Accumulation of Polycyclic Aromatic Hydrocarbons by *Neocalanus* Copepods in Port Valdez, Alaska

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Abstract

Tankers involved in the transport of Alaska North Slope (ANS) crude oil to ports along the west coast of the United States are loaded with seawater as ballast during their return trip to the Alyeska Marine Terminal in Port Valdez, Alaska. As part of these ongoing operations, approximately 10,000,000 gallons of treated ballast-water are discharged daily from the Ballast Water Treatment Facility (BWTF) in Port Valdez. To determine if the treated ballast water effluent affects the zooplankton community, polynuclear aromatic hydrocarbon (PAH) concentrations in water, suspended particulate material (SPM), and *Neocalanus* copepods were determined throughout Port Valdez and in Prince William Sound (PWS) in April 2004. Petrogenic PAH were detected in Port Valdez *Neocalanus* (0.607 to 1.28 μg/g dry weight). Because concentrations in tissue were smaller than those known to cause harm to calanoid copepods by factors of at least 14, current total PAH (TPAH) levels in Port Valdez are probably not injurious to the zooplankton community. *Neocalanus* may acquire PAH from water and or SPM, yet TPAH concentrations in these compartments were below method detection limits (MDL), further suggesting the potential for damage is unlikely. At current rates of discharge into Port Valdez, ballast-water effluent likely has little effect on the plankton community and does not pose a significant toxic risk.

Introduction

Daily discharge of up to 10^8 L of water containing ≤8 mg oil/L into Port Valdez, Alaska, is permissible (NPDES 2004), raising concern for the long-term health of marine biota in the receiving waters. In 1998, the average rate of treated ballast-water discharge (originating from approximately 700 oil tankers) was 10^6 L/day (PWSRCAC 2005). This industrial discharge into

an otherwise sparsely-populated area results from the transfer oily wastewater from tanker holds prior to loading crude oil at the southern terminus of the trans-Alaska oil pipeline. Ballast water, which becomes contaminated because the oil tanks are filled with water to stabilize returning tanker ships, is treated at an onshore Ballast Water Treatment Facility (BWTF) to remove petroleum. About 3.2×10^6 L of oil are recovered per month; treated wastewater is discharged through a diffuser pipe into Port Valdez at a depth of 60 to 80 m (Lysyj et al. 1979). Discharged water mixes with surrounding seawater and is generally trapped below 20 m but occasionally reaches the surface during unstratified conditions (Payne et al. 2002). Beyond the immediate vicinity of the mixing zone, little is known about dilution and hydrocarbon transport within Port Valdez (Payne et al. 2002).

Not all contaminants are removed by the BWTF. Contaminants in discharged water include monoaromatic hydrocarbons, polynuclear aromatic hydrocarbons (PAH), and aliphatic hydrocarbons (Salazar et al. 2002; Payne et al. 2005a,b). Of these, PAH are the most toxic and persist in the water and sediments for longer periods than the more volatile monoaromatics, which are also more easily degraded by microbes (e.g., Wolfe et al. 1994; Atlas 1995; Short et al. 2003; Payne et al. 2005a,b). PAH are present in discharge waters in both dissolved and dispersed phases and have been detected at concentrations on the order of a few parts per trillion (ng/L) outside the diffuser zone (Payne et al. 1998, 2001, 2003; Salazar et al. 2002).

Detection of low PAH concentrations is improved by bioaccumulation, extraction of large water volumes, and passive samplers. Of these approaches, bioaccumulation by living organisms has the advantage of describing natural exposure of the accumulating species, as well as providing a time-integrated response to hydrocarbon exposure (an advantage shared with passive samplers but not with collection of discrete water samples). Bioaccumulation of a

dissolved hydrophobic organic contaminant refers to the increased contaminant concentration within an organism that results from the passive partitioning of the contaminant between the aqueous phase and the lipid compartment of the organism. The extent of bioaccumulation is measured by the bioaccumulation factor (BAF), which is the ratio of the wet-weight concentration of a contaminant in an organism and the concentration in the ambient water (Barron 1994). Ingestion of particulate oil or of oil-contaminated prey by organisms are other potential routes of exposure.

Bioaccumulation of PAH has been demonstrated in zooplankton (Conover 1971; Corner et al. 1976; Lee 1975), including *Neocalanus plumchrus*, a species that is common in the waters of Port Valdez (Cooney 1986a, b; Kirsch et al. 2000; Cooney et al. 2001). The BAF of naphthalene dissolved in seawater by *Calanus helgolandicus* exceeded 36, but the BAF for naphthalene ingested through the diet was much larger, and the depuration rate of naphthalene acquired through the diet was substantially longer that the depuration rate of naphthalene from the dissolved state in seawater (Corner et al. 1976). Following the wreck of the tanker *Arrow* in Chedabucto Bay, Canada in 1970, as much as 10% of the bunker C oil was ingested by zooplankton (Conover 1971), corroborating the experimental work indicating that suspension-feeding zooplankton are especially efficient at accumulating oil associated with small particles. The BAF for dissolved benzpyrene by *N. plumchrus* was about 1,000 (Lee 1975), indicating that these zooplankters act as efficient natural agents for extracting dissolved hydrocarbon contaminants from seawater; larger BAFs are anticipated for hydrocarbons associated with ingestible particles (Corner et al. 1976). These large BAFs facilitate detection of hydrocarbons that may be present in seawater at concentrations that would be difficult to detect by other methods such as extraction of large water volumes.

In addition to facilitating detection of PAH, bioaccumulation may cause harmful internal concentrations of these contaminants in zooplankton, and possibly reduce the availability of zooplankters to the wide variety of predators that prey on them. These predators include species of considerable ecological or economic importance, including forage fishes (e.g. capelin, herring, and smelts) and juvenile salmonids.

Our objective was to determine whether PAH derived from Alaska North Slope (ANS) crude oil is accumulated by zooplankton in Port Valdez. *Neocalanus*, a genus of copepods that includes species that are common in Port Valdez, was selected for study because lipid content is usually high in these species (Båmstedt 1986, Duesterloh 2002). Because we anticipated that the ambient concentrations of PAH would likely be very low, confidence in the detection of PAH accumulated by zooplankters required an explicit determination of the detection limit of the method used for the chemical analysis of hydrocarbons in them. Hence, a secondary objective was to determine explicit method detection limits (MDLs) for PAH in *Neocalanus.* Concentrations of PAH detected in *Neocalanus sp.* were compared with concentrations of dissolved and particle-associated PAH extracted from large volume water samples, to assess the sensitivity of these two methods for monitoring PAH in the waters of Port Valdez.

Study Area

Port Valdez is a narrow fjord located in the northeast corner of Prince William Sound (PWS), Alaska, and is separated from the Sound by a narrow channel and double sill (Fig. 1). West to east, Port Valdez extends about 18 km and is about 5 km wide. The walls of this approximately 100 km^2 basin drop steeply to a nearly flat bottom about 240 m deep; mean depth is about 180 m (Colonell 1980a). The climate is maritime with mild winters (-10 to -4°C) and

cool summers (7 to 13°C; Colonell 1980a). Freshwater input is substantial; mean annual precipitation is158 cm per year (Blanchard & Feder 2000). Tides are large, about 5.6 m maximum and 3.0 m mean. Water stratifies seasonally; surface water temperature ranges from <2.5°C in winter to >11°C in summer (Muench & Nebert 1973). Below 75 m, temperature generally ranges from 3 to 6°C (Muench & Nebert 1973). Surface salinity (upper 20 m) ranges from <1 ppt (summer) to >32 ppt (winter); subsurface salinity is generally between 28 to 32 ppt (Muench & Nebert 1973). In winter, vertical water mixing may extend to the bottom of the fjord (Muench & Nebert 1973). The suspended sediment load is also seasonal; high loads coincide with spring melt and most are discharged by the Lowe River, Valdez Glacier Stream, and Mineral Creek (Sharma & Burbank 1973; Feder & Keiser 1980). Subsurface flow patterns (15 m below the surface) tend to be irregular, with an ill-defined wind-driven western movement (Muench & Nebert 1973).

Three processes influence water exchange between PWS and Port Valdez: tidal flushing, seasonal deep water exchange, and weather-driven events. The tidal prism is about 1.6% of the total volume, suggesting the half-life of a conservative (i.e. degradation-resistant) pollutant is about 22 d in dry weather (Colonell 1980a). Deep water exchange is enhanced during summer and early autumn as surface freshwater outflow is replaced by more saline deeper water from PWS via the Valdez Narrows (Sharma & Burbank 1973; Muench & Nebert 1973; Colonell 1980a). Surges of surface water from PWS into Port Valdez and large outflows at depths >50 m are apparently related to weather systems, and are believed to be the processes with the greatest influence on deep water exchange (Colonell 1980a). Together, these processes suggest the residence time of pollutants is probably no more than a few weeks (Colonell 1980a), but this has not been empirically tested.

Two closely related species, *N. plumchrus* and *N. flemingeri*, are present in Port Valdez (Coyle and Pinchuk 2005). They are mostly sympatric, occupy the same depth range during feeding and growing stages, and were not recognized as separate species until recently (Miller 1988). Adult *N. flemingeri* reproduce in surface waters during late spring and *N. plumchrus* reproduce at depth in January and February (Miller 1988). Adult females of both species release their eggs during late winter at depth, then die (Miller 1988). Developing copepodite stages occur in surface waters of Prince William Sound (PWS) for 2-3 months in spring (Cooney 1986a,b; Kirsch et al. 2000; Cooney et al. 2001). Stage 5 copepodite (C5) abundance of both species peaks in PWS about May 1. Following fertilization of adult female *N. flemingeri* in late May and early June, the adult females migrate to deep water (>300 m depth) and enter diapause until late winter, while the adult males die (Miller 1988). Stage C5 copepodites of *N. plumchrus* migrate to deep water (>300 m depth) and enter diapause at about the same time, and do not develop into adults until late winter when they reproduce and then die (Cooney et al. 2001).

Methods

Copepods, water, and suspended particulate matter (SPM) were collected in 2004 between April 27 and May 1 near the time of peak *Neocalanus* copepodite stage C5 abundance. Five sample stations were occupied within Port Valdez to collect zooplankters and water samples for hydrocarbon analysis (Fig. 1). One of these was located adjacent to the BWTF diffuser and another near the city of Valdez. The remaining three were more remote, including one in close proximity to a long-term environmental monitoring site near Gold Creek (Prince William Sound Regional Citizens' Advisory Council Long Term Environmental Monitoring Program). For reference, additional samples were collected in the Lone Island and Knight Island

area of PWS (Fig. 1). Water characteristics, including thermocline depth, were determined by conductivity-temperature-depth casts at each station. To avoid possible hydrocarbon contamination from the vessel, all engines were shut down during collection and the boat was allowed to drift. Positions were recorded with a Global Positioning System receiver.

Reference *N. plumchrus / N. flemingeri* were collected near Lone Island in Prince William Sound for two purposes, comparison with hydrocarbon concentrations in Port Valdez copepods and to determine method detection limits (MDLs) for the hydrocarbons analyzed in these copepods. Near-surface and below-thermocline water samples were also analyzed for hydrocarbons for comparison with concentrations found in the copepods.

Neocalanus plumchrus / N. flemingeri were collected with a 505 :m-mesh plankton net with a 0.5 m diameter circular opening and 1 L collection bucket with 333 :m mesh at the cod-end, using repeated 50 m vertical casts to achieve sufficient sample sizes. For each hydrocarbon sample, about 500 (range 438 to 620) *N. plumchrus / N. flemingeri* copepodites were collected by forceps (except pipettes were also used at Anderson Bay, the first collection station), placed in hydrocarbon free glass jars, and frozen. Three replicates were collected per station; 14 additional replicates were collected near Lone Island for MDL determination. Additional *Neocalanus* were collected at each station for lipid analysis (n = 100, frozen in liquid nitrogen), species identification (n = 100, preserved in phosphate-buffered formalin), and wet-weight dry-weight estimates (n = 100, frozen). *Neocalanus plumchrus* were presumptively distinguished from *N. flemingeri* in the preserved samples on the basis of the ratio of the cephalosome and prosome lengths of C5 copepodites using criteria given in Miller (1988).

Water samples (3.5 to 3.7 L) were collected from the surface (0 to 1 m) and below the thermocline (9 to 29 m) at each station with a GoFlo Bottle® and processed by vacuum filtration

through 0.7 μm glass-fiber filters (142 mm diameter; Payne et al. 1999). Filtered water samples were spiked with deuterated hydrocarbon surrogate standards and extracted with dichloromethane each evening after sample collection (Short et al. 1996). Filters were frozen for later extraction of SPM.

Hydrocarbon measurement.

Water, tissue, and SPM samples were analyzed for the 44 PAH and 25 aliphatic hydrocarbons listed in Tables 1 and 2, and were processed according to Short et al. (1996). Samples were extracted with dichloromethane after addition of 6 perdeuterated surrogate hydrocarbon standards (Table 3). Water extractions were completed in the field and the extracts were stored at -20°C pending analysis; glass fiber filters (containing trapped SPM) and copepod tissues were macerated and extracted in the laboratory with an accelerated solvent extractor. The SPM was processed as sediment. Isolation and purification of calibrated and uncalibrated hydrocarbons was completed by silica gel/alumina column chromatography followed by size-exclusion high-performance liquid chromatography and fractionation; water samples were only processed through aluminum/silica columns. Extracts of PAH were separated and measured by gas chromatography/mass spectroscopy in the selected ion mode. Calibrated PAH were identified by retention time and two characteristic mass fragment ions, and quantified using a 5-point calibration curve. Uncalibrated PAH homologs (which included alkyl-substituted homologs of naphthalene, fluorene, dibenzothiophene, phenanthrene, and chrysene) were identified by retention times and a single characteristic mass fragment ion. Uncalibrated PAH were quantified by using calibration curves of the most similar calibrated homologs: 2,6-dimethylnaphthalene was used for dimethyl naphthalenes; 2,3,5-trimethylnaphthalene for tri- and

tetramethylnaphthalenes, and 1-methylphenanthrene was used for all the alkylphenanthrene homologs.

Method detection limits were determined experimentally following the procedure given in Section 40 Code of Federal Regulations Part 136, Appendix B. This procedure involves measurement of PAH concentrations in at least seven identical samples, and the measured concentration should be less than five times (and no more than ten times) the estimated MDL. The MDL is the estimated concentration associated with a 1% probability of type I detection error, calculated as the product of Student's $t_{\nu,\forall=0.01}$ and the standard deviation of the PAH concentration measured in the analyzed replicate samples (where $\nu + 1$ is the number of samples analyzed, and $\forall$ is the significance level). Method detection limits for uncalibrated PAHs were not experimentally determined. Consequently, detection limits for these analytes were arbitrarily assumed to be the MDL of the most closely related calibrated PAH analyte (Short et al. 1996).

Eight of the *N. plumchrus / N. flemingeri* samples from Lone Island were homogenized together to ensure uniformity, and then split into 8 aliquots of ~ 1 g each (wet weight). Each aliquot was spiked with 1.1 to 2.6 ng of each of the calibrated PAH, and with 42 to 61 ng of each of the calibrated alkanes per g wet weight of copepod tissue homogenate. These eight spiked aliquots were then processed and analyzed identically as the other samples of *N. plumchrus / N. flemingeri*.

The PAH MDL masses in copepods were about 0.3 to 23 ng (Table 1); sample wet weights and dry weights were near 1 g and 0.2 g respectively. The MDL for a particular PAH in a sample is calculated as the ratio of the MDL mass and the sample aliquot weight. The previously determined MDLs for water and SPM ranged from about 0.00044 to 0.008 µg/L, depending on the specific hydrocarbon in 3.8 L sample aliquots, except for naphthalene, which

had an MDL of 0.056 µg/L. Concentrations below MDL were treated as zero so that total PAH (TPAH) estimates would not be inflated by the inclusion of false positives (Short et al. 1996). The accuracy of the hydrocarbon analyses is estimated as about ± 15% based on comparison with National Institute of Standards and Technology values, and precision expressed as coefficient of variation is usually less than about 20%, depending on the PAH (see Results). Total PAH concentrations were calculated by summing concentrations of 44 individual PAH (Table 1). Relative PAH concentrations were calculated as the ratio of PAH concentration to the TPAH concentration.

Alkanes were measured with a gas chromatograph equipped with a flame ionization detector (Short et al. 1996). Experimentally determined MDLs for alkanes in *Neocalanus* depended on sample weights and were about 18 to 263 ng (Table 2). Concentrations below MDL were treated as zero. The MDLs for alkanes in water were not determined due to their exceedingly low solubility. Total alkanes is the sum of 25 identified alkanes (Table 2); unresolved complex mixture (UCM) concentrations are also reported.

Quality Assurance

Quality control samples were analyzed with each batch of 12 samples to assess the accuracy and precision of the analysis, and to verify the absence of laboratory contaminants introduced during analysis. Included for assessment of accuracy in each batch were two quality control samples prepared from PAH standards from the National Institute of Standards and Technology (NIST) and aliphatics prepared at our laboratory. Precision was assessed by analysis of two NIST standard reference material (SRM) samples analyzed with each batch: SRM 1974a for mussels and SRM 1944 for sediments. Spiked samples were generally included

in batches containing tissue to ensure analytical accuracy. Absence of laboratory contaminants was verified by analysis of one method blank sample with each batch.

Dry Weight Determination

To determine the relationship between wet and dry weights, wet samples were weighed then dried at 70 to 75EC to constant weight. The wet/dry ratio (5.04) was used to convert hydrocarbon data in tissue to a dry weight basis.

Data analysis

For final analysis, biphenyl concentrations were dropped from the water results because they were clearly artifacts in all water samples (76 to 90% of TPAH) and in 4 of the SPM samples (13 to 34% of TPAH; Shoup Bay, Valdez, & Lone Island). Although present as a minor component in petroleum (at part per million levels and 6-12% of TPAH in ANS crude oil), biphenyl is not a major component, and yet it was the dominant PAH in all of the seawater samples examined. The source of this constituent in water is unknown but probably not from the sampling or filtration system because of its sporadic appearance in the SPM samples. Biphenyl concentrations were below MDL in most tissue samples with two exceptions, both at Anderson Bay (6 to 12% of TPAH). This procedural modification did not change composition patterns of the remaining hydrocarbons, and had no influence on interpretation of tissue data. The only substantive change was increased estimates for proportions of other PAH analytes.

Hydrocarbon source identification. Spatial trends in the PAH composition data were visually examined for source characteristics and similarities, by cluster analysis, and by source identification models (Carls, submitted). A dissimilarity matrix relating the hydrocarbon

compositions of samples from the various locations was constructed by calculating the Aitchison distance metric (Aitchison, 1992) between each pair of samples. The Aitchison distance is a quantitative multivariate metric of dissimilarity between two composition patterns *u* and *U* (for the PAH constituents identified in Table 1), calculated as

$$dist\ (u,\ U) = \left\{ \sum_{j=2}^{N} \sum_{i=1}^{j-1} \left[\ln\left(u_i / u_j\right) - \ln\left(U_i / U_j\right) \right]^2 \right\}^{1/2} \qquad (1)$$

where i and j are indices of hydrocarbon analytes, and N is the total number of analytes considered (N = 15). This metric has several advantageous statistical properties, including scale invariance, symmetry (i.e. $dist(u,U) = dist(U,u)$), non-negativity, and the property that $dist(u,u) = 0$. These latter three properties meet the requirements for a dissimilarity metric for statistical cluster analysis.

One limitation of the Aitchison distance metric is the inability to deal with null entries. The PAH accumulated by *Neocalanus* were often near and sometimes just below MDL, and some of the PAH that were just below MDL contained crucial hydrocarbon pattern information. We therefore included PAH that were detected at concentrations that were at least half the MDL in our calculation of the Aitchison distance matrix. These PAH are identified in Table 1.

The hierarchical structure of the resulting matrix was determined from agglomerative and divisive clustering methods using the Cluster Procedure in SAS version 6. Agglomerative clustering employed the "average" method, while the divisive approach employed the "complete linkage" method. The hierarchical structure was visualized by constructing dendrograms, which were inspected for evidence of spatial coherence among the clusters.

Source identification models were used to determine the presence or absence of oil in *Neocalanus* tissue, water and SPM. The results of three models were combined, a first-order loss-rate kinetic weathering model (Short and Heintz 1997), an oil-identification model (Bence and Burns 1995), and a non-parametric model (Carls, submitted). Each model provided two outputs, a non-specific estimate of the presence or absence of petrogenic oil and a specific estimate of the presence or absence of ANS crude oil. These estimates were combined to yield a score for each data record that potentially could range from 0 to 6; each model contributed equally. Scores 4 through 6 were contributed by the ANS-specific submodels; scores 1 through 3 were contributed by the non-specific submodels. No oil was likely where the score was zero; the presence of ANS was highly likely where the score was 6. Scores among stations were analyzed with single-factor Analysis of Variance (all data were normal with equal variance). Treatment means were compared to control means with pairwise contrasts; the Bonferroni inequality (α divided by the number of comparisons) was applied to ensure the probability of incorrect rejection was no more than 0.05 for all comparisons. Similarly, a non-parametric pyrogenic detection model was used to test PAH composition for the presence of pyrogenic sources (Carls, submitted).

Patterns of PAH in *N. plumchrus / N. flemingeri* were compared with dissolved and particulate PAH distributions of the ballast water treatment facility effluent measured in January 2005 (Payne et al. 2005a,b). Hydrocarbons derived from crude oil and associated with particulate oil were determined by the ratio of phytane and TPAH. Phytane is nearly ubiquitous in crude oils but is rarely encountered elsewhere (Dean and Whitehead 1961). Substantive phytane/TPAH ratios indicate the presence of particulate petroleum because phytane is rarely encountered except in crude oil.

Results

The sorted plankton samples contained primarily *N. plumchrus / N. flemingeri*, typically 97 to 98%. The exception was the first sample station, Anderson Bay, where 79% of the sample was *N. plumchrus / N. flemingeri* and the remainder included much smaller species or stages that went unnoticed at the time of collection. These species included *Acartia longiremis, Calanus marshallae, Centrophages abdomina, and Pseudocalanus* spp; none were staged. A change in methods, from collection by pipette to collection by forceps, prevented such mixtures at the other stations. Expressed per unit mass, the Anderson Bay sample was also primarily *N. plumchrus / N. flemingeri* (99%). *Calanus* was present in small quantities at remaining stations (2 to 3%).

Stage C5 copepodites dominated the *Neocalanus* samples, 90 to 98%; the remainder were stage C4 (Table 4). Most of the C5 copepodites were *N. plumchrus*, although the distribution of cephalosome:prosome ratios suggested that some *N. flemingeii* may have been present. Hereafter, these will be referred to as *N. plumchrus*. A third species, *N. cristatus*, are easily distinguished by their much greater size and were not collected. Although not replicated, there were no obvious location-dependent differences in copepodite stage (Table 4).

The estimated mass of stage C5 copepodites was 2.3 mg wet weight[1]. Mass estimates for all copepodite stages (and including up to 3% *Calanus*) ranged from 1.8 to 2.5 mg wet weight (n = 22). Excluded were two unusually low-mass outliers, likely caused by unusual desiccation before measurement. Stage C5 mass estimates, determined by regressing observed mass (dependent variable) against the incidence of stage C5 copepodites (independent variable) and

[1]The observed mass is slightly smaller than estimated by Coyle et al. (1990; 2.86 mg) and Short (unpublished data; 2.97 mg), probably because our specimens were not immediately weighed live and were partially desiccated before wet weight measurement.

extrapolating to 100%, uncovered the same two outliers. Rounded to 1 decimal place, estimated stage C5 copepodite mass was the same (2.3 mg) regardless of inclusion or exclusion of outliers. Percent moisture, estimated in one sample per station, was 80% (range 79 to 81%, $n = 4$ excluding the previously identified outliers).

Thermocline depths varied from 9 to 29 m; temperature changed from about 4.0 to 7.0°C across this boundary (Table 5). The thermocline was deepest in Anderson Bay and shallowest in Shoup Bay.

Hydrocarbons

Quality Assurance Results

Masses of PAH measured in method blanks were usually below equivalent MDLs of *N. plumchrus*, except for naphthalene, the two methylnaphthalene isomers, acenaphthene, fluorene, and phenanthrene. These exceptions were never greater than 3.1 times (for naphthalene in one analysis string) the equivalent MDL, and usually were within a factor of two or less. Contaminant PAH concentrations in the method blanks were subtracted from those in the samples, so these contaminants are not reflected in the final concentrations calculated for the tissue samples. Masses of PAH measured in method blanks of the seawater and the SPM were consistently below (and usually far below) the equivalent MDL.

Most of the calibrated alkane hydrocarbons were not detected in the method blanks of any matrix (83%). The highest signal was for hexadecane in one of the method blanks for water analysis, at an equivalent aqueous concentration of 0.036 :g/L. All the other signals were below equivalent concentrations of 9 :g/L in water, 13 :g/L for SPM in water, and 0.021 :g/g in tissue (dry weight basis). These cannot be compared with equivalent MDLs for the water samples

because MDLs were not determined for water samples owing to the limited solubility of alkanes in water. The alkanes measured in the tissue method blanks were consistently below MDL.

The accuracy of PAH concentration estimates in the ten reference samples (NIST SRM 1491) ranged from 94 to 113% of certified or expected values, and 81 to 109% in three tissue blanks spiked with SRM 1491 except for fluorene (148%) and dibenzothiophene (63%). Analysis of the corresponding samples for alkane hydrocarbons ranged from 91 to 108% in the accuracy-check samples and 84 to 125% in the spiked blank samples.

The median precision of the PAH (including the uncalibrated PAH) in the four SRM 1974b samples analyzed for all the sample matrixes, expressed as the coefficient of variation, was 12%. The precision ranged from 1% to 40%, and was less than 25% for all but five of the calibrated analytes, four of which were 4- or 5-ring PAH. The median precision of the alkanes in tissue and SPM samples ranged from 1% to 9%. Precision was not estimated for dissolved alkanes due to their limited solubility.

Recoveries of surrogate standards typically ranged from >30% to <120% across all sample matrices. Recoveries of surrogate PAH standards were between 30% and 119% in 97% (n = 252) of the estimates. Recoveries of perdueterated naphthalene were as low as 12% but were >30% in 86% of 42 observations. Recoveries of the surrogate alkanes ranged from 43% to 102% (n = 150).

Water and SPM (suspended particulate material)

PAH and alkane concentrations in water and SPM samples were consistently below MDL, except for frequently obvious biphenyl artifacts. Excluding biphenyl and without correction for MDL, aqueous TPAH concentrations were 0.010 to 0.024 µg/L and total alkane

concentrations were 0.011 to 0.073 µg/L (n = 12). Similarly, TPAH concentrations associated with SPMs were equivalent to 0.001 to 0.012 µg/L seawater and total alkane concentrations were equivalent to 0.013 to 0.333 µg/L (n = 12). Because analyte concentrations were below MDL in water and SPM, statistical analyses of the data and source characterization were not attempted. For additional reference, however, PAH and alkane histogram plots for the dissolved and SPM samples are contained in the appendix (with lines indicating compound- and sample-specific MDLs), with the caveat that these further compositional details may simply describe analytical noise. The water and SPM samples were very clean and except for the biphenyl artifact, inadvertent sample contamination above MDL levels during either sample collection or analytical processing was not evident. Composition models did not identify oil in water and SPM samples.

Neocalanus plumchrus

Total PAH concentrations in *N. plumchrus* were greatest in the central and eastern portion of Port Valdez and least at the Lone Island reference station (Table 6). Total PAH concentrations ranged from 0.607 ± 0.248 :g/g to 1.28 ± 0.433 :g/g at the stations within Port Valdez. *Neocalanus plumchrus* collected from the Lone Island station contained 0.409 ± 0.431 :g TPAH per g dry weight, the lowest PAH burden among all stations.

The distribution of PAH in *N. plumchrus* from the Lone Island reference station (Fig. 2a) was substantially different that in those from the ballast water discharge station (Fig. 2b) and all other stations. Naphthalenes and fluorenes accounted for most of the PAH in *N. plumchrus* collected from near Lone Island (Fig. 2a), whereas contributions from phenanthrenes and dibenzothiophenes approached those of the naphthalenes and fluorenes in *N. plumchrus* collected

from near the ballast water discharge (Fig. 2b). Consistent with pyrogenic sources, dibenzothiophene and phenanthrene homologs generally declined in abundance with increasing alkyl substitution in *N. plumchrus* from near Lone Island. In contrast, C2-homolog abundances in these two homolog distributions were greatest near the ballast water discharge, consistent with a petrogenic source.

Composition models suggested the presence of oil-derived hydrocarbons in most Port Valdez *Neocalanus* but not at Lone Island. Petrogenic scores were least at Lone Island; scores were significantly greater at Gold Creek, the ballast-water diffuser, and Valdez (Table 7). The two Port Valdez sites with the smallest petrogenic scores were Shoup and Anderson Bays, the furthest removed from ballast water treatment effluent and the city of Valdez. Conversely, pyrogenic model scores where significantly higher at the reference station than at any other station (Table 7).

The PAH distribution patterns characteristic of *N. plumchrus* from near Lone Island were clustered furthest from those from the ballast water discharge, consistent with composition model results (Fig. 3). The samples from the three locations toward the eastern half of Port Valdez had the closest pattern similarity and may be represented by the pattern evident in *N. plumchrus* from near the ballast water discharge. In contrast, the samples from Anderson and Shoup Bays, on the western side of Port Valdez, had patterns that were more similar to that of samples from Lone Island or were intermediate between the Lone Island and ballast water discharge patterns.

Pristane was by far the most prominent alkane in *N. plumchrus*, accounting for 99.9% of the alkanes listed in Table 2, and 85 – 90% of total alkanes (which include the unidentified alkanes). Concentrations of pristane in *N. plumchrus* within Port Valdez ranged from 7,350 ±

3,310 :g/g at Anderson Bay to 9,240 ± 761 :g/g near the ballast water discharge (Table 8). Pristane concentrations were higher in *N. plumchrus* from Lone Island, at 11,200 ± 243 :g/g. *Neocalanus plumchrus* and *N. flemingeri* produce pristane (Short 2005), thus detection of this biogenic compound was expected. The only other *n*-alkane consistently detected in *N. plumchrus* was docosane (*n*-C_{22}), at concentrations ranging from 0.422 to 1.43 :g/g.

Phytane was not detected in *N. plumchrus* from the Lone Island reference station but was present in almost all of the samples from Port Valdez at average concentrations ranging from 0.123 ± 0.528 :g/g at Anderson Bay to 0.452 ± 0.265 :g/g Gold Creek (Table 8). The presence of phytane indicates petrogenic exposure; this compound is not produced by plankton.

Discussion

PAH in *N. plumchrus* from near Lone Island

The concentration patterns and amounts of hydrocarbons detected in *N. plumchrus* near Lone Island indicate the presence of an area-wide hydrocarbon contaminant burden in seawater that may derive from multiple sources. The absence of phytane in these samples suggests that *N. plumchrus* accumulated this hydrocarbon burden either from hydrocarbons dissolved in the ambient seawater, or from sources of particulate hydrocarbons that contain little phytane such as particulates derived from incomplete combustion of petroleum-derived fuels. The generally declining abundances of the dibenzothiophenes and phenanthrenes with increasing alkyl-substitution, along with the presence of unsubstituted fluoranthene and pyrene also suggest a combustion-derived contribution to this hydrocarbon signature (Barrick et al. 1980). However, the prominence of the naphthalenes is not consistent with a combustion source, and may have derived from a different input source distinct from the inferred combustion source, such as

atmospherically-mediated transport of vapor-phase naphthalenes from distant industrial sources.

The hydrocarbons detected in *Neocalanus* likely reflect those present in ambient seawater. Conceivably, the PAH detected in these copepods were spurious contaminants from shipboard sources that were introduced during sampling but this is very unlikely. No combustion sources on the vessel were operating during collection and samples were exposed to the atmosphere for less than an hour during sorting and counting. The dichloromethane used to extract the seawater samples was also exposed to the atmosphere under similar conditions, but failed to accumulate detectable hydrocarbons (except biphenyl, which was not evident in the copepod samples). The fact that PAH were detected above MDLs, which were measured explicitly for *N. plumchrus* in this study, and substantially exceeded concentrations found in method blanks, provides additional support for the view that these concentrations are not spurious, rather, they amplified hydrocarbon concentrations present in ambient seawater to detectable levels.

If the hydrocarbon burden in *N. plumchrus* did accumulate from the ambient seawater, then the concentrations evident in the copepods imply very low concentrations in the seawater. These copepods typically contain 50% to more than 80% lipid (Båmstedt 1986, Duesterloh 2002), hence PAH concentrations in them may, to a first approximation, be considered as entirely lipid-phase concentrations. Because of their small size, these copepods have a high ratio of surface area to volume, and hence approach equilibrium with contaminants in ambient seawater within a few days of exposure (Lee 1975).

The equilibrium concentration of accumulated PAH with seawater may be roughly estimated from the octanol-water partition coefficient (K_{ow}) of the PAH and the PAH

concentration in the copepod (wet weight basis, see Barron 1994). The K_{ow} is the ratio of a contaminant concentration in octanol and in water. For example, the wet weight concentration of the naphthalene in *N. plumchrus* from near Lone Island was about 0.010 :g/g, and the K_{ow} of naphthalene is ~ 2,200 (Mackay et al. 1992), implying a seawater concentration of 0.0046 :g/L. Much lower dissolved concentrations of other PAH are implied because the K_{ow} increases rapidly with the number of rings and with the extent of alkyl substitution. For example, the K_{ow} of methylnaphthalene is ~7,400, of phenanthrene is ~28,800, and of fluoranthene is ~126,000. Lower ambient concentrations are also implied if PAH are accumulated through the diet from particulates. One recent study with mussels found that particle bound pristane was accumulated to almost a 90-fold greater extent that an equivalent concentration of dissolved pristane (Short 2005), and qualitatively similar results have been reported for naphthalene accumulation by *N. plumchrus* (Corner et al. 1976). Hence, if the copepods collected near Lone Island accumulated PAH through their diet instead of through absorption of dissolved PAH, the implied PAH concentrations in seawater would be well below 0.001 :g/L (i.e. part per trillion). These implied PAH concentrations are consistent with our failure to detect PAH in the water and SPM. In fact, successful detection of hydrocarbons at concentrations well below one part per trillion would require extraction of large seawater volumes (>20 L) or flow-through, solid-phase extraction techniques that would be considerably more expensive than the effort expended here. In any case, such low concentrations are characteristic of the least-contaminated portions of the world's oceans.

PAH in *N. plumchrus* from Port Valdez

The concentration patterns and amounts of hydrocarbons detected in the *N. plumchrus*

samples from Port Valdez indicate a substantial contribution from hydrocarbons associated with particulate material, perhaps in addition to the area-wide particulate or dissolved phase contribution evident in copepods collected from near Lone Island. Detection of phytane in the samples from Port Valdez strongly implicates a particulate-phase association. Phytane is ubiquitous in crude oils and remains in refined oils if not removed during distillation (Dean and Whitehead 1961), it is considerably more resistant to microbial oxidation than are the normal alkanes (Pirnik 1977), and it has very low (<0.5 :g/L) solubility in water, based on comparisons with *n*-alkanes of comparable molecular weight (Sutton and Calder 1974). The ratio of phytane to TPAH in the particulate phase associated with the effluent of the ballast water treatment facility at the Alyeska Marine Terminal may be estimated from data presented in a recent monitoring report on hydrocarbons in PWS (Payne et al. 2004; Payne et al. 2005a,b), and is about 0.5. This ratio is consistent with the ratio for *N. plumchrus* sampled within Port Valdez (Tables 6 and 8). The intermediate TPAH concentrations at Anderson Bay and the close association of the two samples from Anderson Bay that did not contain detectable phytane with the samples from near Lone Island in the cluster analysis (Fig. 3) suggests that contributions from particulate-bound PAH affects only a portion of the *N. plumchrus* within Port Valdez. Consistency between the two clustering procedures demonstrates that the results are robust and cluster results are consistent with differences in composition model scores. Observed clusters, model scores, and phytane distributions are consistent with a local source of particulate-bound PAH within Port Valdez.

The distribution of PAH in the samples of *N. plumchrus* from the ballast water discharge station is consistent with a mixture consisting of contributions from the ballast water discharge and the regional pattern characterized by the hydrocarbon pattern evident in the samples from

near Lone Island (Fig. 2a). The distribution pattern of PAH associated with the particulate phase of the ballast water treatment facility effluent is depicted in Fig. 4, which is derived from data from Payne et al. (2005a,b). This latter distribution pattern accounts well for the differences in PAH distributions of *N. plumchus* from Port Valdez in comparison with those from near Lone Island (compare Fig. 2a and 2b). If all of the increase in TPAH observed in *N. plumchrus* samples from Port Valdez were particulate oil from the ballast water discharge facility, the incremental increase would be between 0.2 and 1 :g/g dry weight (Table 6), or about 0.04 and 0.2 :g/g on a wet weight basis. The bioaccumulation factor for particulate-bound PAH accumulated by these suspension-feeding copepods has not been estimated, but it has been estimated for an alkane hydrocarbon (pristane) accumulated by suspension-feeding mussels (*Mytilus trossulus*) at 175,000 (Short 2005). If copepods are equally efficient suspension feeders as the mussels, then a wet-weight concentration of 0.2 :g/g in *N. plumchrus* would imply an equilibrium concentration of ~ 0.001 :g/L in the ambient seawater for TPAH, and concentrations of individual PAH would be considerably lower.

An additional advantage of sampling *N. plumchrus* for monitoring hydrocarbons in ambient seawater is that the samples provide an average integrated over a considerable effective sampling volume, and hence are more representative of the sampled environment than are discretely collected water samples. For example, one 4 L water sample provides information only on that volume at the time of collection. In comparison, population densities on *N. plumchrus* rarely exceed 1 individual per L in PWS (Short 2005), so collection of 500 specimens provides information on ~ 500 L of ambient seawater, integrated over a few days, and hence are much more likely to detect transient exposures. Having collected 15 such samples from within Port Valdez, each containing approximately 500 copepods dispersed within the water column

swept by our plankton net, we suggest our results provide a more representative indication of the status of seawater in Port Valdez than most other approaches. One exception is a study conducted in 2001 with caged mussels (Salazar et al. 2002), which also effectively sample large volumes of seawater, and the authors of that study also concluded that the PAH contamination of the waters of Port Valdez were in the low parts per trillion, with particle-bound PAH an important component. Hence, our study with *N. plumchrus* and the Salazar 2002 study both imply that the hydrocarbon contamination level of Port Valdez possibly attributable to the ballast water discharge of the Alyeska Marine Terminal is, while detectable, remarkably low, especially in light of the volume of oil that passes through the terminal daily.

Potential for Biological damage

Given the low levels of PAH in water, SPM, and *Neocalanus*, the potential for biological damage in the plankton community of Port Valdez is low. PAH concentrations in water and SPM were below detection limits and well below known damaging aqueous hydrocarbon concentrations. For example, 10 to 80 µg/L crude oil dispersions reduced ingestion and egg viability in *Centropages hamatus*, a calanoid copepod, and swimming activity was reduced at 80 µg/L (Cowles 1983; Cowles & Remillard 1983). The diatom *Ceratualina* sp was killed by 40 µg/L water-soluble fractions of diesel oil (Lee et al. 1977). Naphthalene was toxic to other calanoid copepods (*Eurytemora affinis*, *Acartia* sp, and *Pseudocalanus* sp) in the same range, 10 to 75 µg/L (Lee et al. 1978; Ott et al. 1978). In a recent study that specifically targeted the PAH fraction of ANS, Duesterloh et al. (2002) reported morbidity or death of 5 to 10% of *Calanus marshallae* and *Metridia okhotensis* at 2 µg/L TPAH. Higher toxicity is expected in PAH-specific studies because progressively higher molecular weight aromatic compounds are

increasingly toxic (e.g. Moore & Dwyer 1974; Black et al. 1983; Neff 2002) and much of the earlier research focused primarily on monoaromatic compounds. Similarly low aqueous PAH concentrations are detrimental to fish embryos; lowest observed effective concentration ranged from 0.4-18 μg/L (Marty et al. 1997; Carls et al. 1999; Heintz et al. 1999; Heintz et al. 2000). The maximum TPAH concentration in Port Valdez water (0.02 μg/L, without MDL adjustment) was at least 100 times smaller than levels eliciting toxicity in copepods and >17 times smaller than toxic levels in embryological studies.

Increased toxicity resultant from exposure of *Neocalanus* to the ultraviolet portion of sunlight probably does not pose a significant risk to the zooplankton population in Port Valdez. Absorbance of the ultraviolet portion of sunlight can increase toxicity 2 to 100 times or more when small-bodied transparent or translucent organisms with PAH in their tissues are exposed to sunlight (Pelletier et al. 1997; Cleveland et al. 2000). For example, Duesterloh et al. (2002) reported that *C. marshallae* and *M. okhotensis* mortality at 2 μg/L increased from 5 to 10% under shaded conditions to 100% when exposed to sunlight. However, because ultraviolet light is rapidly attenuated by water (99% attenuation in <1 m; Barron et al. 2000), the potential for phototoxicity in *Neocalanus* is likely small. The mixed surface layer extended down to 9 to 29 m in Port Valdez and expected peak *Neocalanus* abundance is about 50 m subsurface (Cooney et al. 2001), thus most of the population is protected from potential interaction of damaging radiation and PAH sequestered in copepod tissue.

Comparison of TPAH body burdens in Port Valdez copepods to known toxic body burdens also suggests low potential for biological damage. Toxic TPAH concentrations in *C. marshallae and M. okhotensis* tissue (2.1×10^4 to 7.1×10^4 ng/g dry weight; Duesterloh et al. 2002) were more than 14 times greater than those in Port Valdez copepods (493 to 1468 ng/g dry

wt). We were unable to locate other studies that relate PAH toxicity in copepods to body burdens. An indirect approach is to apply critical body residue methods, a technique of admittedly limited usefulness because it assumes a common mode of action (narcosis) for all PAH. Experimentally determined critical body residues for non-polar organic compounds range from 2 to 8 μMol/g wet weight (Lotufo 1998). PAH residues in Port Valdez *Neocalanus* ranged from 0.001 to 0.002 μMol/g wet weight, at least 1000 times smaller than the minimum critical body residue estimate. Even though the critical body residue approach does not account for specific molecular mechanisms, and underpredicts toxicity (Barron et al. 2004), a considerable margin of safety is probable.

Biological communities in Port Valdez have been repeatedly studied since inception of the BWTF diffuser system. Proximal effects are sometimes evident but large scale biological impacts are not. BWTF effluent is acutely toxic; crustacean larvae stopped swimming within minutes of exposure (Rice et al. 1981), carbon fixation by algae was reduced (Alexander & Chapman 1980), mortality of pink and chum salmon fry increased and growth decreased (Wolf & Strand 1973; Rice et al. 1981) and Pacific herring embryos developed abnormally (Wolf & Strand 1973). Increased abundance of opportunistic benthic organisms and decreased abundance of fauna sensitive to hydrocarbons was evident in sediment near the diffuser (Blanchard et al. 2002, 2003), an effect apparently limited to <1 km and inconsistent across years. Positive responses likely reflect an increase in access to carbon; BWTF effluent is a major carbon source in Port Valdez and may contribute about 4% of the total dissolved and particulate organic input (Robertson et al. 1980). Many PAH constituents from BWTF effluent are not immediately degraded (Payne et al. 2005a,b); theoretically 90% of these non-degraded PAH may flush from Port Valdez and enter PWS (Robertson et al. 1980). Broadly dispersed petrogenic hydrocarbons

from the effluent are detectable in much of Port Valdez (Payne et al. 2001, 2004); concentrations in passive samplers and caged mussels were highest in the vicinity of the diffuser and part-per-trillion concentrations of petroleum hydrocarbons in water were widely distributed (Salazar et al. 2002). However, broad-scale negative impacts are not evident. Intertidal faunal variation typically is not related to BWTF effluent (Feder & Bryson-Schwafel 1988; Blanchard & Feder 1997, 2000a,b). The phytoplankton community in Port Valdez is likely not affected by effluent (Colonell 1980b). Effluent-associated changes in the subtidal community have not been reported except around the mixing zone (Hood 1973; Colonell 1980c; Shaw & Hameedi 1988; Blanchard & Feder 2002). The consistent pattern is that although BWTF effluent is directly toxic, dilution quickly reduces hydrocarbon concentrations and the potential for biological effects falls rapidly away from the diffuser.

Collectively, aqueous, SPM, and tissue data all suggest that current TPAH levels in Port Valdez are not injurious to the zooplankton community. Concentrations in tissue were >14 times smaller than those known to cause harm to calanoid copepods. *Neocalanus* had to acquire PAH from water and or SPM and TPAH concentrations in these compartments were below MDLs, further suggesting the potential for damage is unlikely. These results are consistent with previous studies. At current rates of discharge into Port Valdez, BWTF effluent likely has little effect on the plankton community and does not pose a significant toxic risk.

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Table 1. Polynuclear aromatic hydrocarbon (PAH) analytes, abbreviations, deuterated surrogate references, molecular mass, and method detection limits (MDL). Deuterated surrogates were naphthalene-d_8 (1), acenaphthene-d_{10} (2), phenanthrene-d_{10} (3), chrysene-d_{12} (4), perylene-d_{12} (5), and benzo[*a*]pyrene-d_{12} (6). Asterisk indicates PAH used for cluster analysis.

PAH	Abbreviation	Surrogate	Molecular mass (g/mole)	MDL (ng)
naphthalene	N0	1	128.2	3.50
* C-1 naphthalenes	N1	1	142.2	1.91
* C-2 naphthalenes	N2	2	156.2	1.15
* C-3 naphthalenes	N3	2	170.3	0.79
* C-4 naphthalenes	N4	2	184.3	0.79
biphenyl	BP	2	154.2	6.82
acenaphthylene	AC	2	152.2	0.70
* acenaphthene	AE	2	154.2	0.59
* fluorene	F0	2	166.2	1.34
* C-1 fluorenes	F1	2	180.3	1.34
* C-2 fluorenes	F2	2	194.3	1.34
C-3 fluorenes	F3	2	208.3	1.34
C-4 fluorenes	F4	2	222.3	1.34
dibenzothiophene	D0	3	184.2	1.23
* C-1 dibenzothiophenes	D1	3	198.3	1.23
* C-2 dibenzothiophenes	D2	3	212.3	1.23
C-3 dibenzothiophenes	D3	3	226.3	1.23
C-4 dibenzothiophenes	D4	3	240.3	1.23
* phenanthrene	P0	3	178.2	1.70
* C-1 phenanthrenes/anthracenes	P1	3	192.3	3.69
* C-2 phenanthrenes/anthracenes	P2	3	206.3	3.69
* C-3 phenanthrenes/anthracenes	P3	3	220.3	3.69
C-4 phenanthrenes/anthracenes	P4	3	234.3	3.69
anthracene	AN	3	178.2	0.50
* fluoranthene	FL	3	202.3	2.00
pyrene	PY	3	202.3	22.63
C-1 fluoranthenes/pyrenes	A1	3	216.3	2.00
C-2 fluoranthenes/pyrenes	A2	3	230.3	2.00
C-3 fluoranthenes/pyrenes	A3	3	244.3	2.00
C-4 fluoranthenes/pyrenes	A4	3	258.3	2.00
benzo(a)anthracene	AA	4	228.3	0.32
chrysene	C0	4	228.3	0.39
C-1 chrysenes	C1	4	242.3	0.39
C-2 chrysenes	C2	4	256.3	0.39
C-3 chrysenes	C3	4	270.4	0.39
C-4 chrysenes	C4	4	284.4	0.39
benzo(b)fluoranthene	BB	6	252.3	0.56
benzo(k)fluoranthene	BK	6	252.3	0.50
Benzo(e)pyrene	BE	6	252.3	0.78
Benzo(a)pyrene	BA	6	252.3	0.65
Perylene	PE	5	252.3	3.40
indeno(1,2,3-cd)pyrene	IC	6	276.3	0.92
dibenzo(a,h)anthracene	DB	6	278.4	0.42
benzo(ghi)perylene	BZ	6	276.3	0.78

Table 2. Alkanes, abbreviations, deuterated surrogate references, molecular mass, and method detection limits (MDL). Deuterated surrogates were n-dodecane-d_{12} (1), n-hexadecane-d_{16} (2), n-eicosane-d_{20} (3), n-tetracosine-d_{24} (4), and ntriacontane-d_{30} (5).

Alkane	Abbreviation	Surrogate	Molecular mass (g/mole)	MDL (ng)
n-decane	C10	1	142.29	73.85
n-undecane	C11	1	156.31	33.64
n-dodecane	C12	1	170.34	62.25
n-tridecane	C13	1	184.37	22.03
n-tetradecane	C14	2	198.40	48.60
n-pentadecane	C15	2	212.42	34.23
n-hexadecane	C16	2	226.45	25.81
n-heptadecane	C17	2	240.48	25.81
Pristane	PR	2	268.53	44.33
n-octadecane	C18	3	254.50	62.86
Phytane	PH	3	282.56	21.57
n-nonadecane	C19	3	268.53	92.03
n-eicosane	C20	3	282.56	52.48
n-heneicosane	C21	3	296.58	17.76
n-docosasne	C22	4	310.61	59.93
n-tricosane	C23	4	324.64	50.09
n-tetracosine	C24	4	338.67	36.18
n-pentacosane	C25	4	352.69	46.07
n-hexacosane	C26	4	366.72	47.53
n-heptacosane	C27	4	380.75	35.08
n-octacosane	C28	5	394.77	61.47
n-nonacosane	C29	5	408.80	80.41
n-triacontane	C30	5	422.83	48.80
n-dotriacontane	C32	5	450.88	142.40
n-tetratriacontane	C34	5	478.94	263.00

Table 3. Deuterated surrogate polynuclear aromatic hydrocarbon (PAH) standards. Listed are concentrations in spike used for water (a), and SPM and tissue (b). Spike volumes were 500 µL. Spike solvent was acetone for water and hexane for SPM and tissue.

(ng/ml)[a]	(ng/ml)[b]	Surrogate
1000	2000	naphthalene d8
1000	2000	acenaphthene d10
800	2000	phenanthrene d10
800	2000	chrysene d12
1000	2000	perylene d12
1000	2000	benzo[a]pyrene d12

Table 4. Species composition and stage in samples retained for hydrocarbon analysis. Percent *Neocalanus plumchrus / N. flemingeri* (of total sample) is expressed by number and by mass; percent *Calanus* is expressed by number only. Percent at stage is estimated for *Neocalanus* only.

	Percent *Neocalanus*		Percent at stage			
Station	(number)	(mass)	C4	C5	Percent *Calanus*	Total number
1. Anderson Bay	79.4	99.3	6.0	94.0	0.0	126*
2. Shoup Bay	97.1	97.1	8.0	92.0	2.9	103
3. Gold Creek	98.0	98.0	2.0	98.0	2.0	101
4. Ballast-water diffuser	97.0	97.0	9.2	90.8	3.0	101
5. Valdez	98.1	98.1	3.0	97.0	1.9	103
6. Lone Island	98.0	98.0	4.1	95.9	2.0	99

*There were 26 non-*Neocalanus* and non-*Calanus* species in this sample, all much smaller than *Neocalanus*.

Table 5. Thermocline depths and temperature breaks measured at water-column and *Neocalanus* sampling stations in Port Valdez and near Lone Island in Prince William Sound, Alaska.

	Anderson Bay	**Shoup Bay**	**Gold Creek**	**BWD**	**Valdez**	**Lone Island**
Date	04/28/04	04/28/04	04/29/04	04/29/04	04/30/04	04/30/04
Thermocline depth (m)	29	9	14-15	21	19	10
Temperature break (^{o}C)	4.0 to 6.0	4.0 to 6.0	4.4 to 6.7	4.1 to 4.9	4.0 to 7.0	4.6 to 7.0
Surface and below thermocline water sampling depths (m)	0-1 29	0-1 9	0-1 15	0-1 22	0-1 20	0-1 11
Total water depth (m)	79	240	240	233	224	740

Table 6. Total PAH concentrations in *N. plumchrus / N. flemingeri* collected from Port Valdez and near Lone Island in Prince William Sound, Alaska. Totals include all PAH above method detection limits (MDLs), ± 95% confidence interval, in :g per g dry weight.

Anderson Bay	Shoup Bay	Gold Creek	BWD	Valdez	Lone Island
0.607 ± 0.248	0.616 ± 0.0516	1.28 ± 0.433	0.731 ± 0.125	1.01 ± 0.0726	0.409 ± 0.431

Table 7. Combined PAH composition petrogenic scores, pyrogenic scores, and means in *Neocalanus* tissue. Scores in bold lettering were significantly different than at the reference site.

a. Petrogenic scores

	Anderson Bay	Shoup Bay	Gold Cr.	Ballast	Valdez	Reference Lone Is.
	4	2	5	4	4	2
	2	2	4	4	4	2
	4	4	4	4	4	0
mean	3.3	2.7	**4.3**	**4.0**	**4.0**	1.3

b. Pyrogenic scores

	0.93	0.51	0.30	0.08	0.16	2.53
	1.18	0.35	0.28	0.28	0.38	2.21
	0.30	0.38	0.25	0.25	0.28	3.36
mean	**0.80**	**0.41**	**0.28**	**0.20**	**0.27**	2.70

Table 8. Pristane and phytane concentrations (µg/g dry weight) and means for *N. plumchrus / N. flemingeri* by station. ND: Not detected.

	Anderson Bay	Shoup Bay	Gold Creek	BWD	Valdez	Lone Island
a. Pristane						
	7190	7020	7650	8920	9960	11300
	6100	8140	9320	9540	7840	11100
	8750	8810	8750	9260	7880	11200
mean	7990	7990	8570	9240	8560	11200
b. Phytane						
	ND	0.324	0.364	ND	0.507	ND
	ND	0.248	0.421	0.315	0.387	ND
	0.369	0.329	0.571	0.332	0.342	ND
mean	0.123	0.300	0.452	0.216	0.412	ND

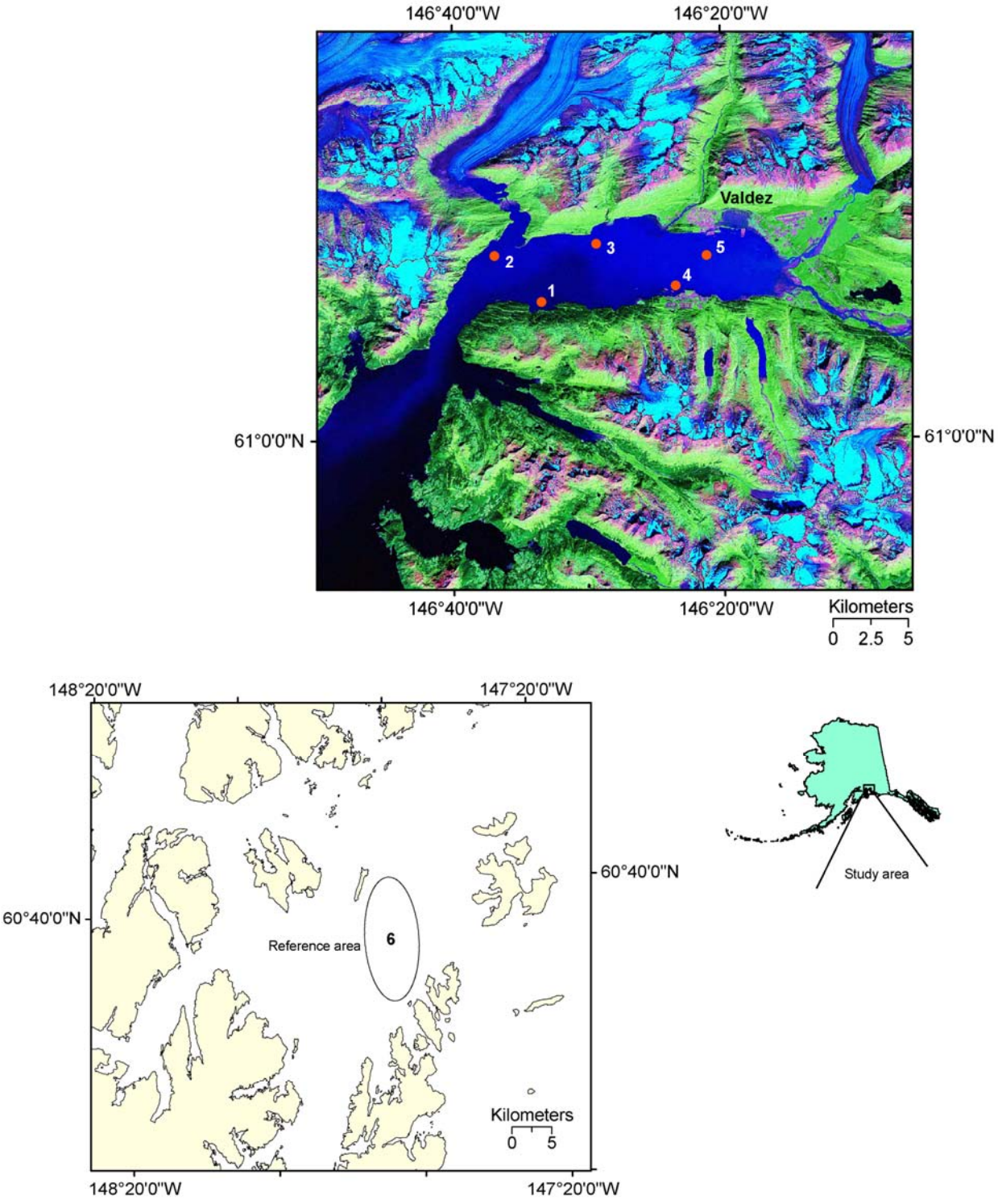

Figure 1. Sample stations in Port Valdez (red circles) and reference area in Prince William Sound (ellipse). Stations were 1) Anderson Bay, 2) Shoup Bay, 3) Gold Creek, 4) ballast-water diffuser, 5) Valdez, and 6) Lone Island. The upper image is a Landsat photograph completed in 2000 or thereafter (NASA).

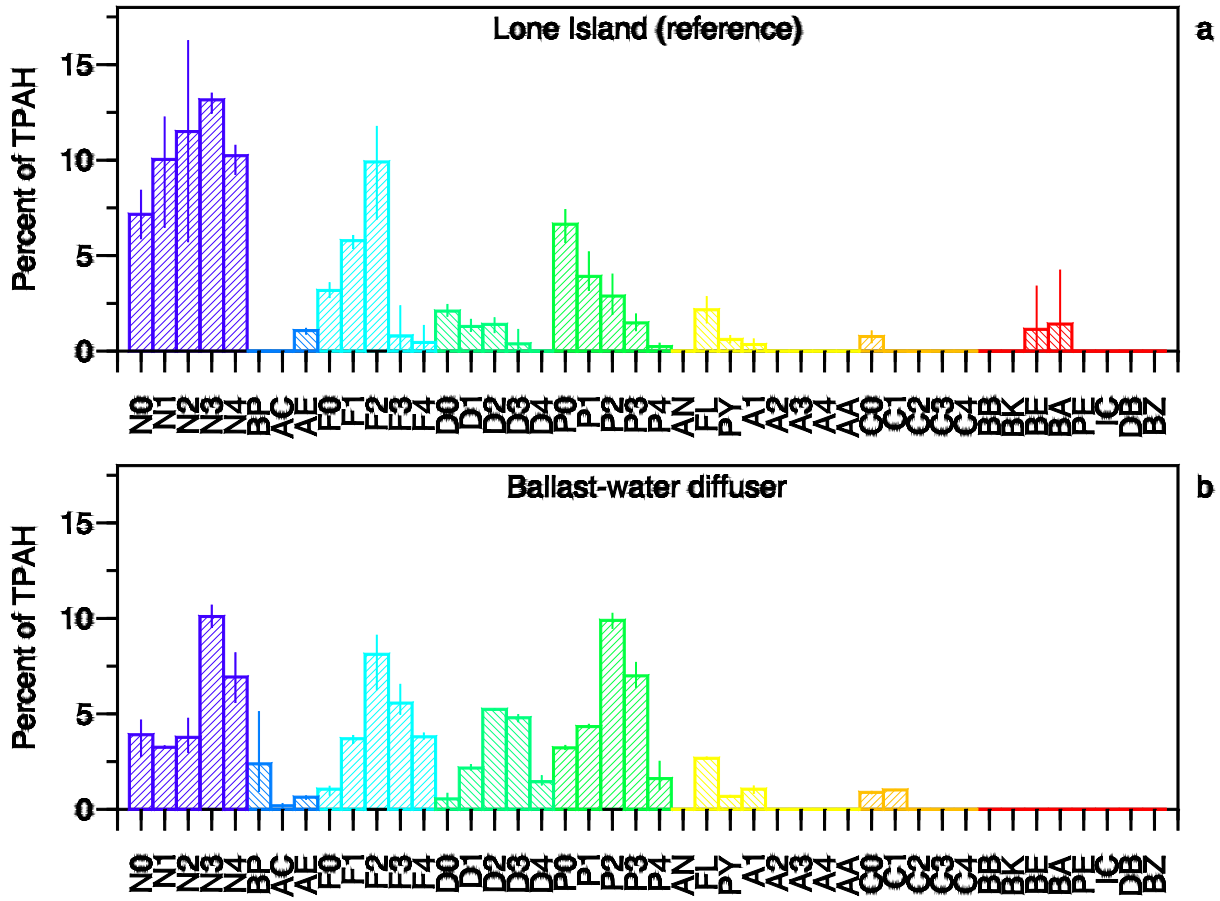

Figure 2. Polycyclic aromatic hydrocarbons in *N. plumchrus* collected near Lone Island in Prince William Sound, Alaska (a), and near the ballast water discharge of the Alyeska Marine Terminal in Port Valdez (b). The bars indicate the proportion of TPAH, and thin vertical lines indicate the range of proportions for the three samples collected. See Table 1 for hydrocarbon abbreviations.

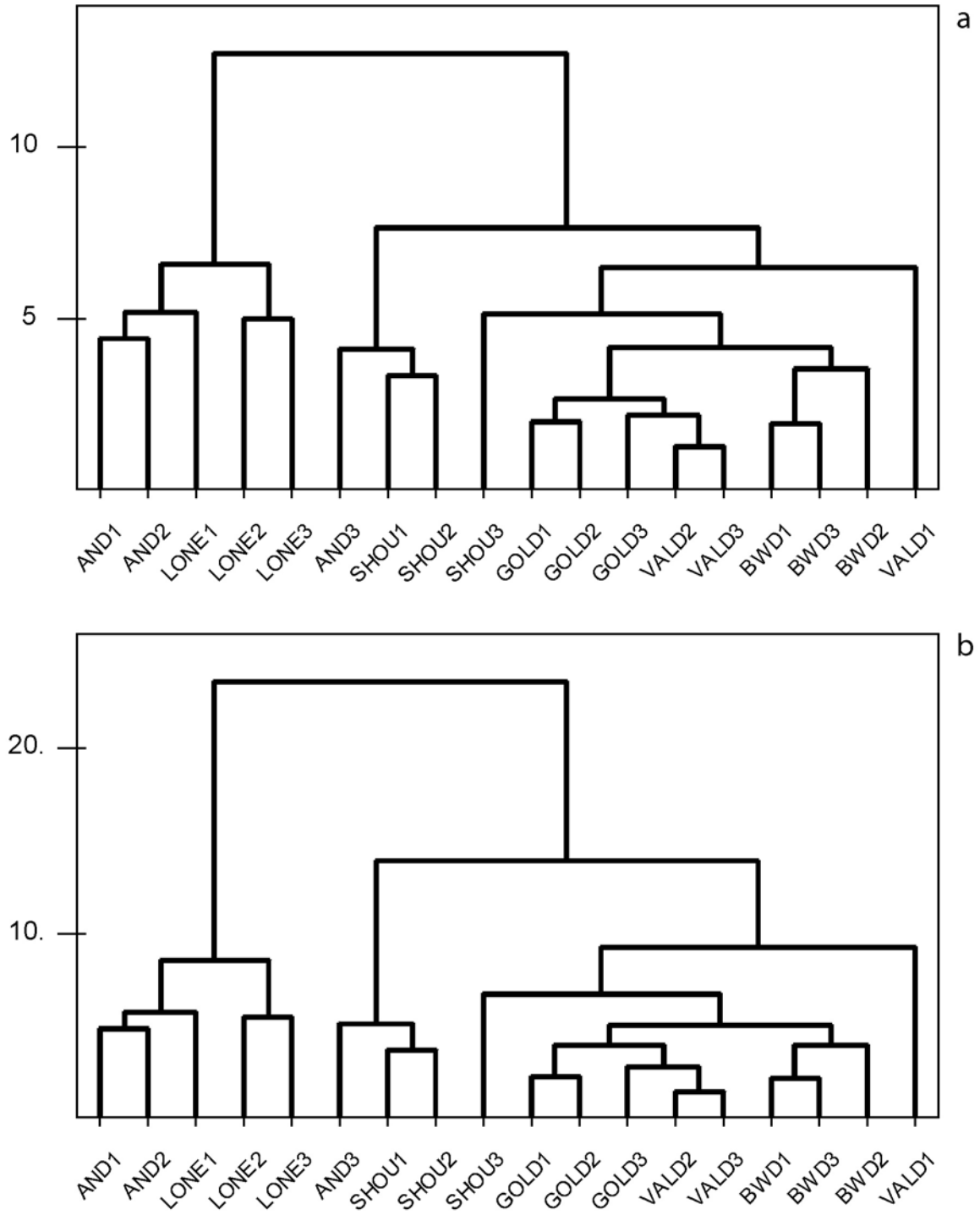

Figure 3. Cluster analysis dendrograms depicting (a) average linkages among patterns of relative PAH abundances in *N. plumchrus* sampled in Port Valdez and near Lone Island in Prince William Sound, Alaska, and (b) complete linkages among patterns. The ordinate is the Aitchison distance metric of pattern differences separating clusters. See Table 1 for PAH used in cluster analyses.

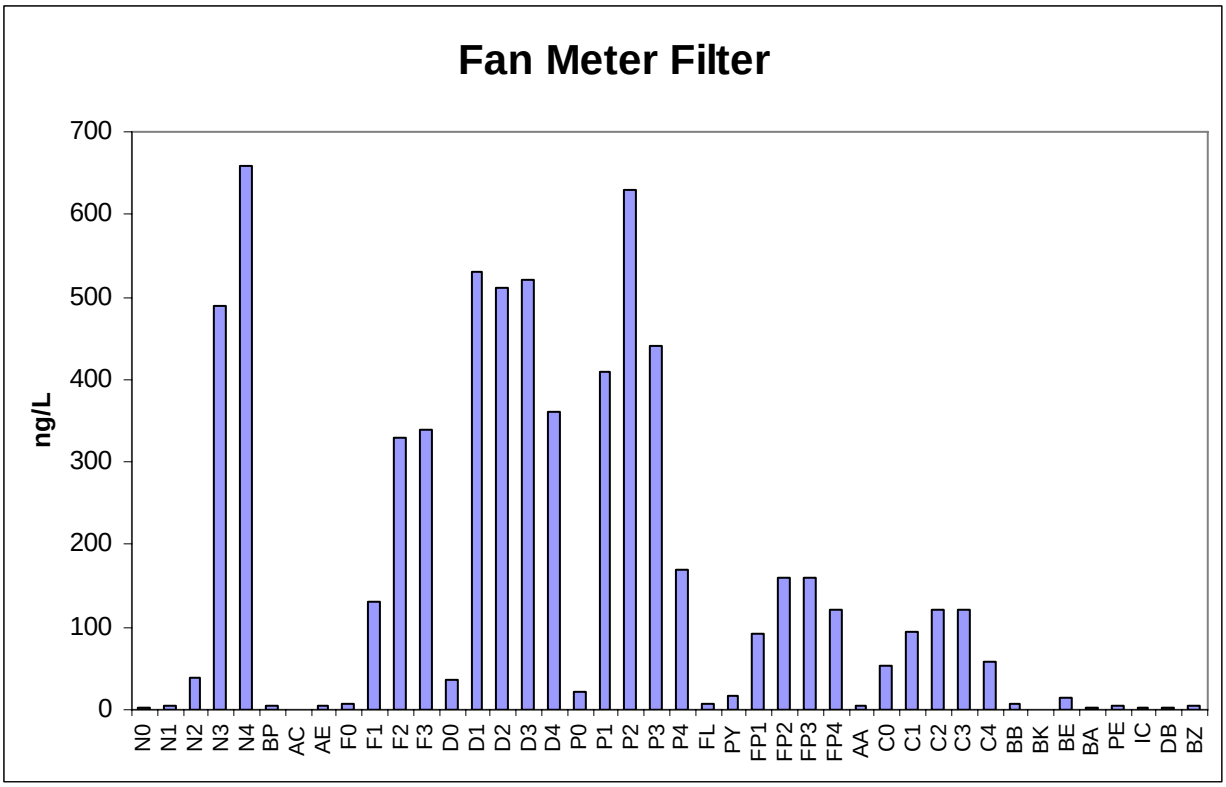

Figure 4. Polycyclic aromatic hydrocarbons in SPM or oil droplet fraction collected from the ballast water discharge facility at the Alyeska Marine Terminal, adapted from Payne et al. (2005a,b). Bars indicate measured concentrations, in ng PAH/L effluent, of PAH associated with particulate material (i.e. whole oil droplets).

Glossary

ANOVA; analysis of variance, an analytical procedure designed to determine if two or more replicated samples are statistically the same or different.

ANS; Alaska North Slope crude oil. The oil spilled by the tanker vessel *Exxon Valdez* was ANS.

BAF; bioaccumulation factor, the ratio of the wet-weight concentration of a contaminant in tissue to the concentration in ambient water.

BWTF; Ballast-Water Treatment Facility, operated by Alyeska to remove oil from ballast water discharged by returning tanker vessels.

Deuterated hydrocarbons are utilized to determine extraction efficiency. Known quantities were intentionally added after sample collection and before processing for hydrocarbons as internal standards. Deuterium is a stable isotope of hydrogen; unlike hydrogen it contains one neutron. The differences in hydrogen and deuterium masses can be discriminated with GC/MS.

FID; flame ionization detector. This technique was used to quantify alkanes emitted from the GC.

GC/MS; gas-liquid chromatography / mass spectroscopy. In gas chromatography, an injected sample is vaporized and transported through a heated glass capillary by inert gas. The column is lined with a thin layer of material and molecules travel through it at different rates, depending on their physical and chemical characteristics. The mass detector discriminates ions emitted from the column by mass. Calibrated PAH are identified by retention time and two characteristic mass fragment ions, and quantified using a 5-point calibration curve.

Kow is the ratio of a chemical in octanol to the same chemical in water at equilibrium. Because the octanol-water partition coefficient is correlated to solubility in water, and because the solubility of organic compounds such as PAH is easier to determine in octanol, this measure is often used as a surrogate for solubility in water.

MDL; method detection limits. Method detection limits were determined experimentally following the procedure given in Section 40 Code of Federal Regulations Part 136, Appendix B.

NIST; National Institute of Standards and Technology, a federal government agency that that works with industry to develop and apply technology, measurements, and standards.

Neocalanus is a genus of large copepods common in Prince William Sound and the Gulf of Alaska.

PAH; see polycyclic aromatic hydrocarbons.

Petrogenic hydrocarbons; petroleum or petroleum-derived material.

N. plumchrus and *N. flemingeri*. Two closely related, mostly sympatric *Neocalanus* species present in PWS and the focus of this study. Most of the C5 copepodites were *N. plumchrus*, although the distribution of cephalosome:prosome ratios suggested that some *N. flemingeri* may

have been present.

Polycyclic aromatic hydrocarbons (PAH). Planar hydrocarbons consisting of two or more benzene rings, such as naphthalenes, fluorenes, and phenanthrenes. The sulfur-bearing heterocyclic dibenzothiophenes are included as PAH in this study. Benzene is a 6-carbon ring with one hydrogen per carbon.

PWS; Prince William Sound, Alaska.

Pyrogenic hydrocarbons. Hydrocarbons derived from pyrolytic sources such as forest fires.

SPM; suspended particulate matter. Matter suspended in seawater and collected by filtration in this study.

SRM; standard reference material, obtained from NIST.

TPAH; total polynuclear aromatic hydrocarbon concentration, the sum of the 44 PAH analyzed in this study after removal of any concentrations below MDL (unless otherwise stated).

Appendix

Polynuclear aromatic hydrocarbon (PAH) and alkane concentrations in water, suspended particulate matter, and *Neocalanus* tissue. All concentration data displayed in this appendix are raw, i.e., they have not been adjusted for method detection limits (MDL).

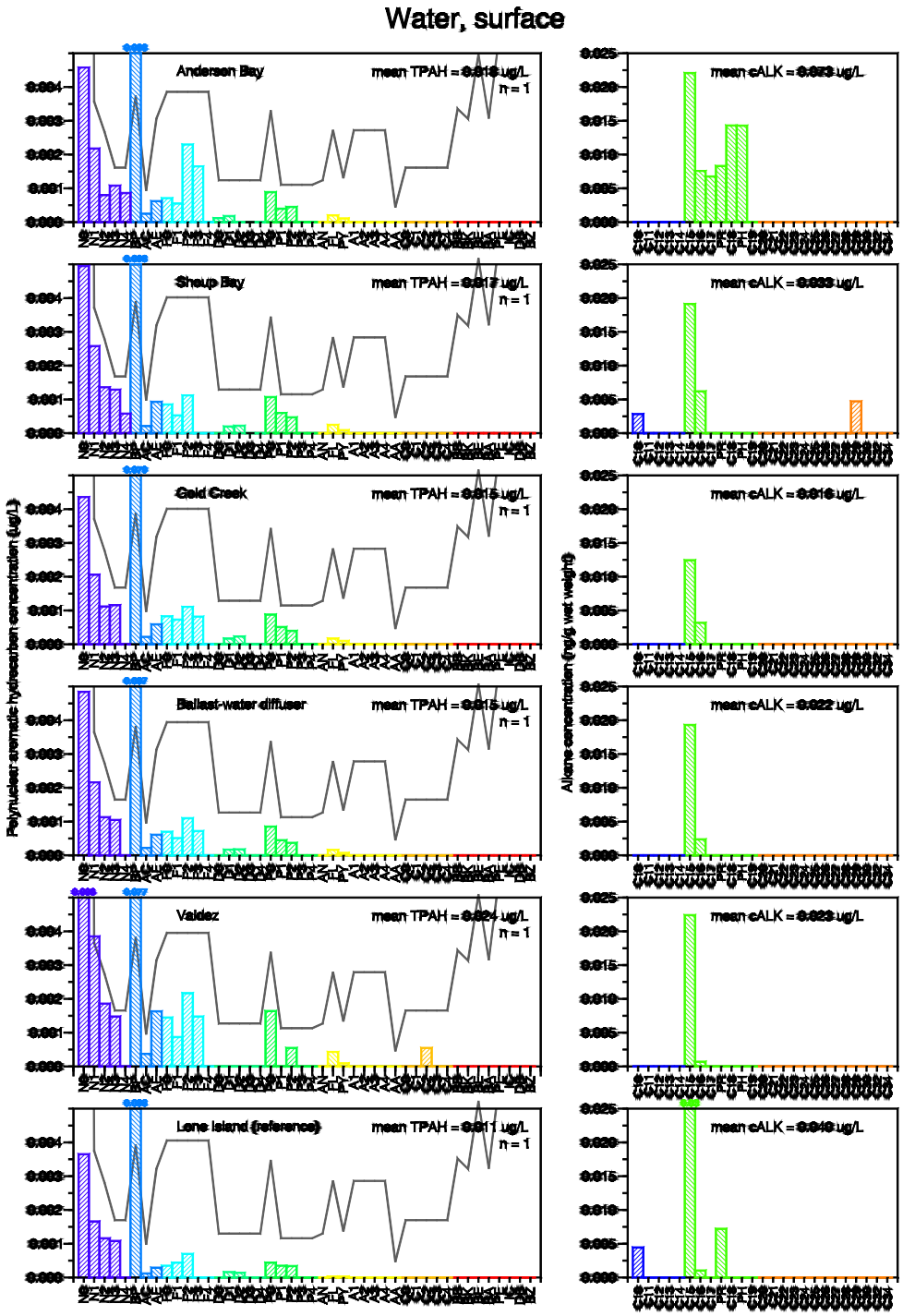

Fig. A1.1. PAH and alkane concentrations in surface water (vertical bars); each bar represents a single observation. Method detection limits are indicated by the gray line for each analyte; bars below this line are below MDL. Biphenyl was the only PAH above MDL in these samples and is an apparent artifact. MDLs have not been estimated for alkanes in water because these compounds are highly insoluble. Analyte abbreviations are explained in Tables 2 and 3.

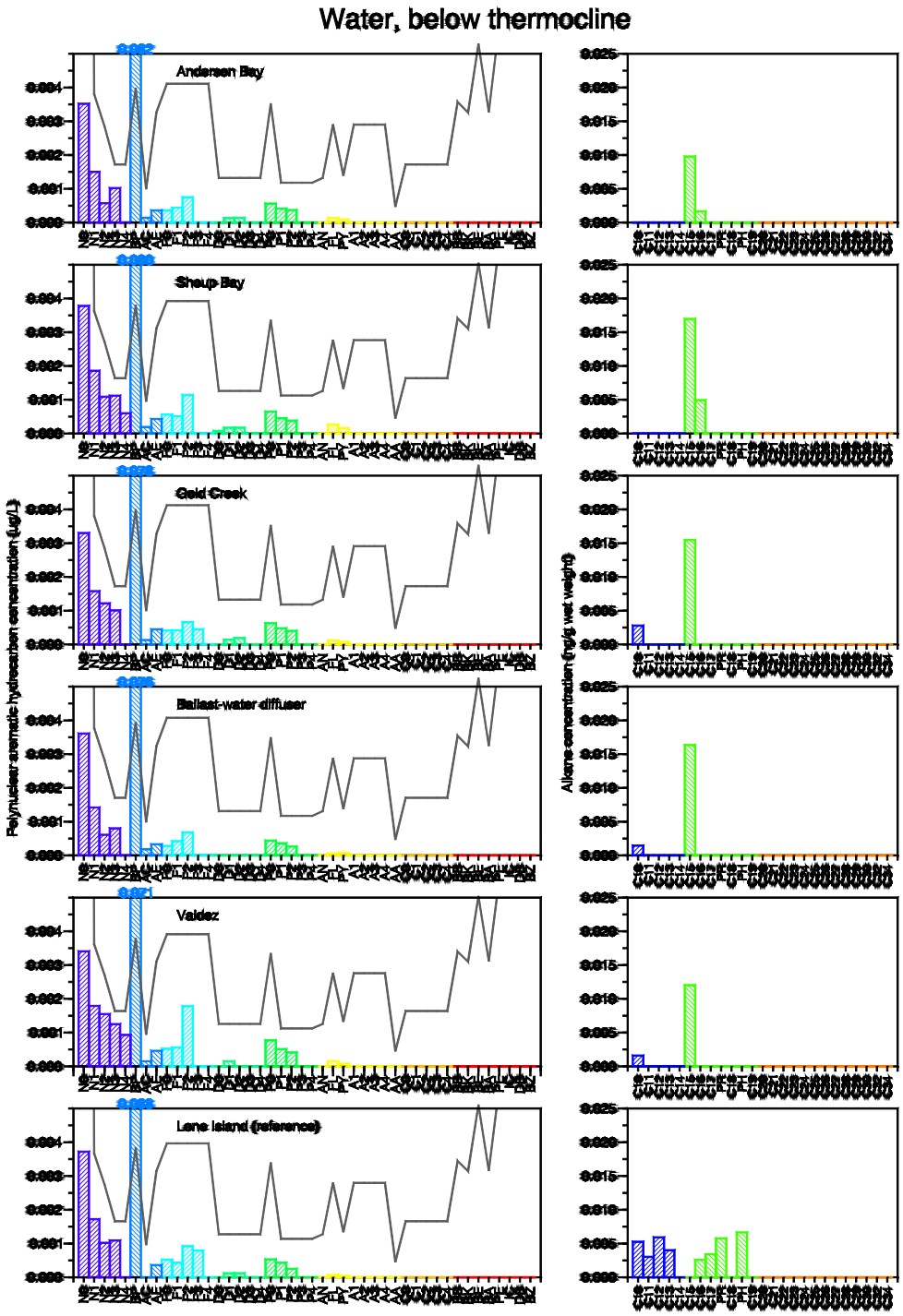

Fig. A1.2. PAH and alkane concentrations in water below the thermocline (vertical bars); each bar represents a single observation. Method detection limits are indicated by the gray line for each analyte; bars below this line are below MDL. Biphenyl was the only PAH above MDL in these samples and is an apparent artifact. MDLs have not been estimated for alkanes in water because these compounds are highly insoluble. Analyte abbreviations are explained in Tables 2 and 3.

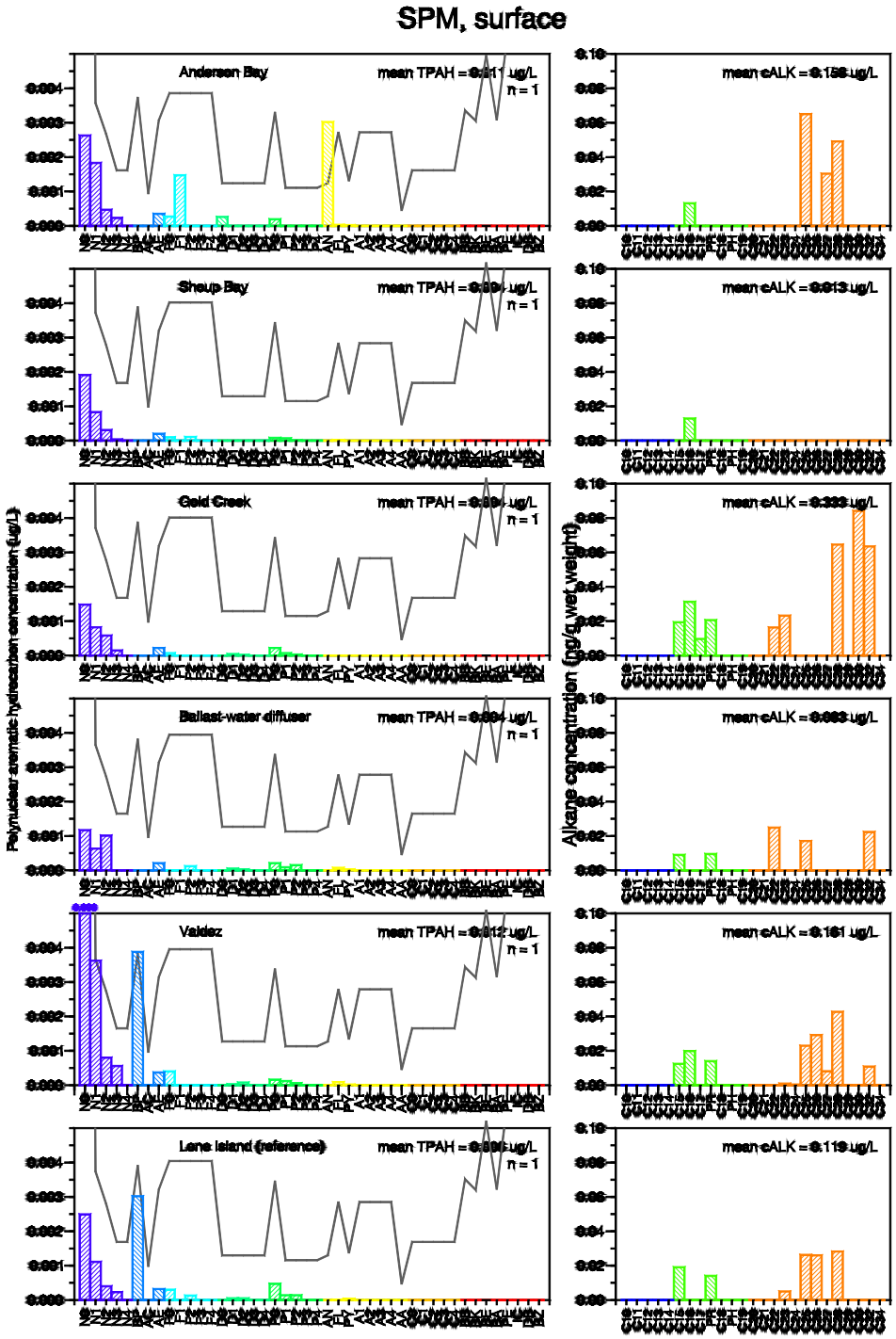

Fig. A1.3. PAH and alkane concentrations in suspended particulate matter at the surface (vertical bars); each bar represents a single observation. Method detection limits are indicated by the gray line for each analyte; bars below this line (all PAH) are below MDL. Because SPM mass was unknown, concentration is expressed as analyte mass (µg) per volume of water filtered (L). MDLs have not been estimated for alkanes in water because these compounds are highly insoluble. Analyte abbreviations are explained in Tables 2 and 3.

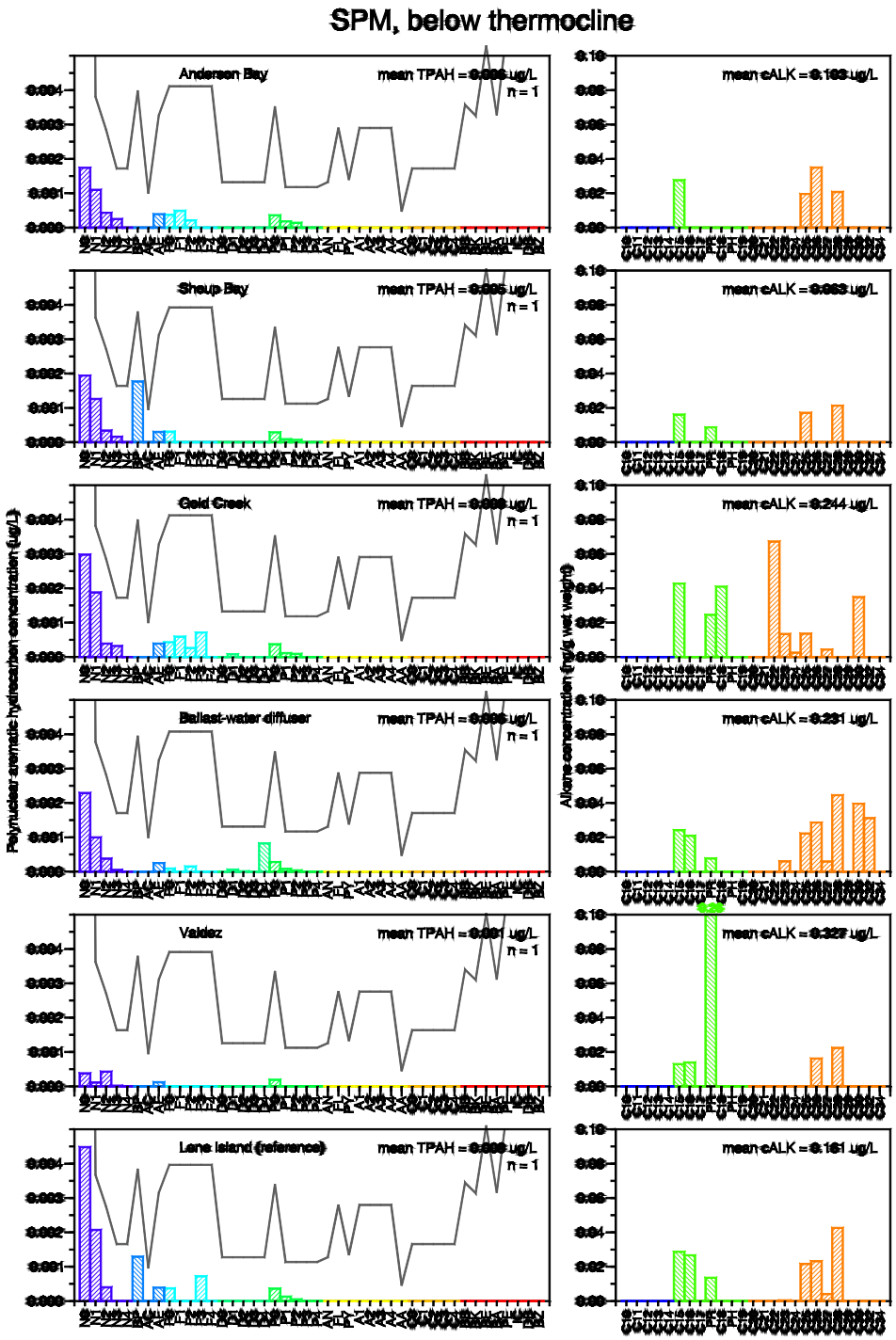

Fig. A1.4. PAH and alkane concentrations in suspended particulate matter below the thermocline (vertical bars); each bar represents a single observation. Method detection limits are indicated by the gray line for each analyte; bars below this line (all PAH) are below MDL. Because SPM mass was unknown, concentration is expressed as analyte mass (µg) per volume of water filtered (L). MDLs have not been estimated for alkanes in water because these compounds are highly insoluble. Analyte abbreviations are explained in Tables 2 and 3.

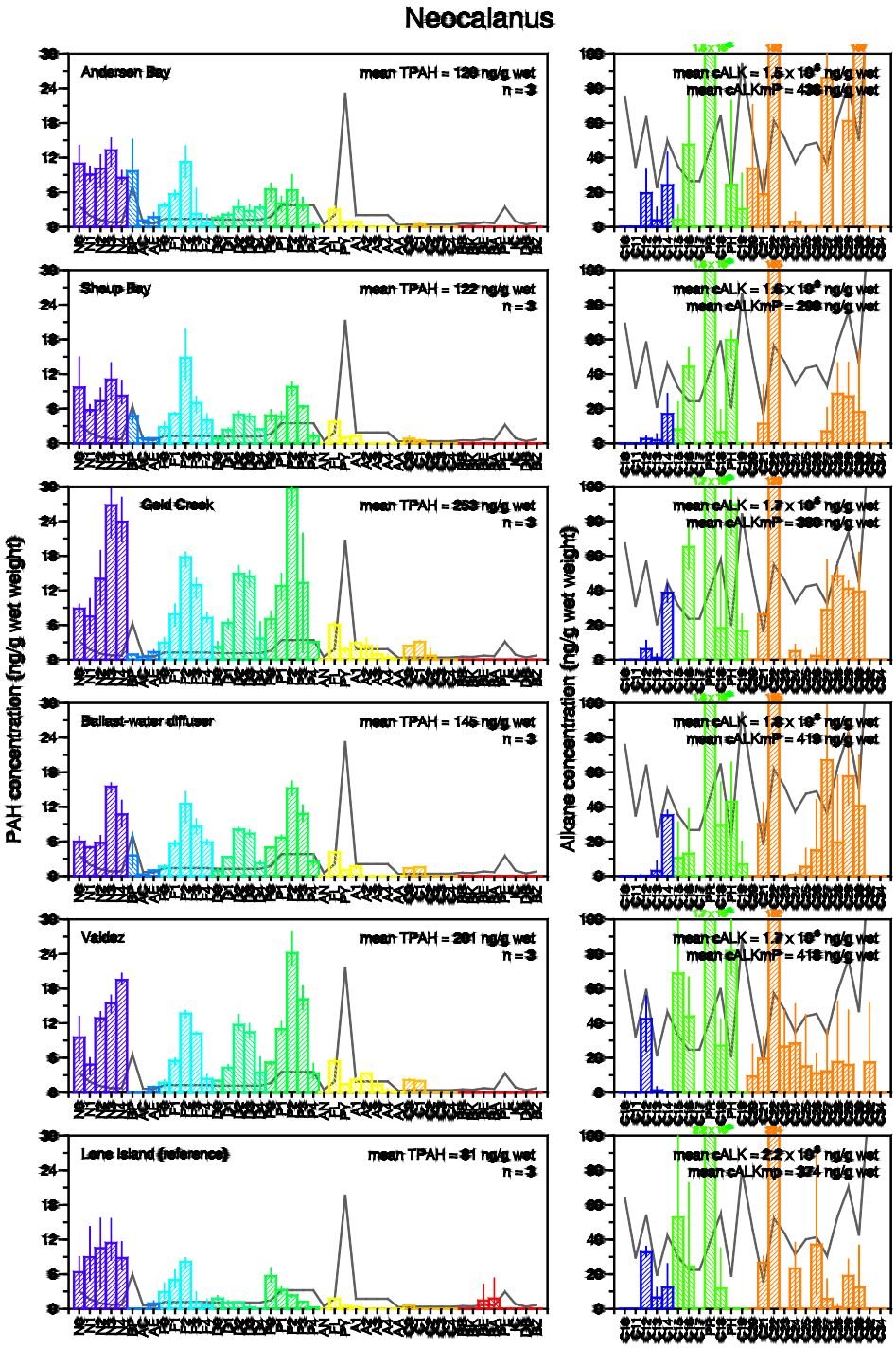

Fig. A1.5. Summary of PAH and alkane concentrations in *Neocalanus* tissue (vertical bars); each bar represents the mean of three samples. Vertical lines indicate sample ranges. Mean method detection limits are indicated by the gray line for each analyte; bars below this line are below MDL. Results filtered by MDL are displayed in Fig. 2. Analyte abbreviations are explained in Tables 2 and 3.