23 | 9.91 | 9.74 | 9.68 | 9.74 | 9.96 | 10.21 | 10.07 | 10.05 | 9.96 | 10.14 |
| Min | 8.56 | 8.29 | 8.01 | 8.13 | 8.13 | 8.28 | 8.39 | 8.50 | 8.39 | 8.34 | 8.35 |
| Max | 14.62 | 14.18 | 13.42 | 13.25 | 13.25 | 13.84 | 14.14 | 14.23 | 14.12 | 13.91 | 14.53 |

Figure 20. Bottom water temperatures during 61-d mussel exposure period.  and gray bar = herring egg deployment site.

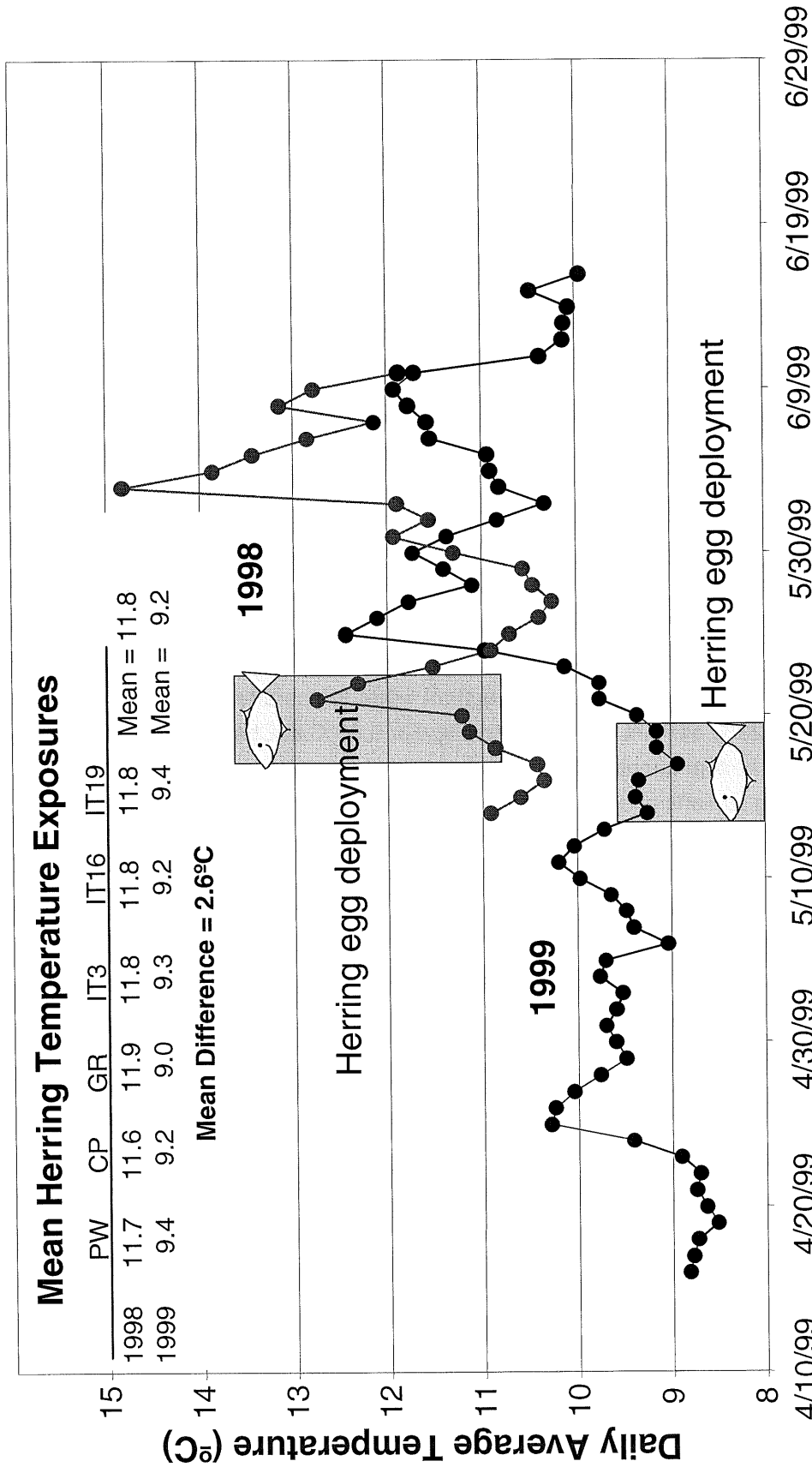
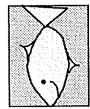


Figure 21. Grand mean for all stations in daily average water temperature: 1999 vs 1998.



= herring deployment period.

The range in daily temperature increased over the course of the mussel deployment period. During April the range in daily temperature was generally between 0.8 and 2°C (Table 17). In both May and June, the range in daily temperature increased at all monitoring locations between 2 and 4°C. During both May and June at some monitoring locations, there were some days where the range in temperature exceeded 4°C. The maximum rate of change was measured at IT-19 (the southernmost station) where the temperature increased from 12.8 to 16.7 °C in 45 minutes. This was an increase of almost 4°C.

The overall average temperatures at each station where temperature was monitored during the mussel deployment period are shown in Figure 22. Although the differences are not great, there is some variability in average temperature within a given site and among sites. The temperatures at the bottom were always lower than at the surface by an average of about 0.25°C. However, the difference in average water temperature for the surface stations was 0.33°C; the difference in average water temperature for the bottom stations was 0.55°C. These data show that not only were water temperatures at the bottom lower than at the surface, but there was more variability in water temperature across stations. As shown in Figure 22, there was a steep decline in average water temperature from PW to CP to GR and the another steep increase to the IT stations. Average bottom water temperatures for these stations ranged from a high of 10.23°C at Point Whitehorn to a low of 9.68°C at Cherry Point to another high at IT of 10.21°. The bottom temperature measurements seemed to be the best indicator of differences among stations and were least influenced by warming by the sun and surface transport of warmer waters from other areas. The results of the one-way ANOVA on daily temperature range over the entire 61-d exposure period indicated statistically significant differences among both the surface and bottom stations (Table 18). The following differences were measured at the surface stations: IT-19 ≠ CP-01, CP-04, CP-07. The following differences were measured at the bottom stations: PW-07 ≠ CP-04, and IT-19 ≠ CP-01, CP-04, CP-07.

Daily shifts in water temperature appeared to be related to tidal cycle at most stations, although absolute temperatures and ranges differed. The most extreme change in temperature occurred at Station IT-19, with a shift of 4°C in 45 minutes that was clearly related to tidal cycle. An example of daily temperature shifts with tidal cycle for Station IT-19 between June 1-5, 1999 are shown in Figure 23.

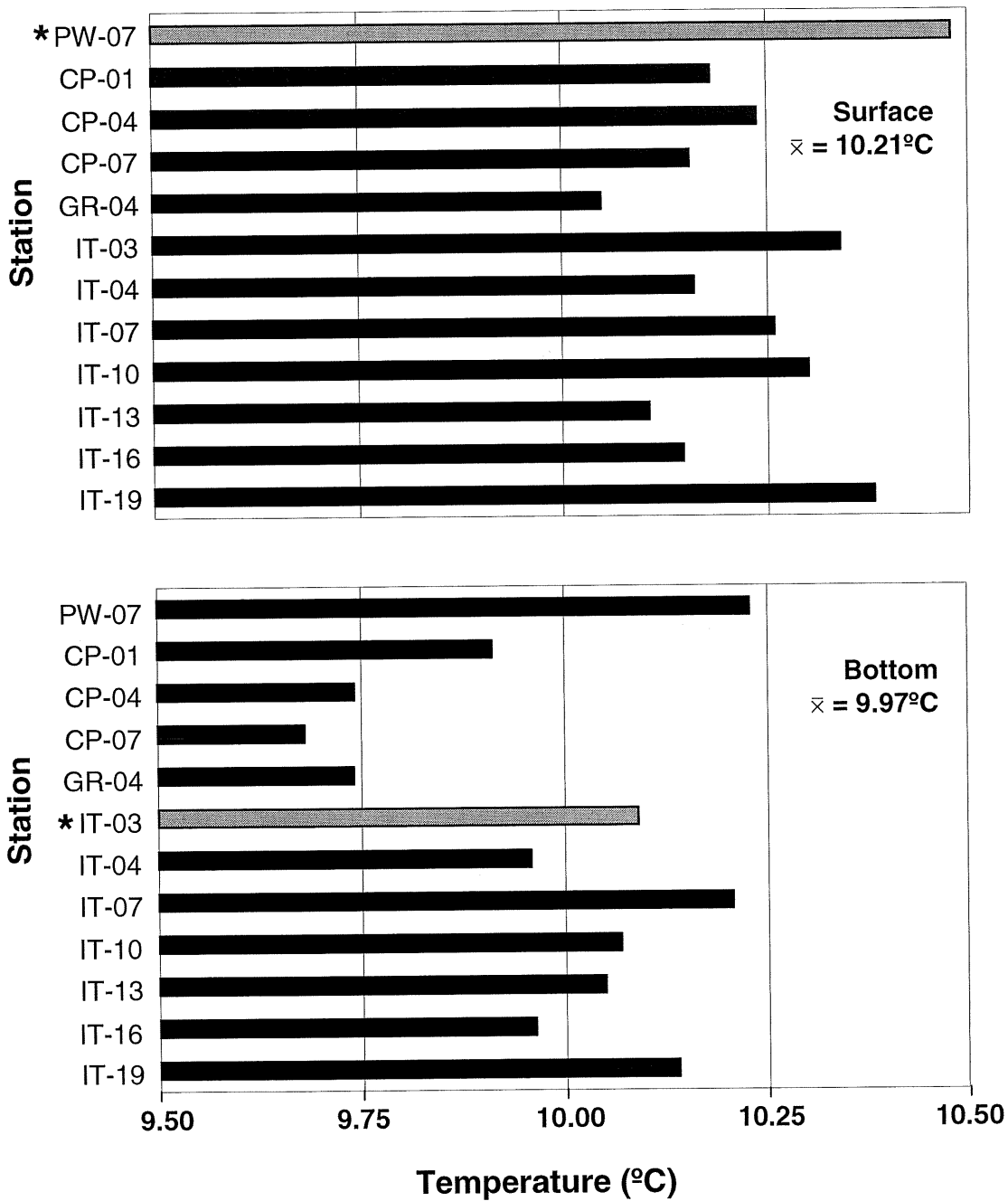


Figure 22. Cherry Point 99 - average water temperature during mussel Deployment. * = average water temperatures for PW-07 surface and IT-03 bottom were calculated by adding or subtracting the average difference between surface and bottom to the actual temperatures measured at the respective stations.

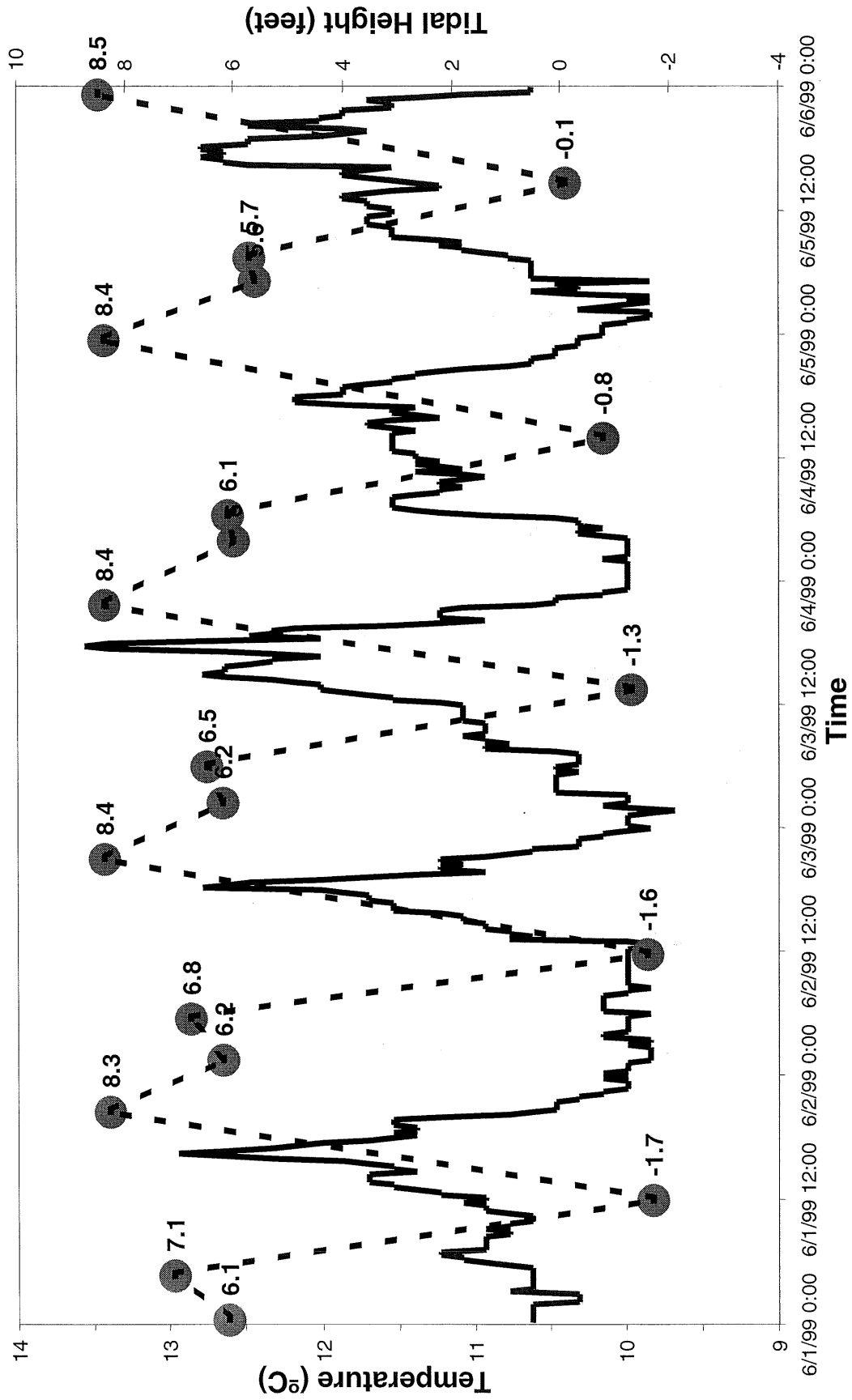


Figure 23. Temperature versus tidal height for Station IT-19: June 1 – 5, 1999.

Table 16. Daily Average Water Temperatures (°C)

| Date | Day | PW7B | CP1S | CP1B | CP4S | CP4B | CP7S | CP7B | GR4S | GR4B | IT3S | IT4S | IT4B | IT7S | IT7B | IT0S | IT0B | IT3S | IT3B | IT6S | IT6B | IT9S | IT9B |
|--------|-----|-------|-------|-------|-------|------|-------|------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 16-Apr | 0 | 8.92 | 8.79 | 8.67 | 8.87 | 8.45 | 8.82 | 8.49 | 8.74 | 8.62 | 8.99 | 8.83 | 8.79 | 8.95 | 9.03 | 8.98 | 8.89 | 8.77 | 8.88 | 8.80 | 8.81 | 9.01 | 8.96 |
| 17-Apr | 1 | 8.76 | 8.75 | 8.74 | 8.88 | 8.56 | 8.79 | 8.54 | 8.73 | 8.59 | 8.90 | 8.74 | 8.74 | 8.85 | 8.95 | 8.87 | 8.84 | 8.69 | 8.82 | 8.73 | 8.76 | 8.91 | 8.91 |
| 18-Apr | 2 | 8.92 | 8.65 | 8.63 | 8.74 | 8.48 | 8.63 | 8.40 | 8.66 | 8.55 | 8.87 | 8.77 | 8.76 | 8.85 | 8.96 | 8.87 | 8.83 | 8.67 | 8.81 | 8.70 | 8.74 | 8.87 | 8.85 |
| 19-Apr | 3 | 8.78 | 8.41 | 8.44 | 8.48 | 8.29 | 8.41 | 8.23 | 8.43 | 8.39 | 8.61 | 8.53 | 8.53 | 8.59 | 8.71 | 8.64 | 8.62 | 8.46 | 8.64 | 8.52 | 8.55 | 8.64 | 8.65 |
| 20-Apr | 4 | 8.72 | 8.57 | 8.53 | 8.67 | 8.44 | 8.58 | 8.33 | 8.54 | 8.44 | 8.78 | 8.65 | 8.65 | 8.73 | 8.83 | 8.75 | 8.73 | 8.56 | 8.74 | 8.59 | 8.64 | 8.79 | 8.76 |
| 21-Apr | 5 | 8.78 | 8.73 | 8.67 | 8.86 | 8.52 | 8.77 | 8.48 | 8.67 | 8.52 | 8.85 | 8.71 | 8.68 | 8.82 | 8.91 | 8.86 | 8.79 | 8.66 | 8.80 | 8.72 | 8.70 | 8.92 | 8.85 |
| 22-Apr | 6 | 9.02 | 8.62 | 8.53 | 8.71 | 8.40 | 8.63 | 8.32 | 8.54 | 8.43 | 8.92 | 8.79 | 8.72 | 8.87 | 8.94 | 8.89 | 8.81 | 8.66 | 8.81 | 8.72 | 8.74 | 8.87 | 8.83 |
| 23-Apr | 7 | 9.27 | 9.07 | 8.85 | 9.12 | 8.70 | 9.00 | 8.68 | 8.65 | 8.54 | 8.98 | 8.80 | 8.77 | 8.91 | 9.01 | 9.03 | 8.90 | 8.86 | 8.90 | 8.86 | 8.85 | 9.18 | 8.97 |
| 24-Apr | 8 | 9.39 | 9.19 | 9.06 | 9.28 | 8.90 | 9.21 | 8.88 | 9.51 | 9.31 | 9.56 | 9.44 | 9.39 | 9.53 | 9.61 | 9.60 | 9.52 | 9.44 | 9.55 | 9.44 | 9.53 | 9.79 | 9.66 |
| 25-Apr | 9 | 9.94 | 10.23 | 10.09 | 10.32 | 9.95 | 10.25 | 9.87 | 10.34 | 10.04 | 10.58 | 10.46 | 10.25 | 10.50 | 10.45 | 10.54 | 10.31 | 10.34 | 10.27 | 10.34 | 10.20 | 10.45 | 10.29 |
| 26-Apr | 10 | 10.17 | 10.22 | 10.01 | 10.32 | 9.85 | 10.17 | 9.72 | 10.24 | 9.89 | 10.53 | 10.38 | 10.16 | 10.48 | 10.42 | 10.51 | 10.30 | 10.35 | 10.28 | 10.35 | 10.17 | 10.50 | 10.26 |
| 27-Apr | 11 | 10.27 | 10.04 | 9.92 | 10.15 | 9.77 | 10.01 | 9.71 | 9.95 | 9.82 | 10.16 | 10.06 | 10.04 | 10.10 | 10.19 | 10.20 | 10.12 | 10.05 | 10.12 | 10.05 | 10.00 | 10.21 | 10.09 |
| 28-Apr | 12 | 9.99 | 9.85 | 9.66 | 9.88 | 9.49 | 9.75 | 9.42 | 9.68 | 9.45 | 9.96 | 9.83 | 9.67 | 9.91 | 9.88 | 9.97 | 9.77 | 9.80 | 9.77 | 9.80 | 9.67 | 9.90 | 9.76 |
| 29-Apr | 13 | 9.88 | 9.63 | 9.42 | 9.68 | 9.22 | 9.61 | 9.12 | 9.35 | 9.13 | 9.71 | 9.50 | 9.37 | 9.60 | 9.55 | 9.63 | 9.47 | 9.44 | 9.45 | 9.44 | 9.36 | 9.73 | 9.54 |
| 30-Apr | 14 | 9.82 | 9.84 | 9.57 | 9.93 | 9.39 | 9.81 | 9.25 | 9.55 | 9.34 | 9.72 | 9.59 | 9.45 | 9.66 | 9.64 | 9.70 | 9.50 | 9.50 | 9.48 | 9.50 | 9.41 | 9.79 | 9.60 |
| 1-May | 15 | 9.98 | 9.85 | 9.73 | 9.92 | 9.53 | 9.85 | 9.39 | 9.68 | 9.49 | 9.80 | 9.68 | 9.59 | 9.74 | 9.76 | 9.79 | 9.69 | 9.61 | 9.68 | 9.61 | 9.60 | 9.78 | 9.71 |
| 2-May | 16 | 9.89 | 9.75 | 9.60 | 9.79 | 9.37 | 9.69 | 9.28 | 9.63 | 9.45 | 9.70 | 9.61 | 9.50 | 9.66 | 9.69 | 9.71 | 9.58 | 9.51 | 9.53 | 9.51 | 9.45 | 9.68 | 9.57 |
| 3-May | 17 | 9.93 | 9.57 | 9.38 | 9.66 | 9.26 | 9.61 | 9.17 | 9.45 | 9.23 | 9.70 | 9.60 | 9.51 | 9.64 | 9.70 | 9.68 | 9.54 | 9.48 | 9.50 | 9.48 | 9.43 | 9.68 | 9.59 |
| 4-May | 18 | 9.90 | 9.70 | 9.55 | 9.83 | 9.38 | 9.72 | 9.29 | 9.56 | 9.32 | 10.15 | 9.95 | 9.66 | 10.03 | 9.90 | 10.03 | 9.71 | 9.80 | 9.68 | 9.80 | 9.64 | 10.16 | 9.88 |
| 5-May | 19 | 9.79 | 9.73 | 9.54 | 9.84 | 9.37 | 9.74 | 9.27 | 9.78 | 9.48 | 10.00 | 9.76 | 9.55 | 9.89 | 9.80 | 9.90 | 9.64 | 9.68 | 9.60 | 9.68 | 9.52 | 10.01 | 9.71 |
| 6-May | 20 | 9.45 | 9.05 | 9.03 | 9.14 | 8.85 | 9.06 | 8.80 | 9.00 | 8.91 | 9.08 | 9.01 | 8.99 | 9.06 | 9.15 | 9.12 | 9.08 | 8.98 | 9.09 | 8.98 | 9.03 | 9.11 | 9.08 |
| 7-May | 21 | 9.75 | 9.35 | 9.35 | 9.47 | 9.19 | 9.41 | 9.11 | 9.39 | 9.27 | 9.54 | 9.43 | 9.28 | 9.51 | 9.50 | 9.56 | 9.36 | 9.37 | 9.36 | 9.37 | 9.28 | 9.59 | 9.46 |
| 8-May | 22 | 9.91 | 9.50 | 9.27 | 9.56 | 9.14 | 9.44 | 9.03 | 9.27 | 9.05 | 9.73 | 9.55 | 9.30 | 9.68 | 9.58 | 9.76 | 9.44 | 9.60 | 9.46 | 9.60 | 9.44 | 9.82 | 9.57 |
| 9-May | 23 | 9.69 | 9.57 | 9.29 | 9.65 | 9.18 | 9.61 | 9.10 | 9.65 | 9.24 | 9.91 | 9.76 | 9.42 | 9.91 | 9.70 | 10.01 | 9.55 | 9.83 | 9.59 | 9.83 | 9.48 | 10.19 | 9.72 |
| 10-May | 24 | 9.69 | 9.95 | 9.57 | 10.02 | 9.40 | 9.93 | 9.32 | 9.91 | 9.57 | 10.33 | 10.13 | 9.87 | 10.24 | 10.13 | 10.32 | 10.01 | 10.14 | 10.03 | 10.14 | 9.88 | 10.45 | 10.05 |
| 11-May | 25 | 9.61 | 10.22 | 9.91 | 10.28 | 9.76 | 10.17 | 9.65 | 10.48 | 9.95 | 10.47 | 10.34 | 10.11 | 10.38 | 10.32 | 10.43 | 10.19 | 10.23 | 10.20 | 10.23 | 10.06 | 10.50 | 10.23 |
| 12-May | 26 | 9.67 | 9.94 | 9.79 | 10.03 | 9.66 | 9.98 | 9.62 | 10.30 | 10.04 | 10.27 | 10.14 | 10.03 | 10.15 | 10.17 | 10.17 | 10.09 | 10.00 | 10.07 | 10.00 | 9.98 | 10.18 | 10.09 |

| Date | Day | PW7 B | CP1 S | CP1 B | CP4 S | CP4 B | CP7 S | CP7 B | GR4 S | GR4 B | IT3 S | IT4 S | IT4 B | IT7 S | IT7 B | IT0 S | IT0 B | IT3 S | IT3 B | IT6 S | IT6 B | IT9 S | IT9 B |
|--------|-----|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 13-May | 27 | 9.69 | 9.96 | 9.61 | 9.93 | 9.44 | 9.87 | 9.40 | 9.67 | 9.35 | 9.91 | 9.75 | 9.51 | 9.87 | 9.79 | 9.92 | 9.65 | 9.71 | 9.61 | 9.71 | 9.56 | 9.89 | 9.71 |
| 14-May | 28 | 9.46 | 9.34 | 9.13 | 9.40 | 8.92 | 9.25 | 8.87 | 9.15 | 8.94 | 9.36 | 9.21 | 9.14 | 9.32 | 9.34 | 9.42 | 9.26 | 9.25 | 9.27 | 9.25 | 9.19 | 9.55 | 9.39 |
| 15-May | 29 | 9.64 | 9.50 | 9.26 | 9.56 | 9.06 | 9.45 | 9.01 | 9.27 | 9.10 | 9.56 | 9.36 | 9.27 | 9.47 | 9.51 | 9.51 | 9.42 | 9.37 | 9.42 | 9.37 | 9.34 | 9.57 | 9.47 |
| 16-May | 30 | 9.65 | 9.45 | 9.32 | 9.57 | 9.17 | 9.48 | 9.10 | 9.34 | 9.16 | 9.37 | 9.24 | 9.19 | 9.36 | 9.41 | 9.42 | 9.32 | 9.24 | 9.31 | 9.24 | 9.25 | 9.53 | 9.40 |
| 17-May | 31 | 9.22 | 8.85 | 8.87 | 8.96 | 8.73 | 8.92 | 8.65 | 8.83 | 8.78 | 9.00 | 8.90 | 8.90 | 8.96 | 9.03 | 9.03 | 9.01 | 8.86 | 9.01 | 8.86 | 8.96 | 9.11 | 9.03 |
| 18-May | 32 | 9.13 | 9.05 | 8.98 | 9.14 | 8.85 | 9.06 | 8.77 | 9.12 | 8.95 | 9.36 | 9.18 | 9.12 | 9.27 | 9.31 | 9.30 | 9.21 | 9.10 | 9.20 | 9.10 | 9.14 | 9.43 | 9.30 |
| 19-May | 33 | 9.42 | 9.14 | 9.00 | 9.17 | 8.85 | 9.11 | 8.79 | 8.96 | 8.86 | 9.39 | 9.17 | 9.11 | 9.26 | 9.33 | 9.28 | 9.23 | 9.12 | 9.20 | 9.12 | 9.13 | 9.41 | 9.26 |
| 20-May | 34 | 9.44 | 9.36 | 9.26 | 9.46 | 9.11 | 9.37 | 9.05 | 9.24 | 9.04 | 9.57 | 9.35 | 9.27 | 9.48 | 9.50 | 9.53 | 9.35 | 9.33 | 9.34 | 9.33 | 9.28 | 9.61 | 9.43 |
| 21-May | 35 | 10.09 | 9.97 | 9.54 | 9.91 | 9.42 | 9.86 | 9.34 | 9.42 | 9.09 | 10.14 | 9.88 | 9.68 | 10.01 | 9.97 | 10.00 | 9.76 | 9.76 | 9.72 | 9.76 | 9.64 | 10.06 | 9.82 |
| 22-May | 36 | 10.07 | 9.67 | 9.50 | 9.77 | 9.29 | 9.68 | 9.21 | 9.53 | 9.23 | 10.07 | 9.87 | 9.66 | 10.01 | 9.95 | 10.05 | 9.80 | 9.85 | 9.79 | 9.85 | 9.70 | 10.22 | 9.86 |
| 23-May | 37 | 10.23 | 9.95 | 9.60 | 10.08 | 9.49 | 10.10 | 9.52 | 10.01 | 9.50 | 10.66 | 10.36 | 9.98 | 10.47 | 10.29 | 10.55 | 10.13 | 10.36 | 10.11 | 10.36 | 10.00 | 10.71 | 10.27 |
| 24-May | 38 | 10.68 | 11.13 | 10.60 | 11.02 | 10.31 | 10.81 | 10.22 | 10.94 | 10.44 | 11.39 | 11.13 | 10.71 | 11.27 | 11.09 | 11.35 | 10.87 | 11.06 | 10.86 | 11.06 | 10.90 | 11.64 | 11.12 |
| 25-May | 39 | 11.79 | 12.52 | 12.07 | 12.51 | 11.83 | 12.44 | 11.80 | 12.70 | 12.17 | 13.12 | 12.75 | 12.20 | 12.79 | 12.52 | 12.82 | 12.35 | 12.52 | 12.28 | 12.52 | 12.12 | 12.92 | 12.31 |
| 26-May | 40 | 11.92 | 12.64 | 11.91 | 12.70 | 11.65 | 12.60 | 11.57 | 12.32 | 11.48 | 12.40 | 12.17 | 11.74 | 12.31 | 12.00 | 12.35 | 11.90 | 12.18 | 11.88 | 12.18 | 11.67 | 12.47 | 11.88 |
| 27-May | 41 | 11.72 | 12.31 | 11.54 | 12.27 | 11.30 | 12.27 | 11.22 | 11.85 | 11.04 | 12.23 | 11.91 | 11.25 | 12.12 | 11.66 | 12.15 | 11.42 | 11.86 | 11.38 | 11.86 | 11.21 | 12.36 | 11.51 |
| 28-May | 42 | 11.50 | 11.31 | 10.90 | 11.30 | 10.67 | 11.18 | 10.55 | 10.78 | 10.32 | 11.45 | 11.11 | 10.80 | 11.35 | 11.11 | 11.42 | 10.92 | 11.26 | 10.92 | 11.26 | 10.86 | 11.81 | 11.17 |
| 29-May | 43 | 11.53 | 11.19 | 10.91 | 11.26 | 10.77 | 11.30 | 10.79 | 11.13 | 10.84 | 11.71 | 11.54 | 11.23 | 11.74 | 11.52 | 11.87 | 11.48 | 11.66 | 11.46 | 11.66 | 11.36 | 12.05 | 11.63 |
| 30-May | 44 | 11.63 | 12.01 | 11.60 | 12.06 | 11.45 | 11.96 | 11.38 | 11.71 | 11.35 | 12.05 | 11.83 | 11.51 | 11.91 | 11.76 | 11.92 | 11.60 | 11.75 | 11.58 | 11.75 | 11.45 | 11.92 | 11.68 |
| 31-May | 45 | 11.56 | 11.79 | 11.59 | 11.83 | 11.41 | 11.73 | 11.33 | 11.48 | 11.30 | 11.51 | 11.31 | 11.12 | 11.38 | 11.34 | 11.36 | 11.18 | 11.15 | 11.13 | 11.15 | 11.01 | 11.33 | 11.18 |
| 1-Jun | 46 | 11.33 | 11.07 | 10.67 | 11.07 | 10.49 | 11.00 | 10.43 | 10.85 | 10.55 | 11.08 | 10.92 | 10.72 | 10.97 | 10.94 | 10.97 | 10.80 | 10.77 | 10.76 | 10.77 | 10.71 | 10.99 | 10.85 |
| 2-Jun | 47 | 10.81 | 10.31 | 10.17 | 10.42 | 9.99 | 10.31 | 9.95 | 10.18 | 9.97 | 10.55 | 10.40 | 10.33 | 10.49 | 10.56 | 10.52 | 10.39 | 10.36 | 10.35 | 10.36 | 10.29 | 10.51 | 10.45 |
| 3-Jun | 48 | 10.96 | 11.22 | 10.73 | 11.27 | 10.53 | 11.10 | 10.48 | 10.93 | 10.43 | 11.05 | 10.78 | 10.56 | 10.87 | 10.80 | 10.89 | 10.63 | 10.70 | 10.63 | 10.70 | 10.53 | 11.11 | 10.78 |
| 4-Jun | 49 | 10.95 | 11.38 | 11.05 | 11.38 | 10.86 | 11.23 | 10.74 | 11.09 | 10.75 | 11.06 | 10.85 | 10.65 | 10.92 | 10.88 | 10.88 | 10.70 | 10.69 | 10.66 | 10.69 | 10.60 | 10.97 | 10.77 |
| 5-Jun | 50 | 11.03 | 11.29 | 10.90 | 11.34 | 10.73 | 11.24 | 10.66 | 11.00 | 10.41 | 11.05 | 10.86 | 10.71 | 10.94 | 10.92 | 10.98 | 10.79 | 10.80 | 10.80 | 10.80 | 10.72 | 11.27 | 10.96 |
| 6-Jun | 51 | 11.66 | 11.87 | 11.29 | 11.89 | 11.12 | 11.81 | 11.08 | 11.68 | 11.06 | 11.84 | 11.66 | 11.31 | 11.75 | 11.64 | 11.75 | 11.47 | 11.53 | 11.41 | 11.53 | 11.29 | 11.80 | 11.46 |
| 7-Jun | 52 | 11.93 | 11.79 | 11.19 | 11.84 | 11.04 | 11.85 | 11.03 | 11.67 | 10.80 | 12.09 | 11.85 | 11.20 | 11.96 | 11.58 | 12.03 | 11.35 | 11.81 | 11.33 | 11.81 | 11.18 | 12.05 | 11.42 |
| 8-Jun | 53 | 11.38 | 12.07 | 11.17 | 12.03 | 10.99 | 11.89 | 10.96 | 11.80 | 11.05 | 12.29 | 12.05 | 11.52 | 12.14 | 11.85 | 12.27 | 11.70 | 12.04 | 11.68 | 12.04 | 11.55 | 12.26 | 11.85 |
| 9-Jun | 54 | 12.05 | 12.46 | 11.58 | 12.30 | 11.40 | 12.28 | 11.37 | 12.06 | 11.38 | 12.53 | 12.20 | 11.57 | 12.27 | 11.88 | 12.27 | 11.63 | 12.00 | 11.59 | 12.00 | 11.40 | 12.35 | 11.71 |
| 10-Jun | 55 | 12.02 | 12.38 | 11.37 | 12.19 | 11.02 | 12.03 | 11.00 | 11.43 | 10.62 | 12.28 | 11.99 | 11.29 | 12.13 | 11.69 | 12.15 | 11.45 | 11.87 | 11.40 | 11.87 | 11.25 | 12.38 | 11.57 |

| Date | Day | PW7 B | CP1 S | CP1 B | CP4 S | CP4 B | CP7 S | CP7 B | GR4 S | GR4 B | IT3 S | IT4 S | IT4 B | IT7 S | IT7 B | IT0 S | IT0 B | IT3 S | IT3 B | IT6 S | IT6 B | IT9 S | IT9 B |
|---------|------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 11-Jun | 56 | 10.99 | 10.51 | 10.08 | 10.50 | 9.85 | 10.53 | 9.93 | 10.18 | 9.89 | 10.70 | 10.48 | 10.21 | 10.62 | 10.49 | 10.63 | 10.38 | 10.47 | 10.31 | 10.47 | 10.22 | 10.79 | 10.45 |
| 12-Jun | 57 | 10.50 | 9.87 | 9.77 | 9.96 | 9.64 | 9.94 | 9.60 | 10.15 | 9.97 | 10.41 | 10.24 | 10.15 | 10.37 | 10.43 | 10.40 | 10.26 | 10.19 | 10.22 | 10.19 | 10.13 | 10.46 | 10.34 |
| 13-Jun | 58 | 10.50 | 10.07 | 9.94 | 10.24 | 9.85 | 10.19 | 9.83 | 9.96 | 9.84 | 10.22 | 10.09 | 10.03 | 10.20 | 10.28 | 10.26 | 10.18 | 10.11 | 10.15 | 10.11 | 10.09 | 10.39 | 10.27 |
| 14-Jun | 59 | 10.40 | 9.98 | 9.92 | 10.12 | 9.79 | 10.03 | 9.71 | 9.85 | 9.75 | 10.28 | 10.09 | 10.03 | 10.22 | 10.26 | 10.21 | 10.12 | 10.02 | 10.06 | 10.02 | 10.03 | 10.38 | 10.24 |
| 15-Jun | 60 | 10.22 | 10.29 | 10.20 | 10.37 | 10.05 | 10.29 | 9.98 | 10.37 | 10.12 | 10.74 | 10.61 | 10.48 | 10.75 | 10.81 | 10.75 | 10.64 | 10.52 | 10.53 | 10.52 | 10.46 | 10.70 | 10.64 |
| 16-Jun | 61 | 10.74 | 9.94 | 9.86 | 10.01 | 9.70 | 10.01 | 9.65 | 9.87 | 9.66 | 10.24 | 9.97 | 9.87 | 10.14 | 10.12 | 10.16 | 9.96 | 9.85 | 9.97 | 9.85 | 9.90 | 10.03 | 9.98 |
| Overall | Mean | 10.22 | 10.17 | 9.90 | 10.22 | 9.73 | 10.14 | 9.67 | 10.04 | 9.73 | 10.33 | 10.15 | 9.95 | 10.25 | 10.19 | 10.29 | 10.06 | 10.09 | 10.04 | 10.10 | 9.95 | 10.37 | 10.13 |
| | Min | 8.72 | 8.41 | 8.44 | 8.48 | 8.29 | 8.41 | 8.23 | 8.43 | 8.39 | 8.61 | 8.53 | 8.53 | 8.59 | 8.71 | 8.64 | 8.62 | 8.46 | 8.64 | 8.52 | 8.55 | 8.64 | 8.65 |
| | Max | 12.05 | 12.64 | 12.07 | 12.70 | 11.83 | 12.60 | 11.80 | 12.70 | 12.17 | 13.12 | 12.75 | 12.20 | 12.79 | 12.52 | 12.82 | 12.35 | 12.52 | 12.28 | 12.52 | 12.12 | 12.92 | 12.31 |
| 0-27: | Mean | 9.56 | 9.45 | 9.30 | 9.54 | 9.14 | 9.45 | 9.07 | 9.41 | 9.19 | 9.63 | 9.49 | 9.37 | 9.57 | 9.58 | 9.62 | 9.46 | 9.44 | 9.45 | 9.45 | 9.38 | 9.66 | 9.51 |
| 28-33 | Mean | 9.42 | 9.22 | 9.09 | 9.30 | 8.93 | 9.21 | 8.86 | 9.11 | 8.96 | 9.34 | 9.18 | 9.12 | 9.27 | 9.32 | 9.33 | 9.24 | 9.16 | 9.23 | 9.16 | 9.17 | 9.43 | 9.31 |
| 34-61: | Mean | 11.06 | 11.08 | 10.68 | 11.11 | 10.49 | 11.04 | 10.44 | 10.86 | 10.43 | 11.24 | 11.01 | 10.71 | 11.13 | 10.99 | 11.16 | 10.83 | 10.95 | 10.80 | 10.95 | 10.70 | 11.27 | 10.91 |

Table 17. Range in Daily Water Temperatures (°C)

| Date | Day | PW7 B | CP1 S | CP1 B | CP4 S | CP4 B | CP7 S | CP7 B | GR4 S | GR4 B | IT3 S | IT4 S | IT4 B | IT7 S | IT7 B | IT0 S | IT0 B | IT3 S | IT3 B | IT6 S | IT6 B | IT9 S | IT9 B |
|--------|-----|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 16-Apr | 0 | 0.83 | 0.93 | 0.93 | 0.92 | 1.10 | 1.08 | 0.78 | 1.07 | 1.09 | 0.93 | 1.09 | 1.23 | 1.07 | 1.08 | 1.08 | 1.09 | 1.11 | 1.08 | 1.09 | 1.08 | 1.23 | 1.23 |
| 17-Apr | 1 | 0.55 | 0.77 | 0.78 | 0.93 | 0.83 | 1.09 | 1.24 | 1.07 | 1.08 | 1.39 | 1.23 | 1.24 | 1.39 | 1.39 | 1.24 | 1.39 | 1.39 | 1.23 | 1.24 | 1.24 | 1.54 | 1.54 |
| 18-Apr | 2 | 1.11 | 0.93 | 0.62 | 0.62 | 0.55 | 0.77 | 0.78 | 0.61 | 0.77 | 1.23 | 1.08 | 1.08 | 1.23 | 1.08 | 1.08 | 1.24 | 1.11 | 1.08 | 1.09 | 1.08 | 1.39 | 1.23 |
| 19-Apr | 3 | 0.55 | 0.46 | 0.31 | 0.31 | 0.28 | 0.31 | 0.31 | 0.31 | 0.31 | 0.61 | 0.62 | 0.46 | 0.62 | 0.62 | 0.62 | 0.62 | 0.55 | 0.61 | 0.62 | 0.62 | 0.62 | 0.77 |
| 20-Apr | 4 | 0.55 | 1.24 | 0.93 | 0.92 | 0.83 | 0.93 | 0.78 | 1.23 | 1.08 | 1.39 | 1.39 | 1.54 | 1.55 | 1.39 | 1.39 | 1.39 | 1.39 | 1.23 | 1.39 | 1.39 | 1.54 | 1.54 |
| 21-Apr | 5 | 1.39 | 1.24 | 0.77 | 1.08 | 0.83 | 0.93 | 0.78 | 1.23 | 1.08 | 1.55 | 1.39 | 1.54 | 1.70 | 1.55 | 1.39 | 1.39 | 1.39 | 1.38 | 1.39 | 1.39 | 1.54 | 1.85 |
| 22-Apr | 6 | 1.39 | 0.93 | 0.62 | 1.08 | 0.55 | 0.93 | 0.78 | 1.07 | 1.08 | 1.39 | 1.39 | 1.24 | 1.54 | 1.39 | 1.39 | 1.39 | 1.39 | 1.23 | 1.55 | 1.54 | 1.86 | 1.70 |
| 23-Apr | 7 | 1.69 | 1.23 | 0.93 | 1.39 | 0.83 | 1.24 | 0.77 | 0.91 | 1.08 | 1.55 | 1.54 | 1.23 | 1.70 | 1.55 | 1.70 | 1.39 | 1.39 | 1.55 | 1.39 | 1.39 | 2.01 | 1.85 |
| 24-Apr | 8 | 2.83 | 1.86 | 1.24 | 1.39 | 1.11 | 1.55 | 1.40 | 2.95 | 2.32 | 1.55 | 1.24 | 1.39 | 1.09 | 1.41 | 1.39 | 1.24 | 1.12 | 1.24 | 1.12 | 1.24 | 1.71 | 1.70 |
| 25-Apr | 9 | 1.13 | 1.24 | 1.24 | 1.25 | 1.41 | 1.40 | 1.56 | 1.72 | 2.17 | 1.24 | 1.39 | 1.70 | 1.24 | 1.40 | 1.24 | 2.01 | 1.13 | 1.56 | 1.13 | 1.70 | 0.94 | 1.40 |
| 26-Apr | 10 | 1.41 | 1.09 | 1.40 | 1.09 | 1.41 | 0.94 | 1.40 | 1.72 | 1.56 | 1.24 | 1.54 | 1.70 | 1.09 | 1.72 | 1.09 | 1.39 | 1.13 | 1.56 | 1.13 | 1.70 | 1.55 | 1.40 |
| 27-Apr | 11 | 2.27 | 1.55 | 1.39 | 1.40 | 1.13 | 1.39 | 1.24 | 1.25 | 1.40 | 1.39 | 1.24 | 1.24 | 1.24 | 1.09 | 1.70 | 1.39 | 1.41 | 1.25 | 1.41 | 1.25 | 1.55 | 1.39 |
| 28-Apr | 12 | 1.13 | 1.55 | 1.25 | 1.55 | 0.84 | 1.40 | 1.08 | 1.24 | 1.09 | 1.86 | 1.86 | 1.39 | 2.01 | 1.71 | 1.55 | 1.24 | 1.41 | 1.24 | 1.41 | 1.08 | 1.70 | 1.24 |
| 29-Apr | 13 | 2.26 | 1.55 | 0.78 | 1.39 | 0.84 | 0.93 | 0.93 | 1.71 | 1.56 | 1.24 | 1.39 | 1.24 | 1.39 | 1.25 | 1.23 | 1.08 | 1.40 | 1.09 | 1.40 | 1.08 | 1.39 | 1.24 |
| 30-Apr | 14 | 1.69 | 1.71 | 1.86 | 1.86 | 1.69 | 1.55 | 1.71 | 1.55 | 1.24 | 1.55 | 1.71 | 1.55 | 1.55 | 1.56 | 1.70 | 1.39 | 1.69 | 1.24 | 1.69 | 1.24 | 2.02 | 1.86 |
| 1-May | 15 | 1.41 | 0.77 | 1.09 | 0.93 | 1.12 | 0.78 | 1.24 | 1.24 | 1.24 | 1.40 | 1.23 | 1.24 | 1.24 | 1.40 | 1.08 | 1.24 | 1.12 | 1.24 | 1.12 | 1.23 | 1.24 | 1.24 |
| 2-May | 16 | 1.41 | 0.61 | 0.93 | 0.62 | 1.12 | 0.77 | 0.93 | 1.09 | 1.24 | 1.09 | 1.08 | 1.24 | 1.08 | 1.09 | 1.08 | 1.09 | 1.12 | 1.24 | 1.12 | 1.23 | 1.09 | 1.08 |
| 3-May | 17 | 1.69 | 1.08 | 1.08 | 1.24 | 1.12 | 1.24 | 1.09 | 1.39 | 1.24 | 1.70 | 1.55 | 1.55 | 1.71 | 1.72 | 1.39 | 1.55 | 1.40 | 1.40 | 1.40 | 1.55 | 1.70 | 2.01 |
| 4-May | 18 | 1.97 | 1.39 | 1.40 | 1.40 | 1.40 | 1.40 | 1.09 | 2.02 | 2.17 | 1.70 | 1.85 | 2.02 | 1.70 | 2.02 | 1.70 | 2.01 | 1.69 | 2.02 | 1.69 | 2.01 | 2.17 | 2.17 |
| 5-May | 19 | 2.25 | 1.87 | 1.71 | 2.01 | 1.96 | 1.86 | 1.39 | 1.25 | 1.40 | 2.02 | 1.86 | 1.70 | 1.70 | 2.03 | 1.70 | 1.85 | 1.69 | 1.87 | 1.69 | 2.01 | 2.01 | 2.17 |
| 6-May | 20 | 1.12 | 0.63 | 0.62 | 0.77 | 0.55 | 0.78 | 0.46 | 0.93 | 0.93 | 0.77 | 0.62 | 0.77 | 0.77 | 0.77 | 0.77 | 0.78 | 0.56 | 0.77 | 0.56 | 0.77 | 0.92 | 0.77 |
| 7-May | 21 | 1.12 | 0.93 | 0.93 | 0.77 | 0.84 | 0.78 | 0.93 | 0.77 | 0.93 | 0.93 | 0.77 | 0.93 | 0.93 | 1.09 | 0.93 | 0.92 | 0.84 | 0.93 | 0.84 | 1.08 | 1.39 | 1.39 |
| 8-May | 22 | 1.97 | 2.17 | 1.24 | 2.02 | 0.84 | 1.40 | 1.24 | 1.24 | 0.93 | 2.32 | 2.32 | 1.86 | 2.16 | 2.02 | 2.31 | 1.55 | 1.97 | 1.56 | 1.97 | 1.40 | 2.16 | 2.16 |
| 9-May | 23 | 1.69 | 1.24 | 1.24 | 1.40 | 1.12 | 1.40 | 1.24 | 1.55 | 1.09 | 2.32 | 2.47 | 1.24 | 2.32 | 2.02 | 2.32 | 1.70 | 1.97 | 1.86 | 1.97 | 1.71 | 2.17 | 2.47 |
| 10-May | 24 | 3.41 | 2.63 | 1.71 | 2.64 | 1.69 | 2.32 | 1.71 | 1.87 | 1.55 | 2.93 | 2.63 | 2.31 | 2.79 | 2.49 | 3.24 | 2.63 | 2.84 | 2.80 | 2.84 | 2.79 | 2.63 | 2.62 |
| 11-May | 25 | 1.69 | 1.70 | 1.55 | 1.55 | 1.97 | 1.70 | 1.71 | 1.87 | 1.86 | 1.40 | 1.70 | 1.85 | 1.55 | 1.87 | 1.55 | 1.85 | 1.69 | 1.87 | 1.69 | 1.85 | 1.40 | 1.55 |
| 12-May | 26 | 1.12 | 1.08 | 1.24 | 1.24 | 1.12 | 1.25 | 1.40 | 1.72 | 1.40 | 1.08 | 0.94 | 1.23 | 0.94 | 1.10 | 0.93 | 1.08 | 0.85 | 0.94 | 0.85 | 1.09 | 1.09 | 1.24 |

| Date | Day | PW7B | CP1S | CP1B | CP4S | CP4B | CP7S | CP7B | GR4S | GR4B | IT3S | IT4S | IT4B | IT7S | IT7B | IT0S | IT0B | IT3S | IT3B | IT6S | IT6B | IT9S | IT9B |
|--------|-----|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| 13-May | 27 | 2.26 | 1.70 | 2.01 | 1.86 | 1.97 | 2.16 | 2.02 | 2.02 | 2.33 | 1.70 | 1.55 | 1.55 | 1.55 | 1.40 | 1.70 | 1.55 | 1.69 | 1.56 | 1.69 | 1.55 | 1.55 | 1.71 |
| 14-May | 28 | 1.97 | 1.39 | 1.09 | 1.54 | 1.11 | 1.24 | 1.24 | 1.39 | 1.08 | 1.54 | 1.55 | 1.24 | 1.55 | 1.40 | 1.55 | 1.55 | 1.67 | 1.55 | 1.67 | 1.55 | 1.67 | 1.70 |
| 15-May | 29 | 2.83 | 2.63 | 2.02 | 2.32 | 1.67 | 2.02 | 1.71 | 2.64 | 2.17 | 2.00 | 1.86 | 1.70 | 2.17 | 2.02 | 2.17 | 2.17 | 1.97 | 2.17 | 1.97 | 2.17 | 1.97 | 2.32 |
| 16-May | 30 | 1.40 | 1.55 | 1.40 | 1.40 | 1.40 | 1.40 | 1.39 | 1.55 | 1.40 | 1.39 | 1.24 | 1.24 | 1.40 | 1.41 | 1.23 | 1.24 | 1.12 | 1.39 | 1.12 | 1.39 | 1.12 | 1.70 |
| 17-May | 31 | 1.40 | 0.32 | 0.31 | 0.31 | 0.27 | 0.47 | 0.31 | 0.46 | 0.16 | 0.15 | 0.31 | 0.31 | 0.46 | 0.31 | 0.47 | 0.31 | 0.28 | 0.31 | 0.28 | 0.31 | 0.28 | 0.62 |
| 18-May | 32 | 1.12 | 1.55 | 1.55 | 1.54 | 1.39 | 1.55 | 1.55 | 1.55 | 1.55 | 1.70 | 1.70 | 1.70 | 1.70 | 1.71 | 1.55 | 1.71 | 1.67 | 1.55 | 1.67 | 1.55 | 1.67 | 1.70 |
| 19-May | 33 | 2.25 | 1.55 | 1.40 | 1.39 | 1.39 | 1.56 | 1.55 | 1.86 | 1.86 | 2.16 | 1.86 | 2.01 | 2.17 | 2.03 | 2.01 | 2.17 | 1.69 | 1.71 | 1.69 | 1.86 | 2.94 | 2.78 |
| 20-May | 34 | 1.12 | 1.86 | 1.87 | 1.86 | 1.96 | 1.87 | 1.86 | 2.02 | 2.17 | 2.32 | 2.01 | 2.01 | 2.32 | 2.33 | 2.48 | 2.32 | 2.24 | 2.17 | 2.24 | 2.32 | 2.78 | 2.78 |
| 21-May | 35 | 1.41 | 1.85 | 1.86 | 1.86 | 1.69 | 1.71 | 1.87 | 2.02 | 2.02 | 2.94 | 2.94 | 2.63 | 3.25 | 2.95 | 3.08 | 3.09 | 2.83 | 2.48 | 2.83 | 2.63 | 3.10 | 3.25 |
| 22-May | 36 | 2.56 | 1.71 | 0.93 | 2.01 | 1.40 | 1.71 | 1.09 | 1.86 | 1.71 | 3.72 | 2.32 | 1.86 | 2.32 | 2.02 | 2.32 | 1.55 | 1.97 | 1.56 | 1.97 | 1.71 | 2.64 | 1.86 |
| 23-May | 37 | 3.15 | 2.47 | 2.01 | 2.48 | 1.97 | 2.01 | 1.87 | 3.10 | 2.32 | 2.94 | 2.00 | 1.85 | 2.16 | 2.02 | 2.77 | 2.31 | 2.55 | 2.48 | 2.55 | 2.16 | 3.26 | 2.78 |
| 24-May | 38 | 2.57 | 4.62 | 4.81 | 4.48 | 4.31 | 4.34 | 3.88 | 4.19 | 3.88 | 4.79 | 4.63 | 4.02 | 4.48 | 4.35 | 3.71 | 4.49 | 3.46 | 3.88 | 3.46 | 3.72 | 4.18 | 3.71 |
| 25-May | 39 | 2.31 | 2.16 | 1.55 | 2.48 | 2.31 | 2.18 | 2.47 | 2.47 | 2.32 | 2.63 | 2.94 | 3.09 | 3.10 | 2.80 | 3.09 | 3.25 | 2.94 | 3.25 | 2.94 | 2.94 | 3.10 | 3.09 |
| 26-May | 40 | 2.64 | 1.70 | 2.63 | 2.01 | 2.31 | 1.72 | 2.63 | 2.63 | 2.94 | 3.24 | 2.93 | 3.40 | 3.71 | 3.56 | 3.85 | 3.87 | 3.47 | 3.57 | 3.47 | 3.56 | 3.71 | 4.33 |
| 27-May | 41 | 2.61 | 3.09 | 2.48 | 2.94 | 2.60 | 2.95 | 2.63 | 3.25 | 2.94 | 3.24 | 2.93 | 3.40 | 3.71 | 3.56 | 3.85 | 3.87 | 3.47 | 3.57 | 3.47 | 3.56 | 3.71 | 4.33 |
| 28-May | 42 | 2.90 | 3.25 | 3.10 | 3.10 | 3.15 | 3.26 | 2.79 | 3.56 | 2.94 | 3.25 | 3.10 | 2.94 | 3.10 | 3.27 | 2.94 | 3.10 | 2.57 | 2.96 | 2.57 | 2.94 | 3.10 | 3.24 |
| 29-May | 43 | 2.31 | 2.63 | 2.79 | 2.48 | 2.87 | 2.48 | 2.63 | 2.48 | 2.63 | 2.94 | 3.25 | 2.79 | 3.10 | 3.12 | 3.55 | 3.10 | 3.16 | 2.95 | 3.16 | 2.95 | 3.41 | 3.40 |
| 30-May | 44 | 2.33 | 1.85 | 1.85 | 2.01 | 2.01 | 2.01 | 2.01 | 2.17 | 1.86 | 2.94 | 2.94 | 2.94 | 3.10 | 2.95 | 3.09 | 3.10 | 2.88 | 3.10 | 2.88 | 3.25 | 3.25 | 3.40 |
| 31-May | 45 | 2.31 | 1.85 | 1.70 | 2.01 | 1.72 | 2.01 | 2.01 | 2.16 | 2.16 | 2.16 | 2.16 | 2.01 | 2.48 | 2.32 | 2.16 | 2.17 | 2.00 | 2.02 | 2.00 | 2.17 | 2.79 | 2.79 |
| 1-Jun | 46 | 1.43 | 1.55 | 1.85 | 1.40 | 1.70 | 1.23 | 1.54 | 2.17 | 2.17 | 2.48 | 1.85 | 1.86 | 2.17 | 2.17 | 2.16 | 2.01 | 2.00 | 2.17 | 2.00 | 2.64 | 2.95 | 2.78 |
| 2-Jun | 47 | 2.59 | 2.16 | 2.32 | 2.17 | 1.98 | 2.16 | 2.01 | 2.17 | 2.16 | 2.78 | 2.78 | 2.79 | 2.95 | 2.95 | 2.94 | 2.79 | 2.57 | 2.65 | 2.57 | 2.79 | 2.94 | 3.10 |
| 3-Jun | 48 | 3.75 | 3.24 | 2.94 | 3.25 | 2.85 | 3.10 | 2.95 | 3.26 | 3.10 | 2.62 | 2.63 | 2.63 | 2.63 | 2.80 | 2.78 | 2.78 | 2.57 | 2.65 | 2.57 | 2.79 | 3.88 | 3.87 |
| 4-Jun | 49 | 2.29 | 1.54 | 1.86 | 1.40 | 1.71 | 1.07 | 1.54 | 1.71 | 1.86 | 1.86 | 1.70 | 1.70 | 1.87 | 2.03 | 1.86 | 1.86 | 1.71 | 1.86 | 1.71 | 1.86 | 2.18 | 2.32 |
| 5-Jun | 50 | 2.59 | 1.69 | 2.48 | 2.02 | 2.56 | 2.33 | 2.64 | 2.48 | 2.64 | 2.47 | 2.47 | 2.47 | 2.63 | 2.34 | 2.31 | 2.33 | 2.28 | 2.34 | 2.28 | 2.33 | 2.94 | 2.63 |
| 6-Jun | 51 | 2.90 | 2.16 | 2.17 | 2.63 | 2.29 | 2.47 | 2.64 | 2.33 | 2.64 | 2.63 | 2.79 | 2.63 | 2.63 | 2.64 | 2.94 | 2.95 | 2.59 | 2.95 | 2.59 | 2.95 | 2.48 | 3.25 |
| 7-Jun | 52 | 3.47 | 2.31 | 2.95 | 2.63 | 3.16 | 2.47 | 3.25 | 2.79 | 2.79 | 3.09 | 3.56 | 3.71 | 3.25 | 3.41 | 3.09 | 3.72 | 3.19 | 3.57 | 3.19 | 3.41 | 3.09 | 3.25 |
| 8-Jun | 53 | 4.03 | 2.61 | 2.48 | 2.63 | 2.59 | 2.63 | 2.79 | 3.72 | 3.25 | 2.94 | 3.40 | 3.56 | 3.09 | 3.58 | 3.09 | 3.42 | 2.90 | 3.41 | 2.90 | 3.56 | 3.09 | 3.40 |
| 9-Jun | 54 | 3.79 | 2.62 | 3.57 | 2.94 | 3.46 | 2.79 | 3.41 | 2.48 | 3.10 | 2.47 | 2.16 | 2.79 | 2.62 | 2.49 | 2.47 | 2.63 | 2.60 | 2.64 | 2.60 | 3.09 | 2.78 | 3.08 |
| 10-Jun | 55 | 4.07 | 3.39 | 3.57 | 3.40 | 3.74 | 3.40 | 3.72 | 3.11 | 2.94 | 3.39 | 3.40 | 3.72 | 3.40 | 3.72 | 3.25 | 3.72 | 3.19 | 3.57 | 3.19 | 3.71 | 3.10 | 3.56 |

| Date | Day | PW7 B | CP1 S | CP1 B | CP4 S | CP4 B | CP7 S | CP7 B | GR4 S | GR4 B | IT3 S | IT4 S | IT4 B | IT7 S | IT7 B | IT0 S | IT0 B | IT3 S | IT3 B | IT6 S | IT6 B | IT9 S | IT9 B |
|---------|------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 11-Jun | 56 | 4.95 | 3.40 | 1.86 | 2.79 | 1.69 | 2.63 | 1.86 | 2.95 | 2.64 | 3.56 | 3.09 | 1.86 | 3.71 | 2.80 | 4.17 | 2.78 | 3.74 | 3.11 | 3.74 | 2.33 | 4.03 | 2.48 |
| 12-Jun | 57 | 3.46 | 1.55 | 1.09 | 1.24 | 1.13 | 1.40 | 1.09 | 3.42 | 2.32 | 3.40 | 2.31 | 2.17 | 2.94 | 2.96 | 2.63 | 2.63 | 2.56 | 2.49 | 2.56 | 2.64 | 3.72 | 3.86 |
| 13-Jun | 58 | 4.65 | 2.46 | 2.01 | 2.48 | 1.98 | 2.63 | 2.47 | 2.64 | 2.48 | 3.10 | 2.94 | 2.95 | 3.26 | 3.11 | 2.94 | 2.79 | 2.85 | 2.64 | 2.85 | 2.64 | 3.26 | 3.40 |
| 14-Jun | 59 | 3.46 | 2.00 | 1.70 | 2.17 | 1.69 | 2.32 | 2.47 | 2.80 | 2.64 | 3.40 | 2.78 | 2.79 | 3.41 | 3.27 | 2.79 | 2.78 | 1.99 | 2.49 | 1.99 | 2.49 | 3.72 | 3.71 |
| 15-Jun | 60 | 0.85 | 2.16 | 2.16 | 2.02 | 1.98 | 2.01 | 2.01 | 2.18 | 2.16 | 2.94 | 2.63 | 2.63 | 3.10 | 3.11 | 3.09 | 3.10 | 2.87 | 3.27 | 2.87 | 3.25 | 3.10 | 3.56 |
| 16-Jun | 61 | 2.88 | 1.39 | 0.78 | 1.40 | 1.13 | 1.40 | 1.25 | 1.09 | 0.78 | 1.24 | 1.09 | 0.93 | 0.93 | 1.09 | 1.08 | 0.78 | 0.85 | 0.79 | 0.85 | 0.46 | 0.78 | 0.94 |
| Overall | Mean | 2.17 | 1.78 | 1.66 | 1.78 | 1.65 | 1.72 | 1.70 | 1.96 | 1.86 | 2.13 | 2.02 | 1.96 | 2.12 | 2.09 | 2.09 | 2.06 | 1.94 | 1.99 | 1.95 | 2.02 | 2.30 | 2.30 |
| | Min | 0.55 | 0.32 | 0.31 | 0.31 | 0.27 | 0.31 | 0.31 | 0.31 | 0.16 | 0.15 | 0.31 | 0.31 | 0.46 | 0.31 | 0.47 | 0.31 | 0.28 | 0.31 | 0.28 | 0.31 | 0.62 | 0.62 |
| | Max | 4.95 | 4.62 | 4.81 | 4.48 | 4.31 | 4.34 | 3.88 | 4.19 | 3.88 | 4.79 | 4.63 | 4.02 | 4.48 | 4.35 | 4.17 | 4.49 | 3.74 | 3.88 | 3.74 | 3.72 | 4.18 | 4.33 |
| 0-27: | Mean | 1.57 | 1.29 | 1.14 | 1.27 | 1.11 | 1.22 | 1.14 | 1.38 | 1.33 | 1.48 | 1.45 | 1.40 | 1.46 | 1.47 | 1.45 | 1.41 | 1.37 | 1.38 | 1.37 | 1.40 | 1.58 | 1.59 |
| 28-33 | Mean | 1.83 | 1.50 | 1.30 | 1.42 | 1.21 | 1.37 | 1.29 | 1.58 | 1.37 | 1.49 | 1.42 | 1.37 | 1.58 | 1.48 | 1.50 | 1.53 | 1.40 | 1.45 | 1.40 | 1.47 | 1.91 | 1.80 |
| 34-61: | Mean | 2.84 | 2.33 | 2.26 | 2.37 | 2.28 | 2.30 | 2.34 | 2.61 | 2.48 | 2.91 | 2.72 | 2.64 | 2.90 | 2.84 | 2.86 | 2.82 | 2.63 | 2.72 | 2.63 | 2.75 | 3.10 | 3.12 |

**Table 18. Statistical Results for Daily Average Water Temperatures
And Daily Water Temperature Ranges**

| | Surface | Bottom |
|--|---|---|
| <i>Daily Average Water Temperatures</i> | | |
| Mussel Exposure | No Significant Differences (p = 0.8187) | Significant Differences (p = 0.0043): PW-07≠CP-07 |
| Pre-herring | No Significant Differences (p = 0.7224) | Significant Differences (p = 0.0005): PW-07≠CP-07 CP-04≠IT-07 CP-07≠IT-19 |
| Herring | No Significant Differences (p = 0.1643) | Significant Differences (p < 0.0001): PW-07≠ CP-01, CP-04, CP-07, GR-04 CP-04≠IT-07, IT-10, IT-13, IT-19 CP-07≠IT-07, IT-10, IT-13, IT-16, IT-19 GR-04≠IT-07, IT-19 |
| Post-herring | No Significant Differences (p = 0.8606) | Significant Differences (p = 0.0134): Post-hoc test did not identify different stations |
| <i>Daily Water Temperature Ranges</i> | | |
| Mussel Exposure | Significant Differences (p = 0.0054): IT-19≠CP-01, CP-04, CP-07 | Significant Differences (p = 0.0001): PW-07≠CP-04 IT-19≠CP-01, CP-04, CP-07 |
| Pre-herring | No Significant Differences (p = 0.2491) | Significant Differences (p = 0.0001): PW-07≠CP-01, CP-04, CP-07 IT-19≠CP-01, CP-04, CP-07 |
| Herring | No Significant Differences (p = 0.9708) | No Significant Differences (p = 0.7996) |
| Post-herring | Significant Differences (p < 0.0001): CP-01≠IT-03 IT-19≠CP-01, CP-04, CP-07 | Significant Differences (p = 0.0001): IT-19≠CP-01, CP-04, CP-07 |

Herring Deployment Temperatures

Water temperatures measured during the herring deployment have been highlighted in Figures 19, 20, and 21, and in Tables 16 and 17. During the 5-day herring deployment, surface water temperatures ranged from about 8.8 to 10°C. Temperatures were fairly constant with isolated

excursions of 2°C or less. The average surface water temperature during the herring exposure was 9.2°C, as compared to the average of 11.8°C measured in 1998 (Figure 21).

Post-Mussel Deployment Temperatures

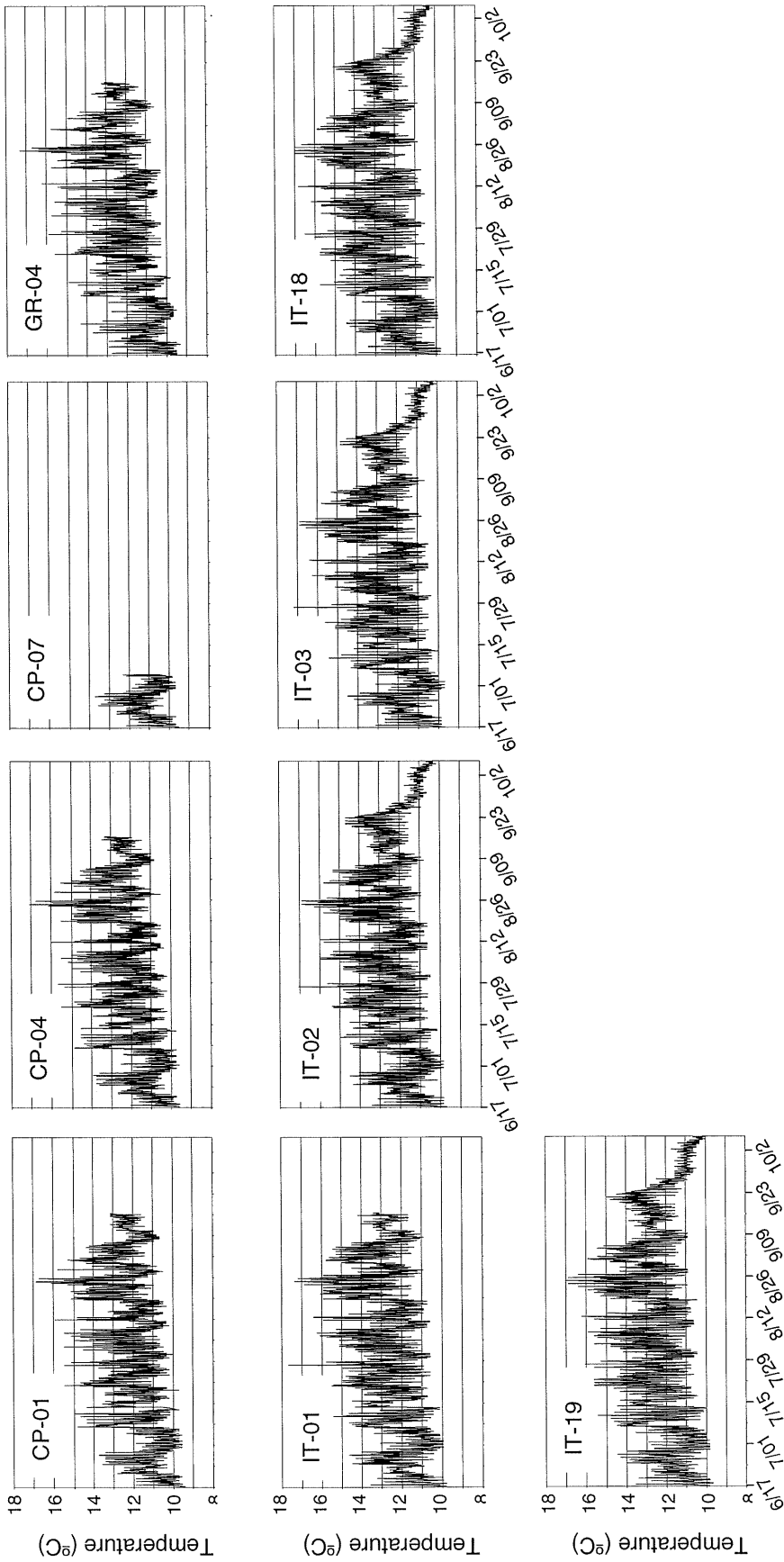
Temperature probes were retrieved from 9 surface and 11 bottom locations approximately three months after the mussels were retrieved. This temperature information was used to characterize temperature conditions along Cherry Point during the summer months. The temperature profiles for the surface locations are shown in Figure 24; the profiles for the bottom locations are shown in Figure 25. The temperature profiles at some stations do not span the entire deployment period because the probes at these stations were an older model which did not have as enough memory to collect data for an additional three months.

The temperature profiles (Figures 24 and 25) show that shortly after retrieval of the mussel cages, average water temperatures increased up until the beginning of September at which time they started to decrease. The fluctuations in temperature over the short time intervals most likely represents both tidal activity and input from upland sources.

4.6 Analysis by Region: Synthesis of Results

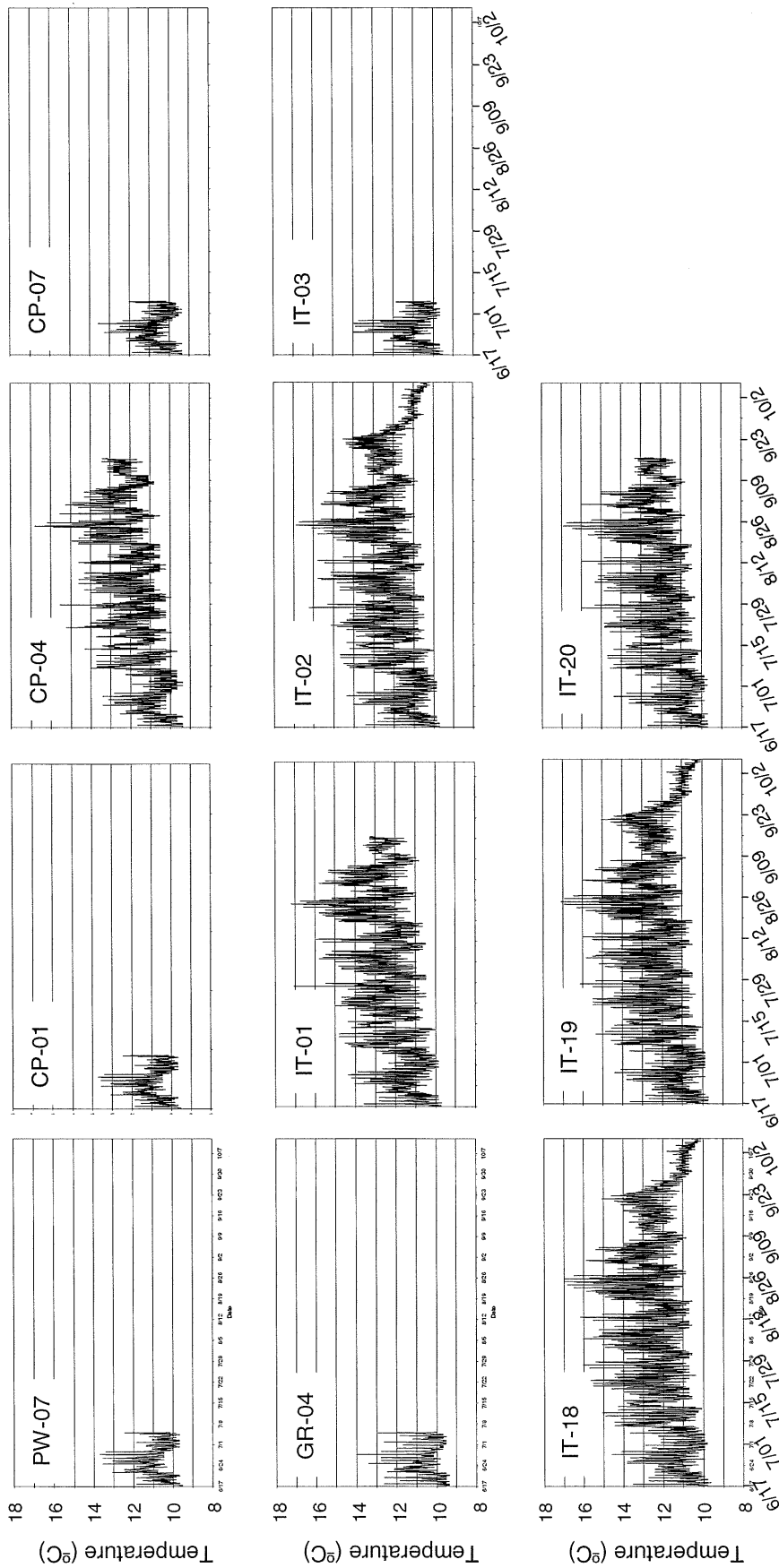
Since the comparison of somewhat arbitrarily chosen stations and sites may have been misleading, particularly at the Intalco-Tosco site due to the large number of stations, the Intalco-Tosco site was divided into regions of approximately 7 stations each. Dividing the Intalco-Tosco site into three regions (i.e., Intalco, stations closest to the Intalco Pier; Mid-Pier, stations mid-way between the two piers; and Tosco, stations closest to the Tosco Pier) provides a different perspective on the results. Only results for TPAH_{o-ND} are provided because using "0" for non-detects is the most realistic approach. The effects and temperature data will be assessed with respect to those TPAH results. Emphasis will also be placed on EOT tissue weights because it was the most discriminating growth metric.

Analyzing the data by region shows that TPAHs increase from Point Whitehorn to Gulf Road and then decrease from the Intalco Pier to the Tosco Pier (Figure 26A). Bottom water temperatures decrease from Point Whitehorn to Gulf Road, increase at the Intalco Pier and then stay about the same south to the Tosco Pier (Figure 26B). EOT tissue weights decrease from Point Whitehorn to the Intalco Pier and then increase from the Mid-Pier to the Tosco Pier (Figure 26C). Although there appears to be a relationship between these three measurement endpoints (TPAH, temperature, and EOT tissue weights) a multiple linear regression analysis did not detect a statistically significant relationship and the variables only explained about 30% of the variance in the data.



| Station | CP-01 | CP-04 | CP-07 | GR-04 | IT-01 | IT-02 | IT-03 | IT-18 | IT-19 |
|---------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Mean | 11.87 | 11.96 | 10.95 | 11.93 | 12.63 | 12.25 | 12.25 | 12.47 | 12.31 |
| Min | 9.42 | 9.59 | 9.53 | 9.34 | 9.82 | 9.70 | 8.32 | 9.79 | 9.68 |
| Max | 16.87 | 17.08 | 13.71 | 17.31 | 17.67 | 17.05 | 17.19 | 16.97 | 17.02 |

Figure 24. Surface water temperatures during 90-d, 147-d post-mussel exposure period.



| Station | PW-07 | CP-01 | CP-04 | CP-07 | GR-04 | IT-01 | IT-02 | IT-03 | IT-18 | IT-19 | IT-20 |
|---------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Mean | 10.76 | 10.76 | 11.65 | 10.51 | 10.41 | 12.23 | 11.98 | 10.79 | 12.01 | 11.94 | 11.77 |
| Min | 9.53 | 9.53 | 9.39 | 9.37 | 9.37 | 9.72 | 9.71 | 9.53 | 9.64 | 9.59 | 9.57 |
| Max | 13.71 | 13.71 | 16.80 | 13.56 | 14.02 | 17.21 | 16.87 | 14.02 | 16.98 | 17.09 | 16.90 |

Figure 25. Bottom water temperatures during 90-d, 147-d post-mussel exposure period.

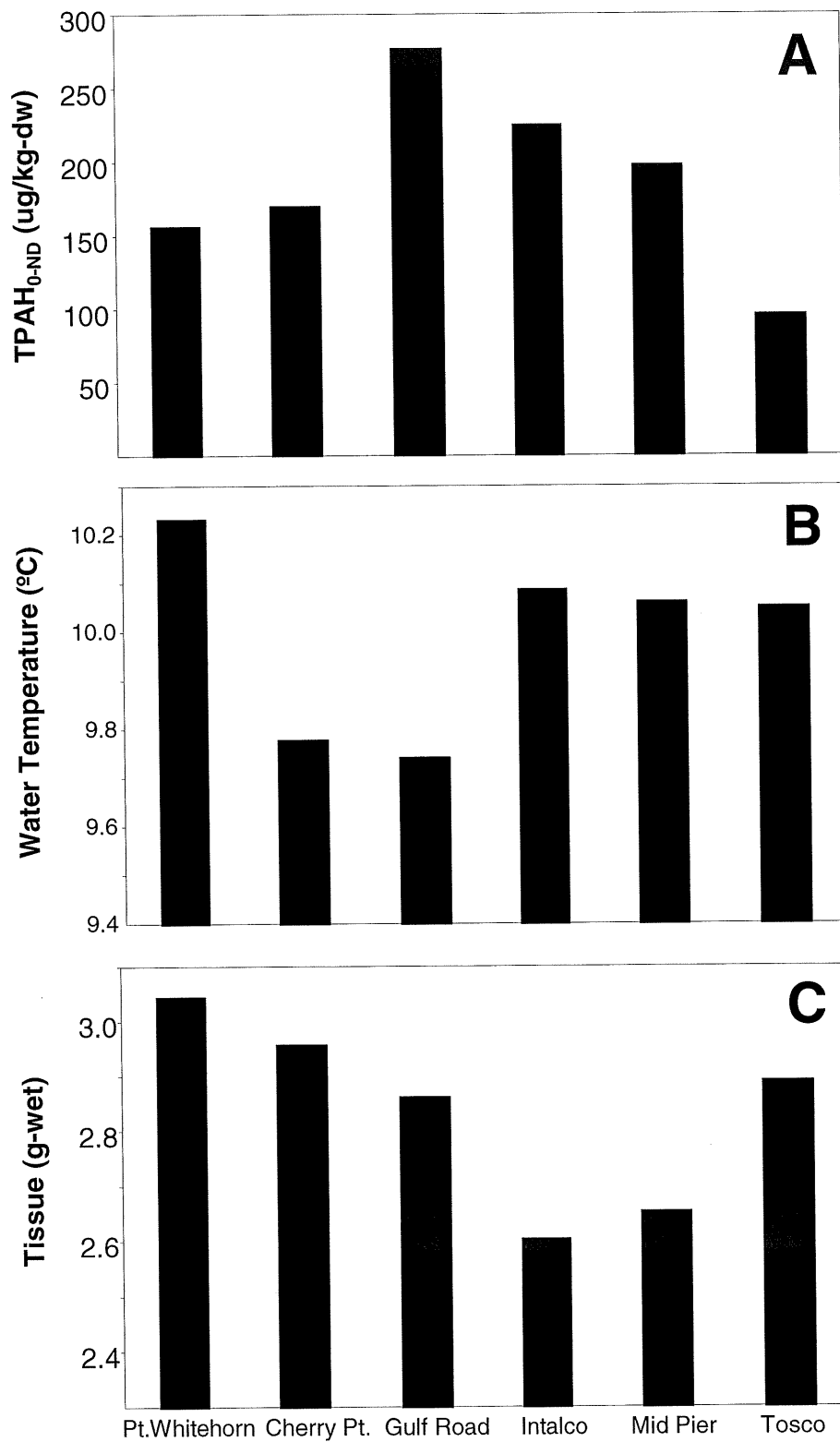


Figure 26. Analysis by Region. A = TPAH, B = Bottom water temperature, C = EOT tissue weight (g-wet)

Nevertheless, it appears there are some relationships between EOT tissue weight and TPAHs as shown in Figure 27. Including only PW, CP, MP, and Intalco in the regional analysis provides almost a straight line ($r^2=0.95$). The real outliers include Tosco and Gulf Road. If the Tosco stations where “zero” PAHs were detected (different from non-detects) are excluded from the analysis the regression between those five stations is also statistically significant ($r^2 = 0.77$). This leaves the only real outlier as Gulf Road. Gulf Road had the highest TPAHs and relatively high tissue weights. Clearly there are other factors affecting mussel growth rate in the Cherry Point reach. Nevertheless, the most important information gained from this analysis is that the gradients in PAH distribution are not as clear as originally expected. It appears that PAHs from the effluent diffusers may be transported by currents, eddy mixing, and longshore transport to provide a patchy distribution onshore and to the herring eggs. Based on both EOT tissue weight and TPAH, the Tosco Pier is more similar to the northern stations than the other “southern stations.” This is consistent with the relative volumes discharged between the ARCO, Intalco, and Tosco piers. There is a statistically significant relationship between the average of monthly flow rates for each outfall and the concentration of TPAHs measured in mussel tissues (Figure 28) although there are only three data points included ($r^2 = 0.99$).

The relationship between water temperature and EOT tissue weight is also not statistically significant ($r^2 = 0.007$). As shown in Figure 29, the stations tend to fall into groups. Gulf Road and Cherry Point are clearly the group with the lowest water temperatures and very similar EOT tissue weights. The Intalco Pier and Mid-Pier have intermediate temperatures but the lowest tissue weights. The next closest grouping with respect to EOT tissue weights and temperature are Point Whitehorn and Tosco. Point Whitehorn had the highest temperatures and the highest EOT tissue weights while Tosco had somewhat lower temperatures and lower EOT tissue weights. These three groupings are perhaps best visualized in the 3-dimensional surface plot shown in Figure 30.

A statistically significant difference was found when comparing the pooled data for the three stations with the highest TPAHs (Gulf Road, Intalco, and Mid-Pier) against the three stations with the lowest TPAHs (Point Whitehorn, Cherry Point, and Tosco) (Figure 31A). Similarly, a statistically significant difference was found when comparing the two stations with the lowest bottom temperatures (Cherry Point and Gulf Road) with the four stations with the highest bottom temperatures (Point Whitehorn, Intalco, Mid-Pier, and Tosco) (Figure 31B). Finally, a statistically significant difference was found when comparing the two stations with the lowest EOT tissue weights (Intalco and Mid-Pier) against the four stations with the highest EOT tissue weights (Point Whitehorn, Cherry Point, Gulf Road, and Tosco) (Figure 31C).

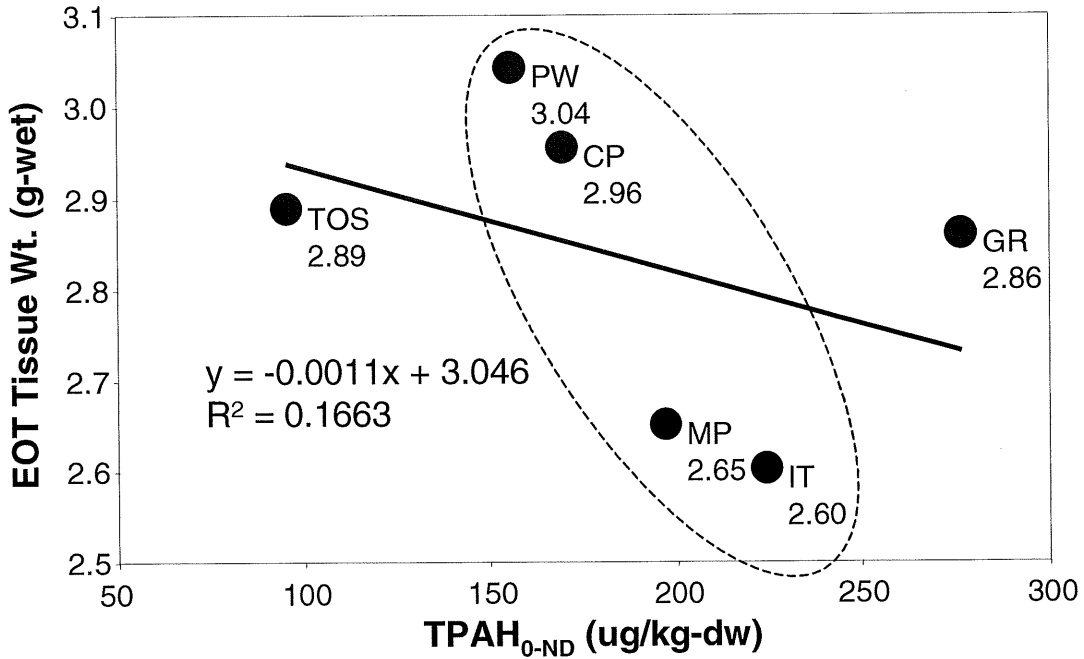


Figure 27. EOT TPAH vs EOT tissue weight for six different regions.

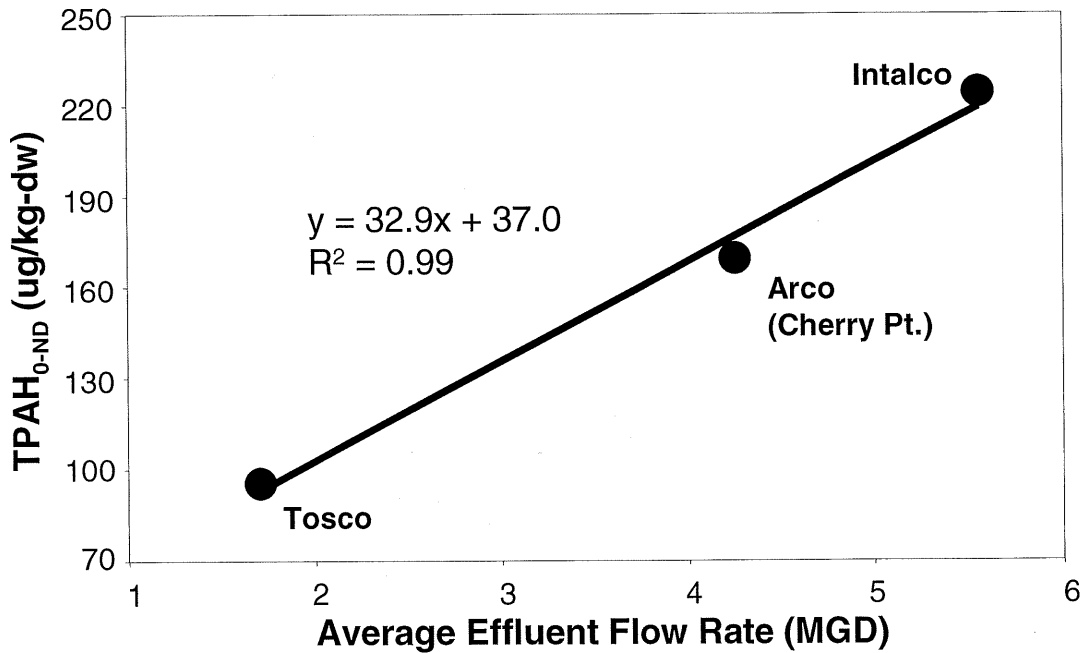


Figure 28. Average effluent flow rate (mgd) for Intalco, Arco, and Tosco versus TPAH_{0-ND} in mussel tissues.

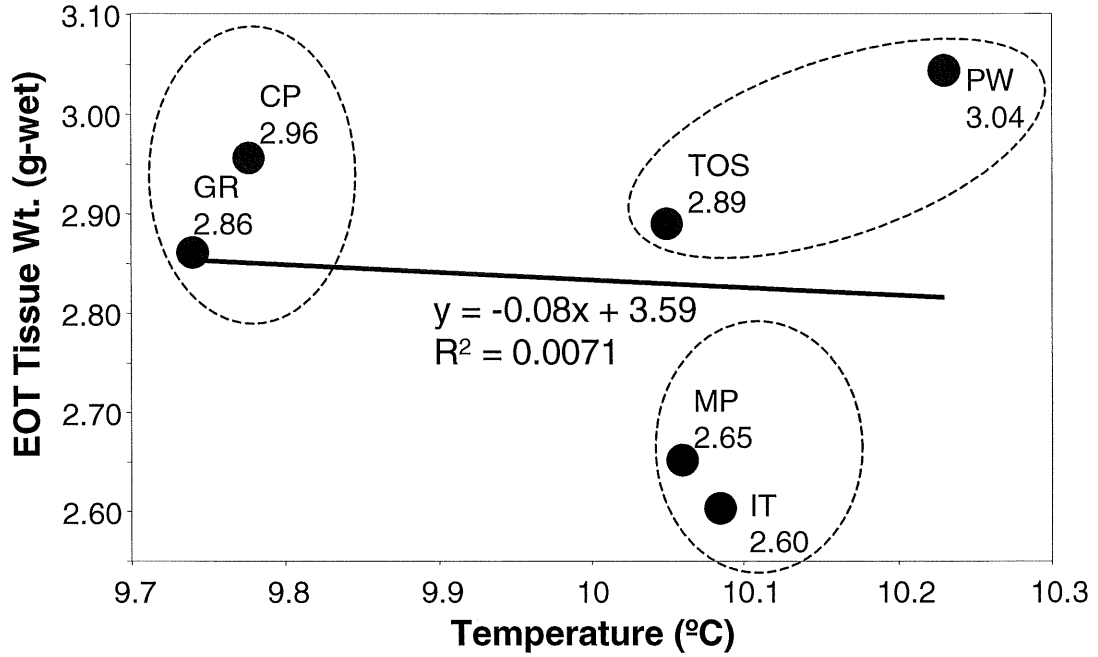


Figure 29. Temperature vs EOT tissue weight for six different regions.

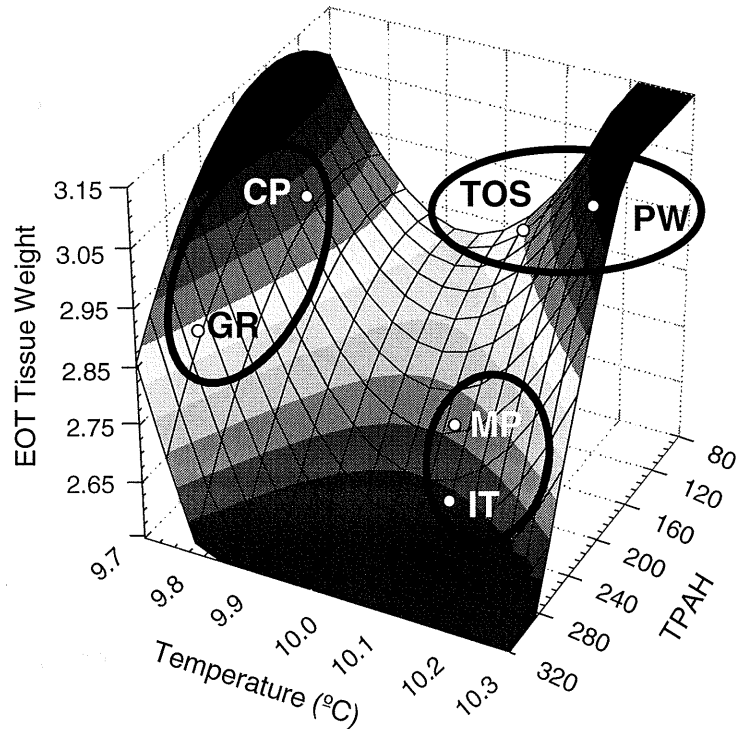


Figure 30. Three dimensional surface plot of $TPAH_{0-ND}$, bottom water temperature and EOT tissue weights. Regions are shown with white dots and groupings indicated by ellipse.

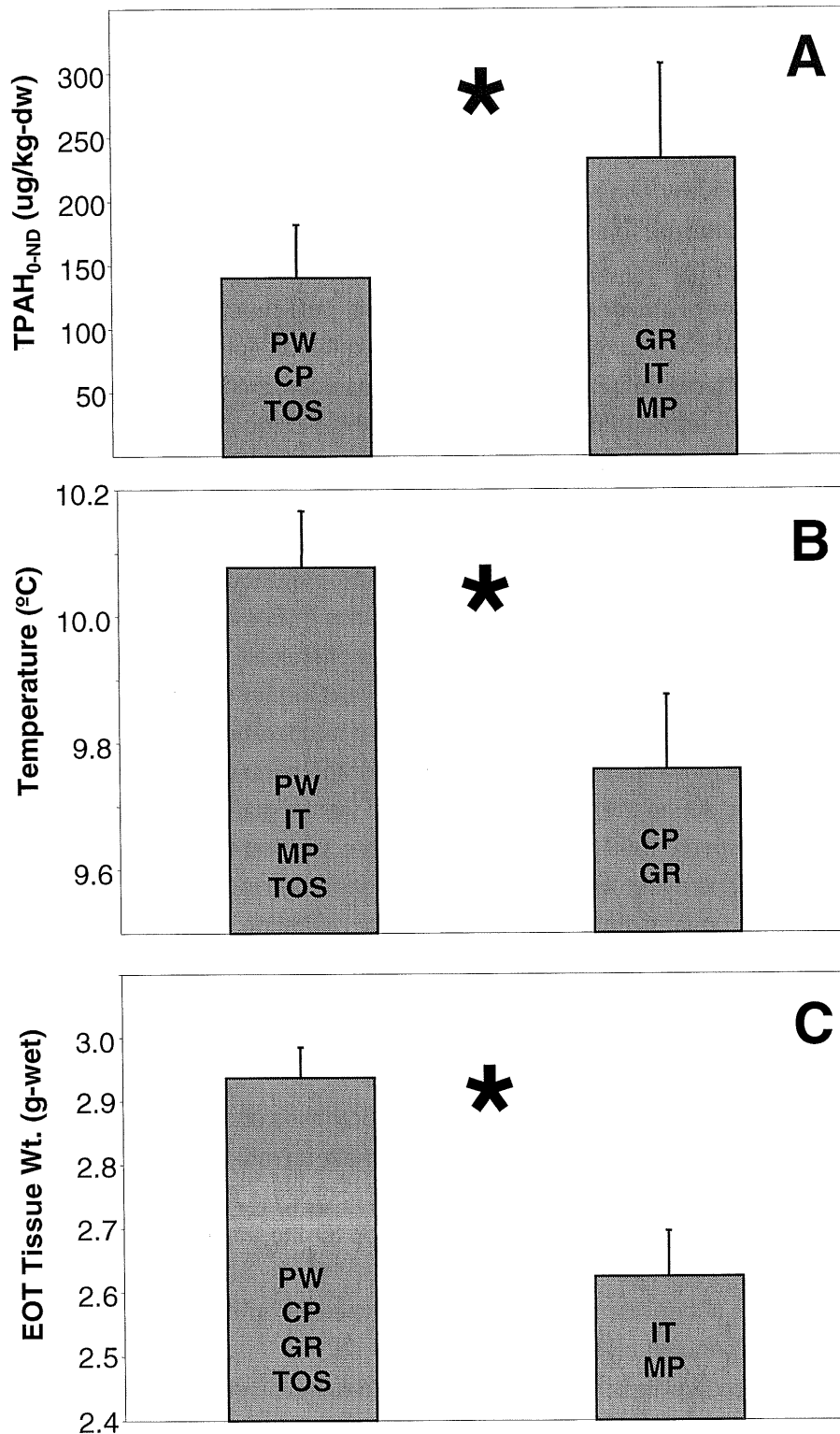


Figure 31. Analysis by Pooled Region. A = TPAH, B = Bottom water temperature, C = EOT tissue weight (g-wet). * = statistically significant difference.

5.0 DISCUSSION

An in-situ caged mussel study was conducted between April 14 and June 17, 1999 to assess the chemical bioavailability and associated effects from exposure to PAHs in the Cherry Point reach. This was a follow-up to a similar study conducted in 1998 that included field exposures of caged mussels and herring eggs. More emphasis was placed on bioaccumulation in 1999 by increasing the number of stations (cages) and reducing the number of mussels per cage. Results of the 1999 study showed that mussels accumulated concentrations of PAHs found to be associated with adverse effects on herring egg development in other studies (Brown et al. 1996), although these elevated concentrations of PAHs had no consistent effect on mussel survival or growth. There was no consistent correlation between PAHs accumulated by mussels and effects on herring egg development by sites or stations although the regional analysis showed correlations similar to those shown in the herring egg results in the last several years (Applied Biomonitoring 1999b; Kocan et al. 1998; Hershberger and Kocan 1999). Understanding both temporal and spatial characteristics of PAH bioaccumulation is crucial for determining potential environmental impacts of these chemicals on the Cherry Point herring population. A significant effort was also directed toward further evaluation of water temperature as a potential natural stressor. To characterize water temperature during the warmest summer months, temperature monitors were re-deployed after the mussels were retrieved in June, 1998. Temperatures during the 1999 herring spawn were significantly lower than the herring spawn in 1998. However, 1999 summer temperatures suggest that summer temperatures during the 1998 El Nino event were probably 2°C higher than in 1998. The 1999 water temperature profiles cover events such as pre-spawn migration, spawning and development, and post-hatch and larval development. As in 1998, all questions addressed by the 1999 study were not answered conclusively, but results provided important information to help WDNR in the decision making process regarding potential impacts on the Cherry Point herring stock.

The following answers were provided for the null hypotheses characterizing effects:

- There were no significant differences in whole-animal weight or shell length among stations (1 cage) or among sites (7 cages pooled for the Point Whitehorn, Cherry Point and Gulf Road sites; 23 cages pooled for the Intalco-Tosco site) at the beginning of the test.
- There was a significant change in mussel metrics among sites after the 61-d exposure period; i.e., the animals grew.
- There were significant differences in whole-animal wet weight, EOT tissue weight, and EOT shell weight among sites after the 61-d exposure period.

The following answers were provided for the null hypotheses characterizing exposure:

- There was significant accumulation of total PAH (TPAH) concentrations in mussel tissue after the 61-d exposure period.

- There were significant differences in TPAH concentration in mussel tissue among sites and regions.

The following answers were provided for the null hypotheses for comparing the potential effects of temperature among stations:

- There were no statistically significant difference in daily average temperature among sites.
- There were no statistically significant difference in daily temperature ranges among sites.

The stations between the Intalco and Tosco piers were initially considered as one site in the original experimental design. The purpose of this approach was to examine gradients of PAH exposure in the vicinity of the Intalco-Tosco piers. However, a detailed evaluation of the exposure and effects data demonstrated that this approach was misleading. The presence of microhabitats in the Intalco-Tosco reach required a shift in emphasis to evaluating TPAH_{0-ND}, temperature, and EOT mussel tissue weights in regions of approximately seven stations each. Actual PAH exposure in the Cherry Point reach is probably patchy and there is a mixed gradient in this area. PAH distribution may be driven primarily by ocean currents and longshore transport from the offshore effluent diffuser. Differences within the Intalco-Tosco stretch may also be associated the presence of physical structures (i.e., pier pilings) which result in microhabitats of very different composition. It was surprising to find the lowest PAH exposure at the Tosco Pier since the 1998 study suggested that the highest PAH exposures were at the southern stations. However, this finding suggested that exposure and the Intalco and Tosco Piers was very different and they should be evaluated as separate regions.

The most important findings of the 1999 study were the following: 1) PAHs and temperature were confirmed as potentially significant stressors for herring egg development; 2) mussels accumulated PAHs to concentrations shown to affect herring egg development in previous studies; 3) the Cherry Point reach should be evaluated in terms of regions rather than gradients, particularly between the Intalco and Tosco Piers; 4) PAH exposure was highest at Gulf Road and lowest at the Tosco Pier; 5) nearly all effects indicators for mussels (i.e., shell growth in length, whole-animal wet-weight growth, increases in tissue weight) were lowest at the Intalco Pier and suggested that mussels there were under more stress than at other sites; 6) significant differences in absolute water temperature and ranges in water temperature were found between 1998 (the El Nino year) and 1999 that could affect the Cherry Point and other herring stocks; 7) based on stressors and effects measurements (i.e., mussel growth) there are significant differences in the microhabitats in the vicinity of the piers at Intalco, Arco, and Tosco that are consistent with the relative volume of discharges at those piers; 8) in-situ field studies provided valuable information with respect to monitoring and assessments of stressors to herring in the Cherry Point reach that could not have been achieved with traditional methods;

and 9) although credible evidence has been provided by the in-situ herring egg deployments conducted by Kocan and Hershberger to suggest that stock effects are the major causes for the decline of herring stocks in Puget Sound, the data collected from the caged mussel study suggest that site effects may be equally or more important than stock effects.

Results from the 1999 caged mussel study confirmed that the Cherry Point Reach is a very complex area with many different regions. It is not possible to characterize the entire stretch as a unit; each of these regions must be evaluated separately and independently of the others to identify potential stressors and associated effects on the herring stock. One of the most controversial areas is that between the Intalco and Tosco piers. The results of this study demonstrate the presence of at least three microhabitats or regions in this 1-mile area. This confounded the interpretation of exposure and effects data from 1998 and 1999 with respect to site-specific stressors on herring. This study was successful in characterizing PAH exposure over a wide geographical area, which can be used in conjunction with existing data and those collected later to identify potential stressors to herring stocks and perhaps even rank their relative importance and discriminate between “site” and “stock” effects.

5.1 Mussel Bioaccumulation of PAHs

Based on increases compared to measurements at the beginning of the test, mussels accumulated PAHs during the 61-d exposure period at approximately 80% of the stations (79% based on concentration, 83% based on content). The highest concentrations were measured at Gulf Road and the lowest concentrations were measured at the Tosco Pier. These results were surprising because PAHs in mussel tissues were low at Gulf Road and high at the Tosco Pier in 1998. Stations in the vicinity of the Intalco Pier, and the region between the Intalco and Tosco Piers had concentrations similar to those measured at Gulf Road. The 1999 tissue chemistry results are more reliable than in 1998 because the mussels were in better condition, the exposure period was longer, and there was significantly more replication at each station to normalize the statistical influence of low and high PAH measurements. In the 1998 study, only one station was used to represent all areas north of the Intalco Pier, except the Arco Pier (2 stations), the Intalco Pier (2 stations), Mid-Pier area (1 station), and Tosco Pier (2 stations). The more intense distribution of stations over the region (i.e., 7 stations each for Point Whitehorn, Cherry Point and Gulf Road; 8 stations for the Intalco Pier; 6 stations for Mid-Pier, and 7 stations for the Tosco Pier) was a primary goal of the 1999 study, and results indicate that the approach was successful.

5.1.1 Patchy PAH Distribution

The expected gradient in TPAH exposure was not found in the 1999 study. Instead, the concentrations decreased with distance from Gulf Road, to the north and to the south. It was

surprising to see that PAH exposure at the Tosco Pier was the lowest and this exposure was more similar to PAH concentrations in mussel tissues from Point Whitehorn and Cherry Point than mussels from the Intalco Pier or the Mid-Pier area. The concentration of PAHs measured in mussel tissues is also consistent with the relative volume of effluent discharged from the Intalco, Arco, and Tosco diffusers. There is a statistically significant relationship between the average monthly flow rates from 1997 to 1999 for each outfall and the concentration of TPAHs measured in mussel tissues although there are only three data points included ($r^2 = 0.99$). The monthly average for the Arco outfall (Cherry Point) ranges from 2.6 to 5.9 MGD (mean = 4.25 MGD), Intalco ranges from 3.9 to 4.7 MGD for outfall 1 and 0.1 to 2.4 MGD for outfall 2 (combined mean = 5.6 MGD), and Tosco from 0.9 to 5.5 MGD (mean = 1.7) (Kim Wigfield, Washington State Department of Ecology, personal communication). Although the regression uses only three data points and the relative concentration in each effluent would have to be characterized to confirm any conclusions drawn from these data, the concentrations of TPAHs measured in mussel tissues reflected the relative volume of each discharge.

A comparison of the 1998 and 1999 TPAH exposures (Figure 32) shows that TPAH concentrations measured in mussel tissues from Point Whitehorn, Cherry Point, and Intalco are very similar. Interestingly, those measured at Intalco are virtually identical. TPAHs measured at Mid-Pier are reasonably close but those at Gulf Road and Tosco differ by almost a factor of three. While these differences could be indicative of temporal variability, the shorter exposure period in 1998, or the poor health and perhaps associated unreliability of the tissue chemistry measurements in 1998, the results suggest a patchy distribution of TPAHs in the Cherry Point reach. The intense spatial coverage of the 1999 study suggests that this patchy distribution of PAHs is real and perhaps not unexpected given the distance of the source of the PAHs in the effluent diffuser several kilometers offshore. It seems reasonable to assume that PAH distribution onshore is driven by dilution, currents, eddy diffusion, and longshore transport. The north-south gradient suggested from the 1998 study is not as straightforward as expected.

5.1.2 PAH Fingerprinting by Alkylated Homolog Analysis

The reason for Gulf Road having the highest TPAHs is unclear. It is possible that the PAHs originated upland and were discharged to the area through non-point sources. This hypothesis is supported by the alkylated homolog analysis which shows that the Gulf Road "fingerprint" is clearly more petrogenic than the fingerprints for Intalco, Mid-Pier, or Tosco (Boehm et al. 1998; Page et al. 1995; Short and Babcock 1996; Short and Harris 1996; Short and Heintz 1997;). Additional characterization of potential upland sources may need to be characterized. In addition to major inputs from runoff and atmospheric fallout from urban areas Barrick and Prah (1987) identified regional sources of combustion-derived PAHs in Puget Sound. Nevertheless herring egg development was more impaired there than at more northern sites (Kocan et al. 1998; Hershberger and Kocan 1999).

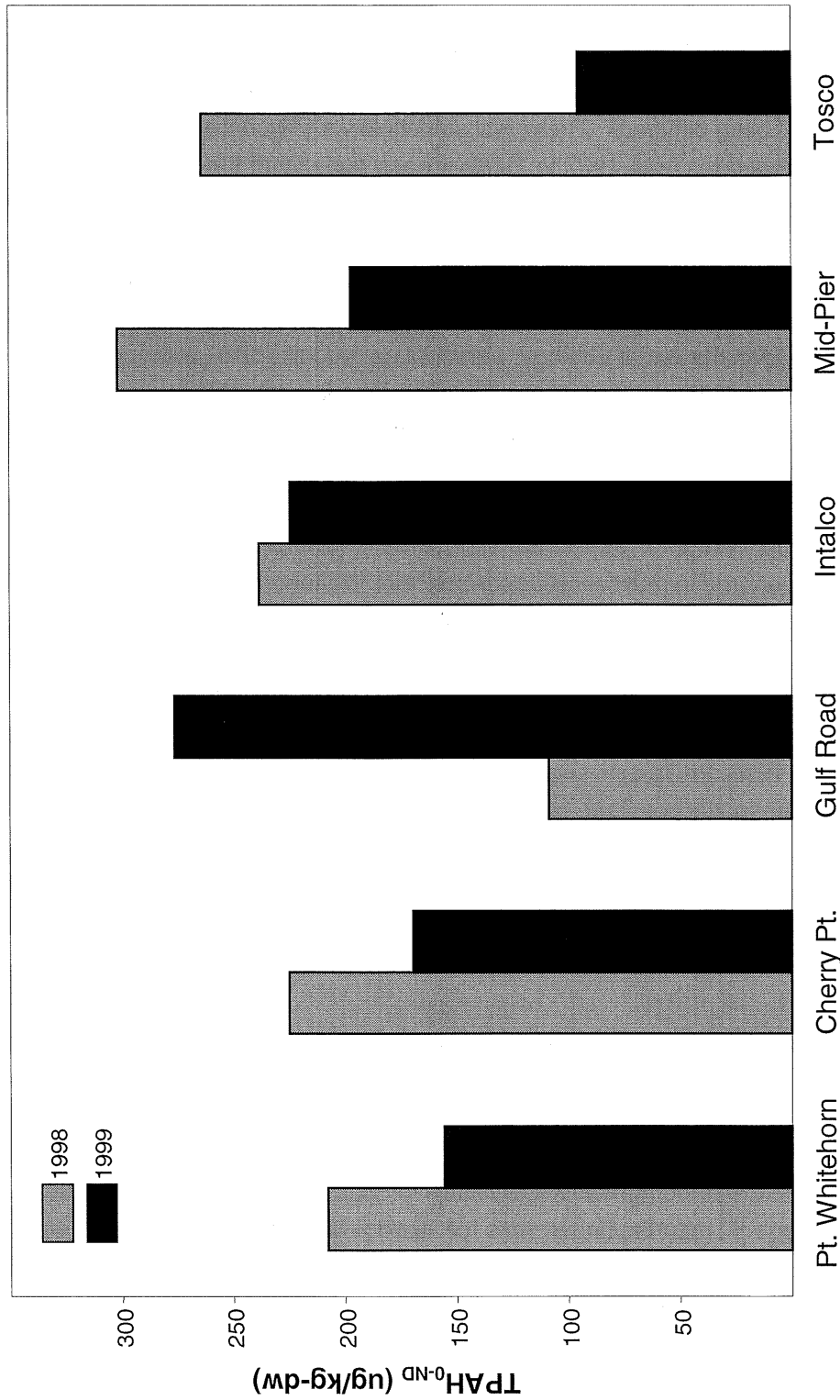


Figure 32. Comparison of TPAH_{0-ND} in 1998 and 1999 for six different regions.

A more thorough analysis of the alkylated homologs provides insight into the sources of the accumulated PAHs in this caged mussel study. A detailed homolog analysis was not conducted on the 1998 bioaccumulation data because of uncertainty associated with the data from tissue weights, loss of lipids, and pre-deployment stress. A homolog analysis of the 1999 data suggested that there were different sources of PAHs along the Cherry Point reach. The chemical analyses of the T₀ mussel tissues showed "0" alkylated homologs. The next lowest ratio of alkylated homologs to parent compounds, 1.90, was detected at Gulf Road. This ratio is more indicative of pyrogenic (combustion products) than petrogenic (petroleum products) sources of PAHs. Interestingly, the three highest ratios, 4.57, 3.65, 3.08, were found at Mid-Pier, Tosco, and Intalco, respectively, and are the most indicative of petrogenic sources of PAHs. The ratios for Point Whitehorn and Cherry Point were intermediate at 2.82 and 2.02, respectively, and suggest mixed pyrogenic or petrogenic sources of PAHs. The highest ratio, indicative of the highest petroleum signal, is at Mid-Pier. This is consistent with the hypothesis that the material may come from offshore diffuser and concentrate between the two physical pier structures.

5.1.3 Sampling and Analysis Protocols affect Comparing Results from Other Studies

One of the most important lessons learned from the 1999 data analysis is that TPAH_{0-ND} is probably the most reliable means to present exposure concentration. This was concluded after analyzing the data four different ways. It is concluded that any analysis using ½ the detection limit for non-detects can be misleading. It may be appropriate to use TPAH_{½DL} data for regulatory purposes when a conservative estimate of effects is required. However, this approach makes it much more difficult to make comparisons among sites because, depending on the frequency of non-detects, the values may be quite similar and biased toward the "high" side due to inclusion of a large number of non-detects. Furthermore, although lipid normalization has its advantages, these results can also be misleading because there has to be a direct relationship between the lipids and the concentration (Hebert and Keenleyside 1995; Meador et al. 1995). The most straightforward and unambiguous analysis uses non-lipid-normalized data with "0" for all non-detects (Boehm et al. 1998; Page et al. 1995; Short and Babcock 1996; Short and Harris 1996).

Comparison of the 1998 and 1999 data also provided insight on proper handling techniques for mussel tissues prior to chemical analysis. The reason for the apparent increase in PAH concentration during the 11 days of exposure in the Marrowstone Laboratory tanks in 1998 can now be explained as an artifact of sampling mussel tissues. In 1998 and 1999, mussels were frozen whole without extracting tissues after the 11-d laboratory holding period and after overnight holding at Samish Bay in 1999. The procedures used in those two events gave an artificially high percent water (less solids) which influenced the dry weight conversions and resulted in artificially high TPAH concentrations. The percent lipids and solids data for both the

Samish Bay mussels from the 1999 study and the Marrowstone Tanks mussels from the 1998 study were curiously low when compared to all other data. Freezing mussels with tissue inside prior to shucking tends to disrupt the cells and associated lipids resulting in an artificially low percent solids and percent lipids. As the mussel tissues freeze inside the shells, the water that is trapped between the shells also freezes and can become incorporated into the disrupted tissues. This trapped water is usually drained off during the shucking process. The percent water and percent lipid values reported for "previously frozen" tissues affects converting the data to a dry-weight basis as well as lipid-normalization. For both the Samish and Marrowstone Tank tissue samples, the TPAH concentrations recalculated based on the expected percent solids were much closer to the expected values. The procedures used during tissue preparation have important ramifications for the apparent elevated PAH concentrations in mussel tissues after an 11-day holding in the Marrowstone Tanks during the 1998 study as well as comparing data sets from different studies. This also has implications for comparisons with traditional Mussel Watch monitoring programs such as the NOAA Status and Trends Program (NOAA 1989) and the California Mussel Watch (State of California 1988).

The concentrations of TPAHs measured in mussel tissues after the 61-d exposure period in 1999 and the 28-d exposure period in 1998 have been associated with adverse effects in herring in other studies. Brown et al. (1996) demonstrated that adverse effects on herring egg development begin to occur at PAH concentrations above 300 ng/g-dw. They measured tissue PAH concentrations in intertidal mussels to estimate exposure to caged herring eggs deployed at some distance away. They also used "0" for non-detects as do other investigators involved in the Exxon Valdez monitoring and assessment program (Boehm et al. 1998; Page et al. 1995; Short and Babcock 1996; Short and Harris 1996). Theoretically, caged mussels co-located with caged herring eggs as in the 1998 and 1999 Cherry Point studies should provide better correlations between PAHs in mussel tissues and associated effects in herring eggs than extrapolating from the tissue concentrations measured in the resident intertidal populations for the herring eggs as in the Brown et al. (1996) study. Kocan et al. (1998) and Hershberger and Kocan (1999) suggest that herring egg development in 1998 and 1999 was depressed along the entire Cherry Point reach and this is consistent with earlier results. The regional analysis presented here however, suggests that a generalized north-south gradient in PAH exposure and effects is more complicated. It is not clear why there was not a better correlation between measured effects in herring egg development and elevated tissue PAH concentrations measured in mussels from the 1998 and 1999 studies unless the stressed mussels did not accurately mimic actual exposure to herring eggs, the stations were too dispersed to establish good correlations, or other stressors such as temperature were involved. Nevertheless, the 1998 and 1999 in-situ studies has provided WDFW/WDNR a baseline set of tissue chemistry data to make better predictions regarding site effects on herring.

5.1.3.1 *Theoretical and Empirical Predictions of Effects*

Methods for predicting adverse effects from tissue chemistry (dose-response) fall into two general categories; theoretical and empirical. Theoretical approaches include those utilizing quantitative structure-activity relationships (QSARs) and equilibrium partitioning theory (EqP). For example, the QSAR approach predicts that acute toxicity will occur at 2-4 $\mu\text{mol/g}$ of nonionic organic chemicals such as PAHs. Chronic toxicity is predicted at 0.2-0.4 $\mu\text{mol/g}$ (McCarty 1991; McCarty and Mackay 1993). Di Toro et al. (in review) utilize the QSAR concept, but add from EqP theory the element of nonionic organic chemicals being primarily associated with lipids in an attempt to refine the predictions. Whereas McCarty and Mackay (1993) advocate the use of whole-body tissue residues, Di Toro et al. (in review) advocate the use of lipid-associated chemicals to make those predictions. This is consistent with using lipid-normalization to reduce variability in the data such as those from the caged mussel study. In either case, using tissue concentrations to predict effects is less ambiguous than concentrations of water or sediment because factors affecting bioavailability are eliminated.

Although these theoretical approaches could be used to predict the environmental significance of the Cherry Point mussel tissue chemistry, each has its own set of drawbacks and limitations, and neither can be as precise as direct, site-specific measurements. One approach that combines theoretical and empirical data was employed by Neff and Burns (1996) to evaluate data from the Exxon Valdez oil spill. They used the tissue residue data from mussels to predict water concentrations and then compared those estimates with ambient water quality criteria from the State of Alaska. The main problem with this approach is that ambient water quality criteria for many chemicals, including PAHs have been questioned recently. Heintz et al. (1999) have suggested that the ambient water quality criteria for PAHs could be as much as an order of magnitude overestimated because they are based primarily on results from acute toxicity tests on insensitive species under laboratory conditions. The same problem exists for tissue residue databases developed by EPA and the Corps of Engineers which are developed for use on the Internet. Most of the results are also based on short-term acute toxicity tests conducted under laboratory conditions. Widdows and Donkin (1992) have used laboratory, field, and mesocosm conditions to measured effects due to PAH exposure directly in mussels. However, mussels are not the real issue at Cherry Point.

5.1.3.2 *Conservative Results*

Conservative estimates of PAH bioavailability and associated effects on herring egg development were produced from the 1998 and 1999 study results. The estimate of PAH bioavailability is considered conservative because of the pre-deployment stress experienced by the mussels in 1998, the short (i.e., 27-d) exposure period, and unexpected increases in PAHs concentrations in mussel tissues while being held in the Marrowstone Laboratory tanks. In

1999 stress was caused by an unusually heavy barnacle set that probably reduced mussel survival, growth, and bioaccumulation of PAHs. As described below, each of the individual stresses in themselves would probably may not cause the mussels to experience stress. But when combined, they likely affected growth and bioaccumulation potential. Most laboratory and field studies, including studies with caged mussels, suggest that chemical equilibrium with PAHs can be reached in 1 to 30 days (Clark and Findley 1975; Pittinger et al. 1985; Meador et al. 1995; Salazar and Salazar 1997). However, other studies suggest that chemical equilibrium is seldom achieved in the field because of ephemeral water column conditions. One recent study has shown that up to 90 days are required to for PAHs to reach equilibrium in mussel tissues, particularly with sediment-sorbed PAHs (Naes et al. 1995a,b).

The relatively short exposure time of the herring eggs in the Cherry Point reach is another reason why the results are considered conservative. While it has been suggested that the first four days of herring egg development are the most crucial for successful development (Kocan et al. 1998) and therefore the most sensitive time for testing, the 4-day exposure used in the 1998 herring egg developmental study and the 7-day exposure period used in 1999 represents an extremely conservative test. Herring development continues after the first four days while the larvae are still sensitive to physical and chemical stressors. In the 1998 and 1999 studies, the herring eggs were removed from these site-specific stressors in the Cherry Point reach and allowed to grow for seven days under less stressful conditions in the laboratory. Therefore, the cumulative effect of continued exposure to site-specific stressors are unknown. Larval herring metamorphose two to three months after hatching (EVS Environment 1999) and may be vulnerable during that entire time period. The effects on herring egg development may be more severe if herring were exposed to the same stressors (i.e., PAHs and temperature) through all stages of their development. If we assume that PAH and temperature stressors are present in the Cherry Point reach, we should also assume that they would have a greater total combined effect on later development stages even though the early developmental stages may be the most sensitive. Furthermore, during the summer the potential stress of elevated temperatures and rapid changes in temperature could be a potential stressor at Cherry Point and other stocks in Puget Sound, depending how late in the season they spawn and associated temperatures.

5.2 Mussel Bioaccumulation of Metals

Mussel bioaccumulation of metals was not evaluated in 1998 because it was felt that PAHs were the most potentially significant chemical stressor. Although there were no comparisons possible between 1998 and 1999, comparisons among sites in 1999 showed statistically significant differences among sites and concentrations that were all lower than at the beginning of the test. However, the magnitude of actual concentrations was relatively low and the environmental significance is unclear. While it is possible that the metals could act in a synergistic way to stress mussels and or herring the measured concentrations appear low enough that they would

not exert any direct effects by themselves (Salazar 1997, Jarvinen and Ankley 1999, US ACOE 1999). It should be stressed however that most tissue residue effects databases are based primarily on acute effects by measuring mortality endpoints that could underestimate potential chronic effects from long-term exposures to low metal concentrations in the field.

5.3 Temperature as a Stressor

Temperature was identified as a potentially significant stressor in both 1998 and 1999 that could have accounted for some of the adverse effects measured in the herring egg experiments or perhaps acted synergistically with existing PAH stressors. As with PAHs, analyzing the water temperature data by region instead of by site or by station gave a different perspective on the relationship between temperature and north-to-south position. Temperatures measured at Cherry Point and Gulf Road in 1999 were significantly lower than at all other regions, although there was a slight trend toward decreasing temperature from north to south. It is possible that large masses of warmer water (i.e., Birch Bay and Lummi Bay) influence both Point Whitehorn and the Intalco-Tosco reach. This is also evidenced by the effects of tidal cycle on daily temperature ranges

A cursory evaluation of the temperature data for 1998 and 1999 indicated conditions in the Cherry Point reach were different than expected, particularly with respect to herring temperature tolerance. Both absolute temperature and daily temperature ranges were near those associated with adverse effects found in previous studies. Although there was no clear relationship between the temperature results and the results of the herring egg study across stations, the most logical explanation for impaired development and survival at Gulf Road in 1998 was elevated temperatures. However, since the 1999 PAH data suggest that Gulf Road could have herring eggs could have been exposed to much higher concentrations than measured in 1998, PAH effects provide another possible explanation. Daily average temperatures in 1999 demonstrate that temperatures were potentially more stressful in regions closest to Birch Bay and Lummi Bay. The most dramatic shift in temperature occurred at IT-19 (Tosco Region) in August with a change of 4°C in 45 minutes. Similar changes were found at most other stations but usually over a period of hours. This was similar to changes in June of 1998 with changes of 4.5°C in about 4 hours at Gulf Road. In 1998 Gulf Road also the lowest in mean percent total hatch, mean percent live hatch, and percent reproductive success (Kocan et al. 1998). Collectively the temperature and PAH data from 1998 and 1999 suggest that temperature alone could have accounted for some of the effects on herring egg development in 1998 but that PAH exposures in 1998 probably underestimated potential exposure and effects for herring eggs. Herring generally have a high tolerance range for salinity, but this tolerance diminishes at higher temperatures (EVS Environment 1999). Short- and long-term temperature changes, such as El Nino events and the Pacific Interdecadal Oscillation, have been associated with declines of certain herring stocks and other fish species. Temperature extremes have also been associated

with larval mortality and abnormal development in laboratory studies and in the field. Egg and larval stages are the most sensitive to changes in temperature, and they inhabit nearshore environments where natural temperature extremes frequently occur as shown in the temperatures measured during the caged mussel study.

The screening level risk assessment (EVS Environment 1999) outlines a general trend of increasing temperature in the Cherry Point vicinity over the past 20 years as well as short-term increases in El Niño years. Data are presented from Active Pass on Vancouver Island, BC, that show a mean sea surface temperature of 18.3°C during August, 1998 (IOS 1999). These mean temperatures do not address the possible adverse effects of extremes at either Active Pass or Cherry Point. Seven sea surface temperatures above 20°C were measured at Active Pass during the month of August. These alone could have an adverse effect on herring. Considering that herring egg development takes two to three months and even higher temperatures would be expected at the Cherry Point reach than at Active Pass, the herring eggs that remained at Cherry Point for complete development (as opposed to those removed for testing) would continue to be exposed to high summer temperatures and PAHs. More work needs to be done to identify the location of developing herring eggs and juveniles relative to these PAH and temperature stressors. For example, it has been established that organic chemicals such as PAHs concentrate in the surface microlayer and that this is also where the highest temperatures occur. Potential exposure to both PAH and temperature stressors could also be important as herring enter the area to spawn and when the young begin to leave. Clarifying these issues could help distinguish between site and stock effects on herring population declines in Puget Sound.

The 1998 caged mussel study demonstrates the need and utility of in-situ temperature monitoring. Without deploying such devices, it would have been impossible to characterize the high temperatures occurring along the Cherry Point reach during the El Niño year or the dramatic extremes in temperature on a short-term, daily basis. These data are critical to understanding the temperature impacts on the herring population. Similarly, without deploying the temperature monitors in the Marrowstone Laboratory tanks, the temperature extremes experienced by the mussels would have gone undetected.

5.4 Mussel Effects Measurements

The effects portion of this study was a secondary objective, yet the effects results provided valuable insight into the bioaccumulation results. One of the primary uses of survival and growth data is to provide a means to calibrate the tissue chemistry data. Without these effects data, the ability to interpret the PAH data would be even more limited. Growth represents an integration of all internal biological processes and is one of the most important factors affecting bioaccumulation of PAHs and other chemicals. The most discriminating endpoints were EOT

tissue weight and EOT shell weight (Bayne et al. 1985; Salazar and Salazar 1998; Widdows and Donkin 1992). However, since mussel soft tissue is the primary physiological indicator of health due to energy storage and flux, the discussion will emphasize EOT tissue weights. EOT tissue weight showed that tissue weights were significantly higher at the two northernmost stations, Point Whitehorn and Cherry Point, than at the southernmost station, Intalco-Tosco. It is difficult to put Gulf Road into a “northern” or “southern” category because the EOT tissue weights for Gulf Road were statistically similar to both Intalco-Tosco and Cherry Point.

Analyzing these data by region instead of by site or by station also provided a different perspective on the relationships with geographic position, PAH exposure, and water temperature. EOT tissue weights were significantly lower at the Intalco and Mid-Pier regions compared to all others. These results are almost a mirror image of the TPAH concentrations in that there was a decreasing gradient of tissue weights from Point Whitehorn to Gulf Road and an increase in tissue weights from Intalco to Tosco. This suggests a relationship between tissue weights and TPAH but statistical analyses could not confirm a significant relationship. Other studies have shown that the highest concentrations of TPAHs measured in mussel tissues could affect herring eggs and perhaps even mussels. There was no apparent relationship between EOT tissue weight and temperature, and the range in temperatures measured in the course of the study should not of had an effect on mussel growth.

Although there were significant increases in total soft tissue weight after the 61-d exposure, these apparent increases in tissue weight were not high enough cause growth dilution, i.e., the dilution of chemical concentrations in tissues by the addition of tissue mass during the exposure period. Biological processes such as growth rate can influence PAH accumulation significantly (Meador et al. 1995; Applied Biomonitoring 1999a,b). Therefore, environmental factors such as temperature, oxygen content, pH, and salinity which affect growth rate can also affect bioaccumulation (Meador et al. 1995; Applied Biomonitoring 1999a,b). Some of these factors may also cause additional stress on mussels.

5.4.1 Survival

The caged mussel study was terminated after 61 days because of concerns regarding a sufficient number of surviving individuals to provide enough tissue for chemical analysis and the effects of barnacle settlement on bioaccumulation and growth. High mortality would preclude obtaining necessary growth and bioaccumulation information. In this study, survival served as an effects endpoint and as a criterion for a successful test. On average, mussel survival was lower than in most previous in-situ field bioassays, where survival normally exceeded 80% (Salazar and Salazar 1999). The relatively low survival in the 1998 and 1999 studies suggests that the mussels were affected by factors other than chemical stress, although chemical stress could have been a contributing factor. It seems most likely here that mussel survival was most

directly affected by pre-deployment stress and predators. The conservative survival criterion of 50% (Applied Biomonitoring 1999a) was not met at four stations and is the first indication that many factors may have affected mussel survival. There was no correlation between mussel survival and development of herring eggs as measured in the concurrent study. If anything, survival appeared to be inversely related to percent abnormal larvae, which seemed to show an increasing trend from north to south (Kocan et al. 1998).

Survival has never been a sensitive indicator of effects and it should mainly be used as an indicator of test acceptability as in laboratory toxicity tests. Survival in bivalves, or any other species, is generally not considered a very sensitive endpoint for evaluating effects because of the bivalve's ability to close and avoid exposure to adverse conditions. However, in a preponderance of evidence approach, survival sometimes provides useful corroborative information. The primary issue associated with the survival measurements for this study was the high degree of predation. It is unclear whether the measured survival is primarily a result of pre-deployment stress, predation, or exposure to site-specific conditions. In 1998, pre-deployment stress included high air temperatures during the pre-sort at Taylor United Mussel Farm, lack of food and extreme temperature shifts in the Marrowstone Laboratory tanks, and loss of tissue mass and lipids as indicated by the tissue chemistry results. In 1999 the primary natural stressor appeared to be the barnacle set.

5.4.2 Growth

The growth metrics were somewhat more sensitive indicators of effects than the survival endpoint or the corroborative endpoints of percent lipids and percent water. However, the most important use of all effects data may be to indicate general trends across stations and station groupings. The 61-d growth rates were small, but tissue weights showed that most energy was directed toward increases in tissue mass. The growth results are not fully explainable with the available physical/chemical data, nor exactly as expected. For example, it was not clear whether growth would be enhanced near the diffusers 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 in the Cherry Point reach, 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 PAHs. Some of our 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 1997, EVS 1996). In this study, both tissue weight and shell weight were the most discriminating growth metrics. However, there was no direct evidence that tissue concentrations of PAHs directly affected any of the mussel growth metrics. It should be re-emphasized however that assessment of effects was not the primary purpose of the caged

mussel study. The primary purpose of the caged mussel study was to quantify exposure to herring eggs and the primary purpose of the herring egg study was to evaluate potential site-specific effects.

There are three primary reasons for measuring growth: as an effects endpoint, to calibrate bioaccumulation, and as a criterion for successful test. In this in-situ field 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. However, the growth data have limited application because: 1) the low survival reduced the ability to detect statistically significant differences among stations; 2) there was insufficient replication to make definitive statements about differences among stations; and 3) the exposure period was too short for differences in growth to manifest themselves even if they had been there. In light of these limitations, differences were documented between BOT and EOT and among stations in growth metrics. In 1998, low survival and pre-deployment stress probably affected the relative sensitivity of different growth metrics and the ability to detect more differences among stations. In 1999 the primary stressor interfering with mussel growth interpretation was barnacle fouling. Previous studies have shown that other metrics, such as tissue weights, are more sensitive indicators of effects when growth rates are low. For example, previous work in San Diego Bay indicated that changes in tissue weights were more discriminating when PAHs were the primary contaminant (Salazar and Salazar 1998). In this study, tissue weights and shell weights had greater discriminating capacity than expected.

5.5 Differences Between 1998 and 1999

The 1999 study was considered successful because mussels survived the exposure period, grew, provided sufficient tissue necessary to allow chemical analysis of soft tissues, and accumulated PAHs. Changes in the experimental design and methods between 1998 and 1999 provided more useful results. Similarities and differences between the 1998 and 1999 caged mussel studies are shown in Table 19. Average mussel survival across stations in 1999 was 57%—slightly higher than in 1998 (47%) even though the exposure period was more than double (61 versus 28 days). The primary factor affecting mussel survival in 1998 was stress induced by holding them in the laboratory tanks for 11 days. Fouling by barnacles may have reduced survival and growth in 1999. Much higher survival was expected in 1999 based on previous caged mussel studies (Salazar and Salazar 1999), but the magnitude of potential effects associated with heavy barnacle fouling was not anticipated. Although the mussels were exposed for a 61-d period, not all of the growth metrics were useful in detecting differences among sites. For shell length, shell weight, and whole-animal wet weight, the percent change was surprisingly similar between 1998 and 1999. Each of these measurements has a “shell” component. Percent change in tissue weight in 1999 was double that of 1998, 78% versus 33%, respectively. One reason why a greater percent change was not observed in the 1999 whole-

animal wet-weights may be because the increased soft tissues are up to 85% water, and the slight increase in tissue material can not be discerned in a whole-body weight measurement.

**Table 19. Comparison of Conditions and Results — 1998 and 1999
Cherry Point Mussel Studies**

| <i>Approach</i> | <u>1998</u> | <u>1999</u> |
|--|--------------------|--------------------|
| Pre-deployment holding strategy | 10-d lab | 1-d field |
| Mussels deployed before herring | no | yes |
| Mussels retrieved with herring | yes | no |
| Cage positioned near bottom | no | yes |
| Predator mesh opening size | 1 inch | 0.5 inch |
| Presorting conditions | sun | shade |
| Mussels ripe and ready to spawn | yes | no |
| Deployment sample grid | diffuse | concentrated |
| Number of stations | 12 | 44 |
| Number of mussels/cage | 90 | 51 |
| Number T ₀ surrogates | 270 | 153 |
| Exposure period | 7- and 28-d | 60-d |
| Extraneous factors affecting survival | predation | fouling |
| Temperature monitor position on deployment array | surface | surface & bottom |
| <i>Results</i> | | |
| Percent Survival | 47 | 59 |
| Maximum EOT TPAH tissue (ug/kg-dw) | 313 | 526 |
| Maximum water temperature (°C) | 16.8 (Jun) | 17.7 (Aug) |
| Maximum Δ temperature = 4.5°C | 4 hours | 45 minutes |
| Percent Δ weight (g) | 25 | 27 |
| Percent Δ length (mm) | 6 | 7 |
| Percent Δ tissue weight (g) | 33 | 78 |
| Percent Δ shell weight (g) | 45 | 59 |

5.6 Problems

The two major problems encountered during the 1999 in-situ mussel study were heavy fouling by barnacles and movement of the deployment arrays. It is likely that survival rates would have been markedly higher had we known about the potential for significant fouling. It would have been possible, although time consuming, to visit each station and brush off any accumulated growth. The fouling by barnacles was so great in some cases that the mussels were likely suffocated. The high density of barnacles around the caged mussels may have been competition for planktonic food causing the mussels to starve. Both the potential for predation, as experienced in the 1998 study, and the potential for fouling demonstrate the need for periodic checks on the caged mussels.

The second major problem encountered in the 1999 study was movement of the deployment arrays. Although extreme care was taken to map the deployment stations using a global positioning system, it is likely that the deployment arrays were moved during periods of strong tidal activity and/or waves, or moved by curious boaters. In areas where in-situ monitoring will occur over a period of two or more years, it is recommended that permanent anchors be positioned at each station. These anchors can be rebar, or other strong material, bent in a "U" shape and buried into the sediment such that the top of the "U" protrudes from the sediment. The deployment array can be attached to this anchoring system by divers. The deployment array can also be tethered to the shore for easy identification and retrieval. An alternative approach is to use a pinger or other locating device. This may be the preferred approach in areas of less ocean energy or areas that are to be monitored infrequently.

One of the major problems encountered in the 1998 study, attempting to coordinate the timing of the in-situ mussel study with the collection and deployment of herring eggs, was eliminated in the 1999 study by having the mussels already deployed when it was time for the herring eggs to be deployed in the field. By using this approach, the health of the mussels was not compromised prior to deployment, and a sufficient window was available for deployment of the herring eggs.

The mussels in the 1999 study did not experience the pre-deployment stress as those in the 1998 study. Extreme care was taken in 1999 to eliminate potential stress associated with exposure to elevated air temperatures during the pre-sort by providing constant shade and keeping the mussels in ocean water when possible. We confirmed with experts from the Taylor United Mussel Farm that the mussels had either spawned or began re-absorbing their gonads. The third approach to eliminate pre-deployment stress was to minimize the holding time and hold the mussels in the ocean rather than flow-through tanks.

5.7 Feasibility and Scientific Value

This study demonstrated that it is feasible to use caged bivalves to monitor water and sediment quality in the Cherry Point reach. Logistical feasibility was demonstrated by successfully collecting, sorting, caging, deploying and retrieving mussels from the desired locations along the 15 to 18-ft depth contour where herring might be expected to spawn. Technical feasibility was demonstrated by establishing some relationships between the concentration of PAHs in mussel tissues by area some differences in growth metrics among station groups. The robust nature of the methodology was demonstrated again with valuable information being provided in spite of an usually high barnacle spawning and settlement that could have adversely affected both mussel health and their ability to accumulate PAHs. Nevertheless, we demonstrated that the mussels were probably healthier than in 1998 and the measured concentrations of TPAHs were probably more reliable than those measured in 1998. We were also able to confirm the potential stress associated with both PAH exposure and temperature along the Cherry Point reach. Null hypotheses in 1998 and 1999 were answered and new hypotheses have been developed for subsequent studies planned for 2000. In the final analysis, the value of in-situ monitoring has been demonstrated by combining the use of caged mussels to characterize chemical exposure and caged herring eggs to characterize effects.

The relative importance of site versus stock effects in affecting herring populations in Puget Sound have not been completely answered. We have presented credible evidence to suggest that temperature and PAH exposure could be affecting herring in the Cherry Point reach and elsewhere. Kocan et al. (1998) and Hershberger and Kocan (1999) have presented credible evidence to suggest that declining herring stocks could be attributable to a "stock effect"; e.g., factors such as overfishing, genetic deficiencies, or long term stressors such as temperature or food in other phases of the life cycle. An integrated monitoring and assessment program that includes controlled experiments in the lab and in the field is the best way to answer the outstanding questions and work is currently underway by Kocan and Hershberger to examine the health of different herring stocks in Puget Sound (including Cherry Point) by evaluating the performance of their eggs under controlled laboratory conditions. We are planning to use caged mussels to examine the PAH and temperature stressors at other spawning locations (including Cherry Point) for herring in Puget Sound along a north-south gradient that should help explain some of the variability in effects measured at Cherry Point.

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 potential exposure and effects on herring eggs. Using caged mussels to monitor environmental conditions and quantify exposure and effects on a site-specific basis will help WDNR to make management decisions on water and sediment quality in the Cherry Point reach 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 be used to predict exposure and effects over space and time. 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.

Caged mussels 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, 1977) 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. Salazar and Salazar (1996) used caged mussels to demonstrate that ship hulls were the major source of tributyltin (TBT) accumulated in mussel tissues since higher concentrations were found near the surface rather than near the bottom. In a recent study on Vancouver Island, mussels were deployed at three depths across six stations to monitor bioaccumulation and effects of pulp and paper mill effluents (Applied Biomonitoring 1998). Results showed a significant gradient in both exposure and effects. These studies are relevant to and support the concept of expanded caged bivalve monitoring in Puget Sound.

6.0 APPLICATIONS TO FUTURE WORK

Results from the 1999 study were instrumental in identifying changes in experimental design, cage design, and station locations for future studies. The survival results from the 1998 and 1999 studies stress the need to identify well in advance, if possible, the potential biological and anthropogenic factors that could affect study results. Predation and fouling could result in low survival. Pre-deployment temperature conditions, reproductive condition, and holding duration prior to deployment are factors that could stress the test organisms and bias test results. Future studies should only be conducted with mussels that had already spawned to eliminate the

stresses associated with spawning activities and the potential to lose lipophilic chemicals with the gametes. The use of a predator mesh with a smaller, 1/4-inch opening was successful at excluding predators such as starfish and crabs, but was ineffective against planktonic fouling organisms such as larval barnacles. Both the 1998 and 1999 studies also demonstrated the need for adequate tethering and anchoring devices in high-energy open ocean areas. For future studies in such areas it is recommended that some type of permanent anchoring device (i.e., a metal hoop partially buried into the sediment, or cement block with a hoop) be set in place prior to deploying the caged mussels. This approach will require divers to attached the deployment arrays to the permanent anchoring structure, but the net benefit will be knowing precisely where the mussel cages are at the end of the test. This approach has proven useful for characterizing physical and chemical conditions in areas of concern, and should be considered by WDNR and other state agencies as a routine monitoring tool in the Pacific Northwest.

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8.0 REFERENCES

- Applied Biomonitoring. 1999a. Final Report. Caged Mussel Pilot Study, Port Valdez, Alaska, 1997. Kirkland, Washington, Report to Regional Citizens' Advisory Council. Contract Number 631.1.97, 96 pp plus appendices pp.
- Applied Biomonitoring. 1999b. Caged Mussel Study: Cherry Point Washington - May 1 to June 10, 1998. Prepared for Washington State Department of Fish and Wildlife. 1 June 1999.
- Applied Biomonitoring. 1998. Draft Final Report. Caged Mussel Pilot Study: Port Alice Mill, EEM Program. 101 pp. plus appendices pp.
- Barrick, R. C. and F. G. Prahl. 1990. Hydrocarbon geochemistry of the Puget Sound region - III. Polycyclic aromatic hydrocarbons in sediments. *Estuarine Coastal Shelf Sci.* 17:187-212.
- Bayne, B. L., Brown, D. A., Burns, K., Dixon, D. R., Ivanovici, A., Livingstone, D. R., Lowe, D. M., Moore, N. M., Stebbing, A. R. D., and Widdows, J. 1985. *The Effects of Stress and Pollution on Marine Animals*. New York, Praeger Special Studies, Praeger Scientific.
- Boehm, P. D., D. S. Page, E. S. Gilfillan, A. E. Bence, W. A. Burns, and P. J. Mankiewicz. 1998. Study of the fates and effects of the Exxon Valdez oil spill on benthic sediments in two bays in Prince William Sound, Alaska. 1. Study design, chemistry, and source fingerprinting. *Environ. Sci. Technol* 32:567-576.
- Brown, E. D., T. T. Baker, J. E. Hose, R. M. Kocan, G. D. Marty, M. D. McGurk, B. L. Norcross, and J. Short. 1996. Injury to the Early Life History Stages of Pacific Herring in Prince William Sound After the Exxon Valdez Oil Spill. *in* S. D. Rice, R. B. Spies, D. A. Wolfe, and B. A. Wright (Editors), *Proceedings of the Exxon Valdez Oil Spill Symposium*. American Fisheries Society Symposium 18. Bethesda, Maryland, American Fisheries Society. p. 448-462.
- Clark, R. C. Jr. and J. S. Finley. 1975. Uptake and loss of petroleum hydrocarbons by the mussel *Mytilus edulis*, in laboratory experiments. *Fish. Bull.* 73(3):508-515.
- Di Toro, D. M., J. A. McGrath, and D. J. Hansen. In Review. Technical basis for narcosis chemicals and PAH criteria. I. Water and tissue. Submitted to *Environ. Toxic. Chem.*
- EVS Consultants. 1996. Data Report - Ketchikan Pulp Company, Annual Bioaccumulation Monitoring Study. Prepared for Ketchikan Pulp Company, Ketchikan, Alaska, June 1996.

- EVS Consultants. 1997. Data Report - Ketchikan Pulp Company, Annual Bioaccumulation Monitoring Study. Prepared for Ketchikan Pulp Company, Ketchikan, Alaska, November 1997.
- EVS Environment Consultants, Inc. 1999. Cherry Point. Screening Level Ecological Risk Assessment. Preliminary Draft. Seattle, Washington, May 1999, Prepared for Washington State Department of Natural Resources pp.
- Gosling, E. 1992. The Mussel *Mytilus*: Ecology, Physiology, Genetics and Culture. Amsterdam, Elsevier.
- Harris, P. M., S. D. Rice, M. M. Babcock, and C. C. Brodersen. 1996. Within-bed distribution of Exxon Valdez crude oil in Prince William Sound blue mussels and underlying sediments. *In*: S. D. Rice, R. B. Spies, D. A. Wolfe, and others ((Eds.)), Proceedings of the *Exxon Valdez* Oil Spill Symposium. Anchorage, Alaska. American Fisheries Society. American Fisheries Society Symposium 18. pp. 298-308.
- Heintz, R. A., J. W. Short, and S. T. Rice. 1999. Sensitivity of fish embryos to weather crude oil: Part II. Increased mortality of pink salmon (*Oncorhynchus gorbuscha*) embryos incubating downstream from weathered Exxon Valdez crude oil. *Environ. Toxicol. Chem.* 18(3):494-503.
- Herbert, C. E. and K. A. Keenleyside. 1995. To normalize or not to normalize? Fat is the question. *Environ. Toxicol. Chem.* 14(5):801-807.
- Hershberger, P. and R. M. Kocan. 1999. Survival Potential of Cherry Point Herring: Larval Abnormalities and Weight at Hatch Following *in Situ* Incubation of Developing Embryos. Seattle, Washington. November 30, 1999, Prepared for Washington Department of Natural Resources, Contract #FY99-092 pp.
- IOS (Institute of Ocean Science). 1999. Daily Sea Surface Temperatures (C) at Active Pass, British Columbia. Sidney, BC (www.ios.bc.ca/ios/osap/data/lighthouse/bcsop.htm), Institute of Ocean Science.
- Jarvinen, A. W., and Ankley, G. T. 1999. Linkage of Effects to Tissue Residues: Development of a Comprehensive Database for Aquatic Organisms Exposed to Inorganic and Organic Chemicals. Pensacola, FL, SETAC.
- Kocan, R. M., P. Hershberger, and T. Mehl. 1998. Herring Embryo-Larval Success Evaluation at Cherry Point: Comparison of *in Situ* Exposures With Laboratory Controls. Seattle, Washington. August 11, 1998, Prepared for Washington Department of Natural Resources, Interagency Agreement #112451 pp.

- McCarty, L. S. 1991. Toxicant Body Residues: Implications for Aquatic Bioassays With Some Organic Chemicals. *In*: M. A. Mayes and M. G. Barron (Eds.), *Aquatic Toxicology and Risk Assessment*. Philadelphia, American Society for Testing and Materials. p. 183-192.
- McCarty, L. S. and D. Mackay. 1993. Enhancing ecotoxicological modeling and assessment. *Environ. Sci. Technol.* 27(9):1719-1728.
- Meador, J. P., J. E. Stein, W. L. Reichert, and U. Varanasi. 1995. Bioaccumulation of polycyclic aromatic hydrocarbons by marine organisms. *Rev. Environ. Contam. Toxicol.* 143:79-165.
- Naes, K., T. Bakke, and R. Konieczny. 1995a. Mobilization of PAH from polluted seabed and uptake in the blue mussel (*Mytilus edulis* L.). *Mar. Freshwater Res.* 46:275-285.
- Naes, K., J. Knutzen, and L. Berglind. 1995b. Occurrence of PAH in marine organisms and sediments from smelter discharge in Norway. *Sci. Tot. Environ.* 163:93-106.
- NOAA (National Oceanic and Atmospheric Administration). 1989. National Status and Trends Program for Marine Environmental Quality. Progress Report: a Summary of Data on Tissue Contamination From the First Three Years (1986-1988) of the Mussel Watch Project.
- Neff, J. M. and W. A. Burns. 1996. Estimation of polycyclic aromatic hydrocarbon concentrations in the water column based on tissue residues in mussels and salmon: an equilibrium partitioning approach. *Environ. Toxicol. Chem.* 15(12):2240-2253.
- Page, D. S., P. D. Boehm, G. S. Douglas, and A. E. Bence. 1995. Identification of Hydrocarbon Sources in the Benthic Sediments of Prince William Sound and the Gulf of Alaska Following the Exxon Valdez Oil Spill. *In*: P. G. Wells, J. N. Nutler, and J. S. Hughes (Eds.), *Fate and Effects in Alaskan Waters*. Philadelphia, American Society for Testing and Materials. p. 41-83.
- Piegorsch, W. W., and Bailer, A. 1997. *Statistics for Environmental Biology and Toxicology*. London, Chapman & Hall.
- Pittinger, C. A., A. L. Jr. Buikema, S. G. Hornor, and R. W. Young. 1985. Variation in Tissue Burdens of Polycyclic Aromatic Hydrocarbons in Indigenous and Relocated Oysters. *Environ. Toxicol. Chem.* 4:379-387.
- Salazar, M. H. 1997. Critical Evaluation of Bivalve Molluscs As a Biomonitoring Tool for the Mining Industry in Canada, *In* *Technical Evaluation of Molluscs As a Biomonitoring Tool for the Canadian Mining Industry*. Ottawa, Ontario, CANMET, Aquatic effects technology evaluation program, pp. 163-248 pp.

- Salazar, M. H. and S. M. Salazar. 1999. Draft Standard Guide for Conducting Field Bioassays with Marine, Estuarine and Freshwater Bivalves. Submitted to ASTM; in Review.
- Salazar, M. H. and S. M. Salazar. 1998. Using Caged Bivalves As Part of an Exposure-Dose-Response Triad to Support an Integrated Risk Assessment Strategy. *In* A. de Peyster and Day. K., Proceedings, Ecological Risk Assessment: A Meeting of Policy and Science, SETAC Special Publication., SETAC Press. p. 167-192.
- Salazar, M. H. and S. M. Salazar. 1997. Using bioaccumulation and growth in caged intertidal oysters to assess oil exposure and effects in Delaware Bay. *In* Proceedings of the Twentieth Arctic and Marine Oilspill Program (AMOP) Technical Seminar. Vancouver, British Columbia. Environment Canada. pp. 661-675.
- Salazar, M. H. and S. M. Salazar. 1996. Mussels As Bioindicators: Effects of TBT on Survival, Bioaccumulation and Growth Under Natural Conditions. *In* M. A. Champ and P. F. Seligman (Eds.), Tributyltin: Environmental Fate and Effects. London, Chapman and Hall. p. 305-330.
- Shigenaka, G. and C. B. Henry. 1995. Use of Mussels and Semipermeable Membrane Devices to Assess Bioavailability of Residual Polynuclear Aromatic Hydrocarbons Three Years After the Exxon Valdez Oil Spill. *In* J. Hughes, G. Biddinger, and E. Mones (Editors), Third Symposium on Environmental Toxicology and Risk Assessment, ASTM STP 1219, Exxon Valdez Oil Spill: Fate and Effects in Alaskan Waters. Philadelphia, American Society for Testing and Materials. p. 239-260.
- Short, J. W. and M. M. Babcock. 1996. Prespill and postspill concentrations of hydrocarbons in mussels and sediments in Prince William Sound. *In*: S. D. Rice, R. B. Spies, D. A. Wolfe, and others (Eds.), Proceedings of the *Exxon Valdez* Oil Spill Symposium. Anchorage, Alaska. American Fisheries Society. American Fisheries Society Symposium 18. pp. 149-166.
- Short, J. W. and P. M. Harris. 1996. Petroleum hydrocarbons in caged mussels deployed in Prince William Sound after the *Exxon Valdez* oil spill. *In*: S. D. Rice, R. B. Spies, D. A. Wolfe, and others ((Eds.)), Proceedings of the *Exxon Valdez* Oil Spill Symposium. Anchorage, Alaska. American Fisheries Society. American Fisheries Society Symposium 18. pp. 29-39.
- Short, J. W. and R. A. Heintz. 1997. Identification of *Exxon Valdez* oil in sediments and tissues fro Prince William Sound and the northwestern Gulf of Alaska based on a PAH weathering model. *Environ. Sci. Technol.* 31(8):2375-2384.

State of California, W. R. C. B. 1988. California State mussel watch marine water quality monitoring program: 1986-87. Water Quality Monitoring Report No. 88-2 WQ. Division of Water Quality .

US ACOE, 1999, Environmental Residue Effects Database Home Page. (Web Page), Available at <http://www.wes.army.mil/el/ered/index.html#misc>.

Whatcom County. 1996. Gateway Pacific Terminal: Draft Environmental Impact Statement and Appendices. Bellingham, WA, Whatcom County Planning and Development Services, Land Use Services Division.

Widdows, J. and P. Donkin. 1992. Mussels and Environmental Contaminants: Bioaccumulation and Physiological Aspects. *In* E. Gosling (Ed.), *The Mussel Mytilus: Ecology, Physiology, Genetics and Culture*. Amsterdam, Elsevier Science Publishers. p. 383-424.

Young, D. R., T. C. Heesen, and D. J. McDermott. 1976. An offshore biomonitoring system for chlorinated hydrocarbons. *Mar. Pollut. Bull.* 7(8):156-160.

Young, D. R., D. McDermott-Ehrlich, and T. C. Heesen. 1977. Sediments as sources of DDT and PCB. *Mar. Pollut. Bull.* 8(11):254-257.

