Exxon Valdez Oil Spill
Restoration Project Final Report

Have wild pink salmon and their habitat recovered from persistent *Exxon Valdez* oil contamination?

Restoration Project 00454
Final Report

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Study history: Determination of pink salmon recovery in Prince William Sound a decade after the *Exxon Valdez* oil spill remained problematic and controversial. As a whole, healthy population levels had been evident for some time, yet there were reports of persistent oil contamination in natal pink salmon streams and adverse biological effects at parts per billion oil concentrations. Thus, there was concern that this contamination continued to inhibit the recovery of wild stocks. In this two year project, we 1) determined if oil-contaminated water could be exchanged between stream banks and stream water, 2) examined the natal habitat of pink salmon in Prince William Sound for evidence of oil contamination in eggs and spawning redds a decade after the spill, and 3) studied the relationship between cytochrome P4501A induction in pink salmon embryos and the biological consequences of exposure to oil, such as abnormal development and reduced growth and survival. The long-term effects of exposure to oil during early development were inferred by combining the experimental results with other brood years (1993, 1995, and 1998). A novel passive hydrocarbon sampler method was developed in conjunction with this field research and is described herein. Some of these results have been published in peer-reviewed journals (Chapter 1), some have been accepted for publication (Chapters 2 and 4) and the remaining is in review pending submission for publication (Chapter 3).

Abstract: A decade after the *Exxon Valdez* spill, intertidal pink salmon natal habitat and groundwater movement in Prince William Sound, Alaska, was examined for evidence of lingering oil, the potential for hydrocarbon transfer to developing eggs, and exposure of eggs to oil. The potential consequences of oil exposure during development were further examined using bioassays. Polynuclear aromatic hydrocarbons consistent with *Exxon Valdez* oil were verified in the water of one of six streams; concentrations increased downslope and cytochrome P4501A in eggs was similarly induced. Because only previously heavily oiled streams were sampled, we infer that most pink salmon spawning habitat in Prince William Sound either has recovered or is recovering. Fluorescent tracer dyes injected into beaches near two of these streams during ebb tides were subsequently observed throughout most of the intertidal portion of each watershed, including surface and subsurface stream water, demonstrating the potential for transfer of dissolved oil constituents from surrounding oiled sediment to developing eggs. Exposure of pink salmon embryos from five brood years to dissolved polynuclear aromatic hydrocarbons demonstrated that cytochrome P4501A induction is related to a variety of lethal and sublethal responses, including abnormalities, reduced growth, poorer predator avoidance, and diminished marine survival. Our results not only demonstrate habitat recovery, they also demonstrate why recovery was not immediate and that the spill likely had a lasting negative impact on wild pink salmon populations in intervening years.

Key words: pink salmon, *Oncorhynchus gorbusha, Exxon Valdez* oil, petroleum hydrocarbon, polynuclear aromatic hydrocarbon (PAH), cytochrome P4501A, intertidal groundwater, habitat damage and recovery

Project data: *Description of data* - Hydrocarbon data are available in the State/Federal trustee council hydrocarbon database 1989-2003 (EVTHD). All other data are archived in spreadsheets.

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EXECUTIVE SUMMARY

Recovery of wild pink salmon (*Oncorhynchus gorbuscha*) and their habitat in Prince William Sound remained unclear and controversial a decade after the 1989 *Exxon Valdez* oil spill. As a whole, healthy pink salmon population levels had been evident for some time, yet there were also reports of persistent oil contamination in natal pink salmon streams and adverse biological effects at part-per-billion concentrations. Thus, there was concern that lingering oil contamination continued to inhibit the recovery of wild stocks. This study was designed to address these concerns.

In this two year project, we 1) determined if oil-contaminated water could be exchanged between stream banks and stream water, 2) examined the natal habitat of pink salmon in Prince William Sound for evidence of oil contamination in eggs and spawning redds a decade after the spill, and 3) studied the relationship between cytochrome P4501A induction in pink salmon embryos and the biological consequences of exposure to oil, such as abnormal development and reduced growth and marine survival. The long-term effects of exposure to oil during early development were inferred by combining the experimental results with other brood years (1992, 1993, 1995, and 1998). A novel passive hydrocarbon sampler method was developed in conjunction with the field research and is also described herein (Chapter 4).

Habitat damage

Intertidal sediment surrounding 31% of the pink salmon (*Oncorhynchus gorbuscha*) spawning streams in western Prince William Sound was contaminated in 1989 by the *Exxon Valdez* oil spill (Brannon & Maki 1996; Geiger et al. 1996; Murphy et al. 1999). Intertidal stream reaches are a critical spawning habitat for pink salmon in Prince William Sound because up to 75% of spawned eggs are deposited intertidally (Helle et al. 1964). Oil often penetrated deep into the gravel beaches these streams typically cross, creating the potential for years of chronic contamination (Michel and Hayes 1993). Although flowing freshwater prevented oil deposition directly in stream channels there was evidence that 1) polynuclear aromatic hydrocarbon (PAH) concentrations were elevated in these streams (Brannon and Maki 1996), 2) that cytochrome P4501A enzyme activity was elevated in alevins from oiled streams, suggesting that pre-emergent fish were exposed to oil (Weidmer et al. 1996), and 3) that fewer eggs survived in oiled streams (Bue et al. 1996, 1998; Craig et al. 2002).

At the time of the oil spill, no one realized that pink salmon spawning habitat was damaged, and no effort was made to monitor hydrocarbon concentrations in these streams. The vulnerability of stream habitat was controversial and some researchers argued that oil concentrations in stream sediment were too low to damage pink salmon (Brannon et al. 1995; Brannon and Maki 1996). Only when Bue et al. (1996) continued to find elevated embryo mortality in the 1990s and Marty et al. (1997) reported toxicity at concentrations much lower than previously recognized was attention refocused on spawning habitat (Rice et al. 2001).

Groundwater exchange between intertidal sediment and streams.

Flow of groundwater from surrounding beaches into stream beds (the hyporheic zone, where pink salmon eggs incubate), provides a plausible mechanism for observed PAH contamination (Heintz et al. 1999). *Exxon Valdez* oil penetrated into sediment throughout much of the mid to upper intertidal area surrounding oiled streams (ADFG 1989). These beaches are periodically covered with water due to tidal cycling, bringing water into contact with oiled substrate. PAH from oil-coated rock in contact with water dissolves into water e.g., Short and Heintz (1997). Because intertidal stream channels are eroded below beach surfaces, gravity-

driven groundwater gradients could potentially transport contaminants from surrounding beaches into stream channels.

The objective of this study was to determine if groundwater from intertidal beaches flows into streambed gravel where pink salmon eggs incubate and the mechanism for this movement should it occur. Groundwater movement was determined by release of fluorescent tracer dyes rhodamine and fluorescein, both highly water-soluble), measurement of groundwater elevation as tides ebbed, and by relating changes in groundwater salinity and temperature to tidal cycles. Subsurface samples were collected from a series of wellpoints (vertical pipes with screened ends) installed in beaches and stream channels before dye release.

Fluorimeteric tracer dyes injected into gravel beaches surrounding streams during ebb tides were rapidly observed throughout most of the intertidal portion of each watershed, including water below stream channels. Within half an hour, dyes were observed upwelling from streambed gravel into stream water. Mean horizontal groundwater flow rates were rapid (4-7 m hr^{-1}), as expected for sand and gravel beaches with high hydraulic conductivity, and were driven by hydraulic gradients within beach groundwater. Dyes were consistently detected in wellpoints located in the hyporheic zone and in surrounding beaches. At Junction Creek, where different dyes were simultaneously released on opposite sides, each dye was detected in the beach opposite release within the first tidal ebb (3 h). Dyes remained detectable in subsequent tidal cycles (observations were discontinued after 2 or 3 cycles) and dye was forced vertically uphill by tidal action (0.5 m elevation increase, the upper extent of observations).

Drainage of groundwater from beaches into surface and subsurface stream water provides a mechanism for contaminant exchange, and together with results from previous publications that demonstrate water flowing through oiled gravel accumulates toxic hydrocarbons, explains how pink salmon eggs in Prince William Sound became contaminated by *Exxon Valdez* oil. The presence of PAH in the hyporheic zone allows pink salmon eggs to accumulate PAH, which explains why Weidmer et al. (1996) observed elevated cytochrome P4501A levels in eggs from oiled streams. The stream substrate characteristics selected by spawning adult pink salmon and required for successful incubation of eggs (including high porosity and water exchange) are the same characteristics that permitted contaminated water ready access to these eggs.

Lingering habitat damage and habitat recovery.

In a second study, we examined pink salmon habitat oiled a decade earlier by the *Exxon Valdez* spill for evidence of either continued oil contamination or habitat recovery. Six anadromous pink salmon streams were chosen for study based on their history of observation (Brannon et al. 1995; Bue et al. 1996, 1998; Craig et al. 2002), oil retention (Murphy et al. 1999), and long-term embryo mortality (Craig et al. 2002). Specific objectives were 1) to sample stream water for the presence of residual *Exxon Valdez* oil with low-density polyethylene membrane sampling devices, 2) to collect sediment and pink salmon eggs from the same locations for hydrocarbon analysis, 3) to examine alevins for induction of CYP1A, and 4) to relate contemporary data to site contamination history. Preliminary analyses (hydrocarbon concentrations in passive samplers) indicated four of these worst-case streams were at or near background levels in 1999, thus tissue analyses and examination was restricted to the two cases with evidence of lingering oil. For comparison, additional data were thoroughly examined from a third stream now at background levels. Sampling was partitioned among four elevation zones, the uppermost located above mean high tide. Increased oil concentrations were anticipated at low intertidal elevations because water contaminated by contact with intertidal sediment drains downward. This study, implemented a decade after the *Exxon Valdez* spill, is the first and only effort to directly measure oil in water and embryos from pink salmon streams.

Consistent indications of low-level oil were observed at one of six previously heavily oiled streams and there were weak indications of remaining oil at a second stream. Oil was not detected in the remaining four streams. PAH consistent with *Exxon Valdez* oil were verified in the water of Sleepy Creek; total PAH concentrations were greatest in the lower intertidal. Similarly distributed total PAH concentrations in water at Junction Creek suggested possible contamination. Concentrations of PAH were elevated or slightly elevated in sediment at the two streams with evidence of PAH in water but were too weathered to be positively identified as *Exxon Valdez* oil. *Exxon Valdez* oil was not identifiable in pink salmon eggs in 1999-2000 potentially exposed to residual oil for roughly 1 month before sampling. However, total PAH concentrations in tissue may have been related to intertidal elevation at Sleepy Bay. Induction of CYP1A in alevins from the two streams with oil was lowest in water above mean high tide and increased downslope.

Two major conclusions emerge from this study, one that supports the habitat damage concept and one that indicates most of the habitat is now either recovered or in the process of recovery. Positive identification of *Exxon Valdez* oil in Sleepy Creek water >10 years after the spill is consistent with drainage of hydrocarbons from surrounding oil-contaminated sediment into pink salmon streams and supports the hypothesis that pink salmon embryos in oiled PWS streams were exposed to oil in earlier years (1989-1993 and 1997; Bue et al. 1998; Rice et al. 2001; Craig et al. 2002). However, our results extend previous time series hydrocarbon data in the vicinity of these streams and demonstrate that total PAH concentrations in sediment had declined by ≥ 99% at the two streams with evidence of lingering oil. Because our samples were all selected from streams that were heavily oiled in 1989, we infer that most pink salmon spawning habitat either has recovered or is recovering.

Cytochrome P4501A induction in salmon embryos predicts reduced long-term survival.

Induction of mixed function oxidase enzymes, such as cytochrome P4501A, in fish tissue as a result of exposure to axenic hydrocarbons has been used as evidence of oil exposure (e.g., Wiedmer et al. 1996) and is useful as a biomarker. How CYP1A induction relates to more serious physiological responses was previously unresolved.

Our goal was was to examine the usefulness of CYP1A induction as a predictor of short, intermediate, and long-term biological consequences of embryonic exposure to oil. To accomplish this, pink salmon eggs were exposed to a series of low and rapidly declining oil doses that began immediately after fertilization and ended at fry emergence. Samples were collected periodically to determine CYP1A activity. Survival was assessed at eyeing, emergence, and periodically for the next 5 months. Emergent fry were examined for gross and cellular abnormalities and cultured 5 months for growth measurements. The relationship between long-term marine survival and cytochrome P4501A induction was addressed by combining our results (1999 brood year) with similar experiments in other brood years (1992, 1993, 1995, and 1998).

Embryonic exposure to oil caused a variety of lethal and sublethal effects including reduced short- and intermediate-term (5 month) survival, increased incidence of abnormalities, histological changes, cytochrome P4501A induction, and reduced growth. The lowest effective aqueous concentration of PAHs causing CYP1A induction (<0.94 μg/L) also caused reduced mass. At aqueous total PAH concentrations < 3.7 μg/L, both length and weight were depressed five months after exposure ended. Marine survival was significantly reduced by embryonic exposure of < 5.2 μg/L. Polynuclear aromatic hydrocarbons are oxidized by cytochrome P4501A (Livingstone et al. 1990; Akcha et al. 2000) and the resultant intracellular water-soluble metabolites are often toxic (e.g, Ma 2001). The resultant potential problems include cell damage,

mutagenesis, teratogenesis, genotoxicity, and cancer (e.g., Longwell 1977; Hose et al. 1981; Neff 1985; Schirmer et al. 1998; White et al. 1999).

We conclude that cytochrome P4501A (CYP1A) induction in pink salmon embryos exposed to crude oil is causally linked to adverse effects at cellular, organismal, and population levels in pink salmon and can be used to predict these responses. Observation of induction in early life stages at similar exposure levels implies long-term negative consequences for the individual and the population.

Passive samples allowed detection of low PAH concentrations in stream water.

A sensitive new passive sampler technology was utilized in the study of intertidal stream water in Prince William Sound and allowed detection of oil at concentrations lower than previously possible, but also verified that the majority of pink salmon streams in Prince William Sound have likely fully recovered from oiling. Low-density polyethylene membranes, typically filled with triolein (SPMD), have been previously deployed as passive environmental samplers designed to accumulate nonpolar hydrophobic chemicals from water, sediments, and air. Our experimental low-density polyethylene membrane device (PEMD) is similar except without triolein. Passive sampling devices have excellent detection sensitivity, integrate aqueous hydrocarbon concentrations over time, do not metabolize accumulated hydrocarbons, and are cheaper and easier to analyze than biological tissue.

Our objective was to verify the effectiveness and reliability of simple PEMDs to sample low concentrations of PAH. We 1) compared PAH accumulation (concentration and composition) in PEMDs to that in treatment water and in pink salmon eggs, 2) monitored PAH retention in PEMDs placed in clean water for 40 d, and 3) compared PEMD and SPMD performance in field environments where intermittent low levels of PAH were expected

These experiments demonstrate that PEMDs are reliable passive sampling devices capable of accumulating PAH from water. Concentrations of total PAH accumulated by PEMDs were highly correlated with concentrations in oil-contaminated water ($r^2 \geq 0.99$) and linear over the range tested ($< 17\ \mu g \bullet L^{-1}$). Hydrocarbons were highly concentrated in PEMDs, improving the detection of PAH that were only present at low levels in water (e.g., chrysenes). Composition of PAH accumulated in PEMDs was consistent with source oil and although larger molecular weight PAH were preferentially accumulated and retained in PEMDs, PAH composition varied with that in water, and was highly similar to accumulations in commercially available SPMDs. The PEMDs retained 78% of accumulated total PAH for 40 d in clean water. The PEMD and SPMD samplers deployed in a field setting clearly provided the same information; TPAH concentrations were correlated and differences in relative PAH composition averaged zero. Thus, a simple plastic membrane can be conveniently used for environmental monitoring, particularly in situations where contaminant concentrations are low (in the parts per billion range), variable, and intermittent.

5. Summary

Results of these studies provide evidence that pink salmon spawning habitat in Prince William Sound was contaminated by *Exxon Valdez* oil, corroborate evidence that pink salmon populations were damaged by the spill, and demonstrate that a decade later most spawning habitat either has recovered or is recovering. Drainage of groundwater from beaches into surface and subsurface stream water provides a mechanism for contaminant exchange. Present at low levels were PAH from the *Exxon Valdez* in the water of a minority of streams a decade after the spill. A mechanism for contaminant transport, high PAH toxicity (drawn from a number of

laboratory assays including the one reported here), and evidence of lingering contamination a decade after the spill supports the hypothesis that wild pink salmon embryos were damaged by exposure to oil (Bue et al. 1996, 1998; Rice et al. 2001; Craig et al. 2002). That pink salmon embryos were exposed to oil was evident from cytochrome P4501A induction (Weidmer et al. 1996). Our results suggest that cytochrome P4501A induction predicts population-level consequences; fish exposed to oil as embryos do not grow as rapidly as their cohorts, are more susceptible to predation, and less likely to survive to reproduction. The good news is that pink salmon habitat in Prince William Sound had largely recovered a decade after the spill, but based on reduced embryo survival in 1997 (Bue et al. 1998) and hydrocarbon levels around streams in 1995 (Murphy et al. 1999), recovery may have required the greater part of a decade.

INTRODUCTION

The main body of this report is subdivided into four chapters; each is a complete study. Some of these results have been published in peer-reviewed journals and the remainder have either been submitted or are being prepared for publication. Here we introduce the topics of each chapter; salient findings are presented in the executive summary. Introductions specific to each study, with appropriate references to previous literature, are included with each chapter.

The purpose of the four studies described in this report are 1) to determine if oil-contaminated water could be exchanged between stream banks and stream water, 2) to examine the natal habitat of pink salmon in Prince William Sound for evidence of oil contamination in eggs and spawning redds a decade after the spill, 3) study the relationship between cytochrome P4501A induction in pink salmon embryos and the biological consequences of exposure to oil, such as abnormal development and reduced growth and survival and 4) describe passive hydrocarbon sampling, a novel method developed to sample low hydrocarbon levels in the environment.

OBJECTIVES

This project had four main themes, each with specific objectives. Chapter(s) that address each objective are indicated in parentheses.

1. Hydrological survey of pink salmon natal habitat in western Prince William Sound (Chapter 1).
    a. Map the physical characteristics of each stream surveyed.
    b. Characterize the sediment structure.
    c. Provide evidence of water exchange between sediment in stream banks, stream water, and salmon redds.

2. Examination of pink salmon natal habitat in PWS for persistent *Exxon Valdez* oil (or lack thereof) and evaluation of current contamination of eggs and alevins.
    a.. *Determine how rapidly the incubating environments are recovering*
        1. Measure oil in banks adjacent to bench-mark streams last sampled in 1995 by fast-screening procedures to extend the recovery model past 1995 (Chapter 2).
    b. *Measure the availability of PAHs in the incubating environment*
        1. Measure oil in stream sediment by gas chromatography and mass spectrometry (GC/MS) to verify there is little or no contaminant directly in the stream (Chapter 2).
        2. Measure aqueous oil contamination in salmon redds with buried semipermeable membrane devices (SPMD's) to verify oil transport interstitially to salmon redds (Chapters 2 and 4).
        3. Verify method sensitivities by measuring oil in a stream with a known natural oil seep (Appendix 1).
    c. *Measure exposure of eggs and fry to PAH*
        1. Directly measure oil concentrations in eggs by GC/MS, and compare to concentrations in SPMD's (Chapters 2 and 4).
        2. Inspect eggs for indirect evidence of exposure to oil using the biomarker cytochrome P4501A as an index of exposure and compare to PAH concentrations in eggs and SPMD's (Chapter 2).

2. Examination of the usefulness of the biomarker cytochrome P4501A as a predictor of the biological impacts of oil exposure (Chapter 3).
    a. *Controlled laboratory test with graded oil doses to establish a dose-response curve at part per billion levels*.
    b. *Influence of exposure level on the prevalence of cytochrome P4501A activity and embryo tissue*
        1. Inspect emergent fry for gross and histological lesions.
        2. Determine P4501A induction in organ tissues.
    c. *Initiation of cytochrome P4501A activity in developing pink salmon*
        1. At one dose, measure P4501A response at four developmental stages to determine the onset of induction.
    d. *Relation between cytochrome P4501A activity and short and long-term effects*
        1. Relate P4501A induction to growth of experimental fish cultured at LPW (brood year 99) from the graded series of oil exposures.
        2. Relate P4501A induction to ocean survival (brood year 98) and reproductive fitness of returning adults to parts per billion exposures from the companion pink salmon toxicity study 99476.

3. Synthesis of this project, and long-term impact data from other projects, to redefine pink salmon recovery in PWS, and provide a status of that recovery (Chapter 2).

Fulfillment of Objectives

Chapter 1. Carls, M.G., Thomas, R.E. Rice, S.D. 2003. Mechanism for transport of oil-contaminated water into pink salmon redds. Mar. Ecol. Prog. Ser. 248:245-255.

Chapter 2. Carls, MG, Rice SD, Marty GD , Nydan D. Accepted pending revisions. Pink salmon spawning habitat is recovering a decade after the *Exxon Valdez* oil spill. Trans Am Fish Soc

Chapter 3. Carls MG, Heintz RA, Marty GD, and Rice SD. In review prior to journal submission. Cytochrome P4501A induction in oil-exposed pink salmon embryos predicts reduced survival potential. Mar Ecol Prog Ser.

Chapter 4. Carls, MG, Holland L, Short JW, Heintz RA, Rice SD. In press. Monitoring polynuclear aromatic hydrocarbons in aqueous environments with passive low-density polyethylene membrane devices. Environ. Toxicol. Chem.

Chapter 1
Mechanism for transport of oil-contaminated groundwater into pink salmon redds

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Abstract

Groundwater movement from oil-contaminated intertidal beaches to surface and subsurface water of salmon streams in Prince William Sound, Alaska, was studied to determine if transport of dissolved petroleum hydrocarbons to incubating pink salmon eggs *(Oncorhynchus gorbuscha*) was plausible. Beaches surrounding 31% of the streams in the Sound were extensively oiled in 1989; salmon egg mortality was elevated even though little oil was observed in stream gravel. In 2000, fluorescent tracer dyes injected into two of these beaches during ebb tides were subsequently observed throughout most of the intertidal portion of each watershed, including surface and subsurface (hyporheic) stream water. Mean horizontal groundwater flow was rapid through the porous gravel (4-7 m hr^{-1}) and was driven by hydraulic gradients within beach groundwater. When different dyes were simultaneously released at ebb tide on opposite sides of a stream, each dye was detected in the beach opposite release within the first tidal ebb. Dye was moved vertically upward at least 0.5 m by subsequent incoming tides. Thus, tidal cycles and resultant hydraulic gradients provide a mechanism for groundwater transport of soluble and slightly soluble contaminants (such as oil) from beaches surrounding streams into the hyoprheic zone where pink salmon eggs incubate.

Introduction

The potential vulnerability of pink salmon (*Oncorhynchus gorbuscha*) habitat to pollution was evident after the 1989 *Exxon Valdez* oil spill in Prince William Sound (PWS), Alaska. Extensive areas of shoreline were coated with oil, including beaches surrounding the intertidal reaches of streams used by these fish (Brannon & Maki 1996, Murphy et al. 1999). These intertidal reaches are a critical spawning habitat for pink salmon; up to 75% of spawned eggs are deposited here (Helle et al. 1964, Heard 1991). Furthermore, approximately 31% of salmon streams in PWS were oiled (Geiger et al. 1996). Oil often penetrated deep into the gravel beaches these streams typically cross, creating the potential for years of chronic contamination (Michel and Hayes 1993). Flowing freshwater prevented oil deposition directly in stream channels. Despite this apparent protection of spawning habitat, cytochrome P4501A enzyme activity was elevated in alevins from streams in oiled areas but not in those from reference streams, suggesting that pre-emergent fish were exposed to oil (Weidmer et al. 1996).

The vulnerability of pink salmon stream habitat is controversial. Because flowing freshwater diverted oil from streams, Brannon et al. (1995) and Brannon & Maki (1996) argue that oil concentrations in stream sediment were too low to damage pink salmon. These researchers reported that mean total polycyclic aromatic hydrocarbon (PAH) concentrations in the sediment of oiled streams were low, 0.5-2818 ng g^{-1} (but greater than in non-oiled reference streams, 0.2-64 ng g^{-1}). [Total PAH is defined as the sum of concentrations of 39 compounds ranging from naphthalene through benzo(ghi)perylene (e.g., Short et al. 1996).] Except in the highest tide zone studied (3.7 m), Brannon et al. (1995) failed to detect oil-related embryo mortality, but data were limited to a single year and included fewer eggs and less stream area

than observed by Bue et al. (1996, 1998) and Craig et al. (1999). Studies by the latter authors had much greater statistical power (Rice et al. 2001) and reached opposite conclusions; that embryo mortality in these streams was elevated through 1993 and again in 1997 and that the key difference was oil. However, for oil to be causative, transport of hydrocarbons from heavily oiled sediment surrounding streams into pink salmon redds is necessary.

Flow of groundwater from surrounding beaches into stream beds (the hyporheic zone, where pink salmon eggs incubate), provides a plausible mechanism for the observed contamination (Heintz et al. 1999). *Exxon Valdez* oil penetrated into sediment throughout much of the mid to upper intertidal area surrounding oiled streams (ADFG 1989). These beaches are periodically covered with water due to tidal cycling, bringing water into contact with oiled substrate. PAH from oil-coated rock in contact with water dissolves into water e.g., Short and Heintz (1997), Marty et al. (1997), and Heintz et al 1999. Because intertidal stream channels are eroded below beach surfaces, gravity-driven groundwater gradients lead to transport of potential contaminants from surrounding beaches into stream channels. Periodic pulses of oil-contaminated water could potentially contaminate the lipophylic salmon eggs buried in stream gravel while the gravel (which is not lipophylic) remains relatively uncontaminated. Similar groundwater flow has been demonstrated in creeks in tidal marshes and sandy beaches, with slower, less extensive flow in the less permeable marsh sediment (e.g. Agosta 1985, Harvey et al. 1987, Li et al. 1999). In marshes and sandy beaches, the hydraulic groundwater head corresponds to beach elevation on ebb tides, causing horizontal subsurface flow either seawards or towards stream channels (e.g. Lanyon et al. 1982, Harvey et al. 1987, Nuttle & Hemond 1988). Tidal movement in areas adjacent to rivers not only causes cross-shore fluctuations in groundwater, but also oscillating flows along the shore which can result in movement of contaminants from groundwater to river water (Li et al. 1999).

The objective of this study was to determine if groundwater from intertidal beaches flows into streambed gravel where pink salmon eggs incubate and the mechanism for this movement should it occur. Groundwater movement was determined by release of fluorescent tracer dyes, measurement of groundwater elevation as tides ebbed, and by relating changes in groundwater salinity and temperature to tidal cycles. Sediment texture was characterized in order to relate porosity and permeability to hydraulic conductivity, thus providing a framework for interpretation of groundwater movement and a way to compare study results with those in other environments, such as saltwater marshes.

Study Area

The study was completed in June 2000 at 2 pink salmon streams in western PWS, Sleepy Creek on Latouche Island and Junction Creek on Chenega Island (Figs. 1.1-1.2). Areas surrounding both streams were heavily oiled in 1989 (Gundlach et al. 1990) and studied by Brannon et al. (1995), Bue et al. (1996, 1998), Craig et al. (1999), and Murphy et al. (1999). Evidence of oil persisted in beaches around both streams through at least 1995 (Murphy et al. 1999), and pink salmon egg survival was monitored through 1997 (Bue et al. 1998, Craig et al. 1999). Dye injection wells were intentionally located in previously oiled beach areas and adjacent to reaches of stream previously monitored for egg survival. Recent maps were compared with preceding maps to describe temporal change. Beach sediment was well aerated at both sites; anoxia was never encountered in numerous pits dug to depths of 1 m.

Sleepy Creek

Sleepy Creek extends roughly 3.9 km south to north on the north end of Latouche Island (Fig. 1.1 inset). The stream leaves a bedrock channel just above the high tide line and angles northeast across a gravel beach at the south end of Sleepy Bay where large amounts of *Exxon*

Valdez oil penetrated sediment to depths of 50-125 cm (Hayes & Michel 1998). Asphalt-covered mousse was observed in intertidal sediment within 52 m of the stream at the time of our study (June 2000). Roughly two-thirds of the sediment was greywacke and one-third was shale. Water flow and volume were 1.0 ± 0.1 m s^{-1} and 1.25 m^3 s^{-1} where the stream entered the intertidal area. The mean stream gradient through the intertidal study area at Sleepy Creek was -2.5 ± 0.6 cm m^{-1} (range -4.1 to -0.9 cm m^{-1}). The stream channel was eroded 1.1-2.6 m below the surrounding beach; elevation differences were least in the lower intertidal area.

Junction Creek

Junction Creek extends primarily north for approximately 2.0 km to exit on the northeastern side of Chenega Island into Knight Island passage (Fig. 1.2 inset). The stream valley is fairly broad and Junction Creek passes through a marshy area (roughly 5000 m^2) as it enters a gravel beach. Oiling immediately surrounding Junction Creek ranged from light to heavy in 1989 and was mapped by the Alaska Department of Fish and Game (ADFG), but there was no visible evidence of this oil at the time of our study. Roughly two-thirds of the sediment was shale and one-third was greywacke. Stream flow and volume were 0.5 ± 0.1 m s^{-1} and 0.26 m^3 s^{-1} near the high tide line. The mean stream gradient through the intertidal study area at Junction Creek was -2.1 ± 0.4 cm m^{-1} (range -3.4 to -0.7 cm m^{-1}). The stream channel was eroded 0.9-2.1 m below the surrounding beach; elevation differences were least in the lower intertidal area.

Methods

Wellpoints were installed in beach sediment and stream channels at Sleepy Creek and Junction Creek to trace groundwater movement (Fig. 1.3). Wellpoints, which had a hardened steel tip followed by a 60 cm screened section, were threaded onto variable lengths of 3.2 cm diameter steel pipe. Polyethylene tubing, 1.3 cm in diameter, was threaded through the top to within approximately 8 cm of the bottom of the screened area. Wellpoint water samples were withdrawn from this tube. A second polyethylene tube to sample intermediate depths was extended along the outside of the pipe and ended 0.3 m below the sediment surface (for beach installations only). The end of this tube was packed in gravel to prevent plugging. Before rising tides flooded the wells, the central tubing was removed and the pipes were capped to prevent water entry from the upper end. Tubing was marked to ensure reinstallation in the original pipes at the proper depth.

Wellpoint installation at Sleepy Creek resulted in considerable local sediment disturbance because boulders were frequently encountered, but sediment disturbance was minimized at Junction Creek. Sleepy Creek pits were dug by shovel as deep as practical and wellpoints were hammered in as far as possible beyond the pit bottom. Pits were partially backfilled with original sediment, enough to cover the intermediate depth tube. Digging at Junction Creek was limited to 0.3 m, enough to place the intermediate collection tubes, and wellpoints were hammered to a constant depth of 1.2 m in beaches or 0.9 m in the stream channel. Pits were backfilled with original sediment. Wells uphill from each injection point did not have wellpoints and were installed by digging to a depth of 0.9 m. Rock was packed around the end of these 2 pipes to prevent plugging and the holes were refilled with original substrate.

Sleepy Creek was the first stream sampled, and the experience gained at this site helped us develop a more comprehensive survey at Junction Creek. Most of the wellpoints at Sleepy Creek were clustered within a 5 m radius, except one pipe was placed 33.7 m downstream of the dye injection point (Fig. 1.1). The total area monitored was 130 m^2, including 29 m of stream channel. There were 4 wellpoints in the stream channel, one on the stream margin, and five in the beach on one side of the stream (including the injection well). Liquid rhodamine WT dye,

473 ml, specific gravity 1.03, was poured into the injection well on a falling tide at 14:10 h on June 14, 2000 (Figs. 1.1, 1.3). (Predicted high tide was 2.7 m at 13:12 h for Latouche Island.) The injection well was located 7.9 m from the high tide line and 4.9 m from the stream. Fluorescence, temperature, and salinity sampling began before injection (at 12:37 h on June 14), and continued periodically through 17:00 h on June 15. Wellpoint and intermediate depth samples were collected by peristaltic pump; surface samples were dipped from water adjacent to pipes (when present). Fluorescence samples were placed in 120 ml glass bottles with Teflon lids, immediately placed in the dark, kept cool, and periodically transferred to a support vessel for analysis. Temperature (± 0.1° C) and salinity (± 0.5%) were measured with a handheld meter. Sampling cycled through all available wells; the rate of sampling was controlled by the rate at which samples could be collected (about 3 min per sample × 3 samples per pipe) for 3-7 h periods (Fig. 1.4).

Both sides of the stream were equipped with wellpoints at Junction Creek and an area of 577 m^2 was monitored (Fig. 1.2). Four wells were placed on the south beach (including a fluorescein injection well), plus one on the stream margin. Five additional wells were installed on the north beach (including a rhodamine injection well). Four wells were placed in the stream channel, spanning a distance of 51 m. Both injection wells were installed at the same elevation, 3.3 m above mean lower low water (MLLW) (Figs. 1.2, 1.3). Liquid rhodamine WT and fluorescein dyes, 473 ml each, specific gravity 1.01-1.03, were simultaneously injected on a falling tide at 03:48 h on June 18, 2000, just after the water level fell below these pipes. (Predicted high tide was 3.6 m at 02:28 h for Chenega Island.) The rhodamine injection well was about 14 m from the high tide line, 4.0 m from the stream, and 2.2 m inside the area identified by ADFG as heavily oiled 1989 (Fig. 1.2). The fluorescein injection well was about 6 m from the high tide line, 7.3 m from the stream, and 0.4 m inside the area heavily oiled in 1989. Wells were periodically sampled as described for Sleepy Creek, except sample times were constrained to 2 falling and 1 rising tide cycle, and no surface samples were collected (Fig. 1.4). Analysis of fluorescence samples from Junction Creek was delayed until return to the laboratory, but they were stored in the dark and kept cool to ensure that the dye did not degrade before analysis.

Groundwater elevations during 2 receding tides were determined in 6 open pits in the north beach of Junction Creek (Fig. 1.2). Measurements were completed to the nearest 3 mm from reference marks of known elevation on pipes placed in each pit. Observations were cycled among accessible pits over a 2.3-2.7 h period starting when the tide began to ebb. Elevations at common times were determined by best-fit linear regression modeling; r^2 ranged from 0.988 to > 0.999 for these estimates, except $r^2 = 0.968$ in one pit on June 18. The first measurement in this pit was apparently in error and was excluded from final analysis. Groundwater elevations at common times (for overlapping observations only) were regressed against corresponding beach elevations; best fit models were accepted. (Regression models included x-ladder of powers, y-ladder of powers, exponential and power).

Sediment grain size was characterized along transects placed near the wellpoints. A 36 m transect at Sleepy Creek was extended parallel to the stream and sediment was collected every 3 m from approximately 0.25 × 0.25 m × 0.25 m holes (n = 12). Each location was photographed prior to disturbance; dimensions of the largest rocks were determined from these photos. Masses of boulders too large for the balance to weigh were estimated by water displacement and a field-determined density of 2.78 g cm^{-3}. Sediment was collected every 5 m along two 25 m transects at Junction Creek, located on opposite sides ($n_{total} = 12$). Wet sediment was sieved through 16, 32, 63, and 100 mm mesh, weighed to the nearest 5 g, and discarded. The smallest fraction (< 16 mm) was weighed (wet), collected, dried, passed through 0.125, 0.25, 0.5, 1, 2, 4, and 8 mm mesh, and also weighed to the nearest 5 g. Dry and wet-weight ratios were determined for wet-sieved data and applied to allow combination of the two data sets. Assuming a potential

maximum mesh size of 500 mm (based on rock size measurements), mid-grain size was estimated as described by Buchanan (1984); geometric means were the retransformed means of logarithmically transformed sizes. Porosity was estimated by placing 600 ml unconsolidated sediment subsamples (< 16 mm fraction) in a 1 L graduated cylinder and measuring the volume of water required to fill the interstitial space. Porosity measures were repeated with consolidated sediment; ~100 ml aliquots of sediment were sequentially tamped into the cylinder until 600 ml were present; roughly 25-33% more sediment was present than in unconsolidated tests. *In situ* sediment packing was not measured. Organic content was determined in the < 16 mm fraction by drying samples at 77°C for several days, then combusting at 500°C for 5 h.

Fluorescent analysis

A filter fluorometer (Model 10AU, Turner Designs) was used to determine the presence of rhodamine WT (Fluorescent FWT Red) and fluorescein (Fluorescent FLT, Yellow/Green) in water samples. Excitation and emission wavelengths were 550 ± 10 nm and > 570 nm for rhodamine, and 486 ± 10 nm and 510-700 nm for fluorescein. Samples were centrifuged at 600 g for 3 min to remove suspended material, decanted into 25 × 150 mm test tubes, and equilibrated to 21° C in a water bath before measurement. pH, determined for each sample at the time fluorescence was measured, averaged 7.8 at Sleepy Creek (range 7.1-8.4), and 7.6 (range 6.8-8.6) at Junction Creek. Rhodamine WT, 5% by weight, and fluorescein, 7.5% by weight, dyes were obtained in liquid form from Kingscote Chemicals. Standard solutions, prepared by serial dilution of stock solutions with distilled water and also equilibrated to 21° C, were used for calibration and to determine when quenching could be expected. Background fluorescence was subtracted from sample fluorescence using blank solutions collected intermittently in the field. Water samples were collected in duplicate at each sampling; paired fluorescence values were averaged before further data analysis. Mean coefficient of variation in paired samples with detectable fluorescence was 25%: concentrations > 0.005 ppb were considered detectable. Only a few intensely colored injection well samples required dilution to avoid quenching. Analysis of samples where both dye colors were present did not result in false detections.

Mapping

Streams were mapped to describe the sites and for comparison with post-*Exxon Valdez* spill conditions (ADFG 1989). Primary mapping was by triangulation from fixed points; mapped points were subsequently verified with a global positioning system including ground-based differential correction (20 cm accuracy; July 2000). Additional upstream detail and high tide positions were digitized from an aerial photograph of Junction Creek (May 2000); scaling was determined by overlaying the map on the photograph. A 1989 Junction Creek map (ADFG 1989) was corrected for an apparent 10% north-south optical distortion (also determined by map overlay) and combined with our map (Fig. 1.2). Our Sleepy Creek field map was similarly overlaid on a 1989 map (ADFG 1989), but correspondence between current and past maps is likely less accurate because not all fixed details could be matched exactly.

Vertical elevations of well points, stream stations, and stream cross-sections were determined with a surveyor's level. The elevation of a fixed reference point was determined with respect to known low tide levels, and periodically rechecked to ensure that the level remained calibrated. Five cross-sections were completed at Sleepy Creek, and seven at Junction Creek The elevation of the lowest points in the stream channel were estimated from cross-section profiles.

Reported distances and areas were determined from final map positions. Estimated areas are based on projection to a flat plane and do not account for changes in surface topography.

Stream gradients (change in elevation / change in distance) were estimated by determining the distance between lowest channel points along cross-sections.

Groundwater flow rates

First arrival times of tracer dyes at known distances allowed estimation of groundwater flow rates. First arrival was recorded exactly for a subset of dye plumes emanating from stream gravel at measured distances from appropriate dye injection points. The time of first dye arrival in fixed well points was also determined, but these rate estimates are conservative because first detection of the dye was dependent on sampling frequency and exact arrival times were unknown. Data were first analyzed by 3-way ANOVA (dye color, stream, and vertical placement). Classifications were simplified for final analysis where initial statistical results were clearly not significant ($p > 0.5$; e.g., vertical placement was dropped at Junction Creek).

Hydraulic conductivity (K), was estimated in 2 ways, from grain size analysis and from tracer dye movement (Krumbein and Monk 1942, Millham & Howes 1995, Baird & Horn 1996). Grain size data were used to compute intrinsic permeability, from which K was estimated: $K = 760\ (GM_d)^2\ e^{-1.31\sigma}\ \rho_w\ g\ \upsilon^{-1}$, where GM_d = the geometric mean grain diameter (mm), σ = the standard deviation in phi units [$-\log_2$(diameter in mm)], ρ_w = density of water (0.999849-0.999947 g cm^{-3}), g = acceleration due to gravity (980.6 cm sec^{-2}), and υ = viscosity of water (0.01386-0.01481 g sec^{-1} cm^{-1}). Observation of dye movement allowed *in situ* calculations: $K = v\ n_c\ (dh/dl)^{-1}$, where v = dye velocity (m hr^{-1}), n_c = consolidated porosity (0.32-0.35, dimensionless), and dh/dl = hydraulic gradient (0.03-0.27, dimensionless). Estimates of dh/dl were based on an observed correlation between beach elevation and groundwater elevation.

Results

Drainage area affected by tracer dyes

Fluorescent tracer dye was detected in groundwater within a single tidal cycle throughout most of the area monitored at both streams, including groundwater in banks opposite of dye release (Figs. 1.1-1.2). Dye upwelled from stream beds into stream water within 0.5 h and colored plumes washed downward into the bays. At Sleepy Creek, rhodamine was detected in all wellpoints in place during the first ebb tide (n = 8). At Junction Creek, rhodamine was detected in 8 of 10 wellpoints during the first tidal outflow but fluorescein was detected in just 3 wellpoints. During the first tidal outflow, fluorescein was also detected in 3 of 10 shallow pits on the bank opposite release and 8 of 12 pits on the same side: rhodamine was also detected in both banks at this time (Fig. 1.5). By the time the first tide cycle (ebb plus flood) was complete at Junction Creek, rhodamine had been detected in 9 of 11 wellpoints, and fluorescein in 10 of 11 wellpoints.

Ultimately, single-point dye releases were detected in groundwater throughout most of the area surveyed for dye presence including groundwater in beaches and groundwater below both stream channels (Figs. 1.1-1.2). In the deeper Junction Creek samples, rhodamine was detected over the entire area monitored, 577 m^2, including all 61 m of monitored stream channel. Similarly, fluorescein was detected over a 438 m^2 area, including 57 m of stream channel. Dyes were frequently detected in groundwater on the side opposite of release at Junction Creek, and generally co-occurred below stream channels. At Sleepy Creek, where the area monitored was smaller and constrained to a single bank plus stream bed, dye was detected in groundwater throughout the entire area monitored, 130 m^2, and extended at least halfway under the stream channel (the furthest extent observed). Dye was also detected at all monitored intermediate and surface depths at this site, indicating vertical water movement. At Junction Creek, dye was usually, but not always, found at intermediate depths where that particular dye was detected in

deeper sediment. Dye was transported vertically at least 0.5 m uphill by incoming tides; uphill concentrations were moderately high (9 ppb rhodamine, 22 ppb fluorescein).

Sediment characteristics

Beach sediment surrounding each stream was coarse (Table 1.1). At Sleepy Creek, cobbles and boulders comprised 46 ± 5% of the mass (n = 12). Estimated mid-grain size ranged from 39-375 mm; little sediment was ≤ 0.25 mm (0.3 ± 0.1%). Mean porosity estimates ranged from 32-40% (± 3%); permeability was $1.6 \times 10^{-5} \pm 5.4 \times 10^{-6}$ cm^2. At Junction Creek, the frequency of cobbles and boulders on the south beach was 34 ± 4% (n = 6), but fewer were present on the north beach (9 ± 2%, n = 6). Estimated mid-grain size ranged from 18-108 mm; little sediment was ≤ 0.25 mm (0.6 ± 0.1%). Mean porosity estimates ranged from 35-46% (± 0.7%); permeability was $2.4 \times 10^{-6} \pm 3.5 \times 10^{-7}$ cm^2.

Groundwater movement was rapid

Horizontal groundwater flow, estimated by timing tracer dye movement, was rapid (1.3 to 12.4 m hr^{-1}) through the porous beach substrate (Table 1.1). Dye began upwelling from stream beds into stream water 23-34 min after subsurface injection into beaches at Junction Creek, and 26 min after injection at Sleepy Creek. The mean flow rate at Sleepy Creek, 4.5 ± 1.2 m hr^{-1} (n = 7), was less than at Junction Creek, 7.0 ± 0.7 m hr^{-1} (n = 11, p = 0.054). The type of dye did not influence rate estimates (p = 0.940). Flow rate estimates from wellpoints and shallow groundwater samples at Junction Creek were clearly similar (p = 0.505) and were combined in this analysis. Flow rate estimates based on seepage into the stream channel at Junction Creek were higher, 9.5 ± 0.8 m hr^{-1} (n = 7, p = 0.059).

Estimates of hydraulic conductivity (a measure of the ability of sediment to transmit water) ranged from 0.05-4.5 cm sec^{-1}. Estimates based on permeability (0.05-4.15 cm sec^{-1}) were very similar to estimates based on dye movement and consolidated sediment porosity (0.06-4.5 cm sec^{-1}). These estimates were within the range predicted by Freeze and Cherry (1979) for clean sand and gravel and indicate great capacity for fluid transmission.

Mechanisms

The primary mechanism of dye dispersal was groundwater movement, evident because dye concentrations were consistently greater in groundwater than in paired water samples collected above the sediment. These paired observations were limited to Sleepy Creek, where dye concentrations in surface water averaged 7 ± 6% of concentrations in corresponding wellpoints (range 0-91%, n = 16). Colored surface water flushed into bays where it was diluted and there was no evidence that dye in surface water recontaminated study areas during subsequent flood tides.

Tidal cycles, the ability of sediment to transmit water, and gravity resulted in hydraulic gradients within these intertidal beaches, which in turn was responsible for lateral water movement. Groundwater elevations declined as tides receded, but were correlated with beach elevations ($0.89 \leq r^2 \leq 0.999$), thus creating a hydraulic gradient (Figs. 1.3 and 1.6). The downslope direction of these hydraulic gradients caused groundwater to flow toward stream channels, as previously illustrated by dye movement. Although not quantified, hydraulic gradients were clearly reversed during flood tides, as demonstrated by the upslope transport of fluorescent dye.

Salinity and Temperature

Salinity and temperature in groundwater increased rapidly with incoming tides and declined with outgoing tides, further evidence of groundwater movement and demonstrating the

dynamic between salt and freshwater sources (e.g. Fig. 1.4). Wellpoint salinity ranged from 0.1-27.6 at Sleepy Creek and 0.4-24.9 at Junction Creek, but was sufficiently site-specific that individual patterns were obscured when all data were combined (for each creek), even when they were grouped by closely adjacent wells. Overall salinity ranges in stream water (0.0-27.6 at Sleepy Creek and 0.0-32.6 at Junction Creek) were slightly larger than in wellpoints, but closely overlapped them. Salinity at high tide at Junction Creek was generally lower 1.2 m below the surface (mean 11 , range 4-22) than at 0.3 m (mean 22, range 19-26) ($p < 0.001$), but at Sleepy Creek salinity tended to be higher in the deepest groundwater than in shallow groundwater or surface water.

Discussion

Tidal motion and hydraulic gradients provide a mechanism for groundwater transport of soluble and slightly soluble contaminants (such as oil) in beaches surrounding streams into streambed water and gravel where pink salmon eggs incubate. Fluorimeteric tracer dyes injected into gravel beaches surrounding streams during ebb tides were rapidly observed throughout most of the intertidal portion of each watershed, including water below stream channels. Within half an hour, dyes were observed upwelling from streambed gravel into stream water. Mean horizontal groundwater flow rates were rapid (4-7 m hr^{-1}), as expected for sand and gravel beaches with high hydraulic conductivity, and were driven by hydraulic gradients within beach groundwater. Dyes were consistently detected in wellpoints located in the hyporheic zone and in surrounding beaches. At Junction Creek, where different dyes were simultaneously released on opposite sides, each dye was detected in the beach opposite release within the first tidal ebb (3 h). Dyes remained detectable in subsequent tidal cycles (observations were discontinued after 2 or 3 cycles) and dye was forced vertically uphill by tidal action (0.5 m elevation increase, the upper extent of observations).

Groundwater elevations, measured only at Junction Creek, increased as sediment elevation increased, providing evidence of the hydraulic gradient responsible for lateral subsurface water movement. This observation is consistent with water table observations in intertidal salt marshes (e.g. Agosta 1985, Nuttle & Harvey 1988, Howes & Goehringer 1994), in sandy beaches (e.g. Ericksen 1970, Lanyon et al. 1982), and unconfined coastal aquifers (e.g. Todd 1964). When a beach surface is flooded, infiltration from an essentially unlimited source of water fills the sediment to capacity and the depth-averaged head becomes equal to the elevation of the sediment surface (Nuttle & Hemond 1988). When the surface is exposed, the hydraulic head corresponds to sediment elevation, thus the greatest horizontal water movement occurs where bank slopes are steepest (Harvey et al. 1987, Nuttle & Hemond 1988). Harvey et al. (1987) found that subsurface flow was primarily horizontal, occurred when marsh surfaces were not flooded, and was directed toward creek banks. Our results, both visual and fluorimetric, clearly indicate lateral and downslope movement, but also demonstrate that groundwater can be transported uphill by incoming tides when the direction of the hydraulic gradient is temporarily reversed.

The rapid horizontal water flow at our study sites (4-7 m hr^{-1}) indicates that potentially all groundwater in each intertidal drainage basin might enter the stream during typical tidal cycles. At Sleepy Bay, estimated drainage distance during a typical tide cycle is 48 m at +3 m MLLW (10.8 h mean emergence time × 4.5 m hr^{-1}). Potential drainage distance at low elevations are smaller, e.g. 22 m at +1.6 m MLLW. This suggests that groundwater throughout the entire intertidal Sleepy Creek drainage basin may potentially flow into the stream as tides ebb, an area > 1300 m^2. Similarly, groundwater can potentially drain from the entire intertidal portion of the drainage basin at Junction Creek into the stream channel during a typical tide cycle. The capacity for groundwater drainage in these gravel beaches ($5 \times 10^{-2} \leq K \leq 4 \times 10^{0}$ cm s^{-1}) is greater

than in saltwater marshes where sediment is fine grained and hydraulic conductivity is correspondingly lower ($2 \times 10^{-6} \leq K \leq 7 \times 10^{-3}$ cm s^{-1}) and drainage may be limited to within 4-15 m of creek banks (Agosta 1985, Nuttle & Harvey 1988, Nuttle & Hemond 1988). The estimated hydraulic conductivity range for sandy shorelines, 2×10^{-2} to 9×10^{-2} cm s^{-1} (Lanyon et al. 1982, Millham & Howes 1995), overlaps the lower portion of our observed range.

Implications for eggs of anadromous salmon

Habitat characteristics selected by pink salmon for spawning are the same characteristics that facilitate exposure of eggs to contaminants in surrounding beaches. The porous gravel beach sediment surrounding the two anadromous salmon streams observed in this study typifies the substrate surrounding anadromous salmon streams in Prince William Sound and is the habitat selected by pink salmon for spawning. For successful incubation, pink salmon eggs require good intragravel flow of oxygenated water, thus coarse gravel with little silt in high-flow areas are utilized (Heard 1991). Pink salmon spawning habitat is remarkably similar throughout western Prince William Sound and hydraulic conductivity is undoubtedly similarly high throughout this habitat. The coarse nature of the spawning substrate, placement of eggs in gravel below stream channels (20-25 cm depths are typical, Helle et al. 1964, Heard 1991), and the high frequency of intertidal spawning place these eggs at risk of exposure to any contaminants that might be contained in surrounding beaches.

The flow of groundwater containing PAH dissolved from oiled intertidal beaches surrounding anadromous streams into the hyporheic zone explains how pink salmon eggs became contaminated after the *Exxon Valdez* oil spill. Although flowing freshwater did not allow oil to directly contaminate stream channels, shorelines surrounding both study streams and many others in western PWS were coated with oil (Brannon & Maki 1996, Bue et al. 1996, 1998, Geiger et al. 1996, Craig et al. 1999, Murphy et al. 1999). Aliphatic and aromatic hydrocarbons dissolve into water surrounding oil-coated rock at rates related to the energy required for escape (Short & Heintz 1997), and the resultant contamination is sufficient to cause toxicity (Marty et al. 1997, Heintz et al. 1999, 2000). Dissolved PAH are most likely responsible for this toxicity, which increases with molecular weight (Rice et al. 1977, Neff 1979, Black et al. 1983, Heintz et al. 1999, 2000). Murphy et al. (1999) detected high molecular weight PAH in sediment surrounding streams in PWS and concluded that concentrations at some streams were likely toxic through 1993. For example, mean total PAH concentrations in intertidal sediments adjacent to seven oiled spawning streams in 1989 ranged from 13-823 $\mu g\ g^{-1}$ (Murphy et al. 1999), greater than concentrations known to damage pink salmon embryos (3.8 $\mu g\ g^{-1}$, Heintz et al. 1999). [Of primary toxic concern are naphthalenes through chrysenes, which dissolve into water in contact with oiled sediment. Larger molecular weight PAH are rare or absent in Alaska North Slope crude oil (e.g., Short et al. 1996) and monoaromatics generally evaporated before shoreline oiling (Wolfe et al. 1994).] The presence of PAH in the hyporheic zone allows pink salmon eggs to accumulate PAH, which explains why Weidmer et al. (1996) observed elevated cytochrome P4501A levels in eggs from oiled streams. Because these eggs are lipophilic, they accumulate much higher hydrocarbon concentrations than are present in the surrounding water (e.g. Heintz et al. 2000). In contrast, stream gravel, which is not lipophilic, is not likely to accumulate dissolved hydrocarbons and this explains why Brannon & Maki (1996) found relatively few contaminant hydrocarbons in gravel from these streams. Reduced embryo survival resulted from exposure through 1993 (Bue et al. 1996, 1998). Mortality between oiled and reference streams did not differ significantly from 1994-1996, but survival was lower again in 1997 (Craig et al. 1999). Possibly shifts in stream courses increased the flow of dissolved hydrocarbons into stream water; ongoing studies in 2000-2001 have continued to find persistent oil at many sites within PWS

(Rice et al. 2001) and *Exxon Valdez* oil was detected in the intertidal hyporheic zone at Sleepy Creek in 1999 (unpublished data).

Shifts in intertidal stream channels are not unusual and increase the potential for transport of contaminants into stream water and stream beds. Michel and Hayes (1993) report that Sleepy Creek constantly shifts position: estimates from their maps suggest 1-11 m annual movement is possible in the vicinity of our study (Michel and Hayes 1994). An anchor installed at +2 m MLLW in Sleepy Creek in the fall of 1999 could not be located in June 2000, apparently because the stream had shifted approximately 4 m and deposited a thick layer of gravel over it. Changes at Junction Creek offered more definitive evidence of short-term channel shifts; anchors placed at +2 m MLLW in the stream center in August 1999 were on the bank in June 2000, indicating that the stream channel had moved approximately 3-4 m. A longer-term change in the Junction Creek channel position is evident by comparing the 1989 position mapped by ADFG with the 2000 position (Fig. 1.2). Large-scale stream shifts may increase the potential for contaminant transport by functionally increasing the area of the drainage basin. At smaller scales, reworking of contaminated sediment may bring such material in direct contact with the stream channel and mechanical movement may expose fresh surfaces on oil-coated material, accelerating the weathering process and increasing downstream and hyporheic contaminant concentrations.

Conclusions

Groundwater in intertidal gravel beaches surrounding streams drains into stream water and the hyporheic zone below streams during ebb tides. Direct evidence that groundwater from intertidal beaches enters stream water and the hyporheic zone was provided by tracer dyes. Secondary evidence of groundwater water movement included 1) lack of anoxia in beach sediment, 2) the cyclic presence and absence of subsurface water, and 3) cyclic changes in temperature and salinity of groundwater corresponding to tidal cycles. Lateral groundwater flow, driven by hydraulic gradients related to watershed topography and tidal cycles, may extend throughout the entire intertidal portion of drainage basins during each ebb tide because hydraulic conductivity is high in gravel beaches, hence flow rates are rapid. Drainage of groundwater from beaches into surface and subsurface stream water provides a mechanism for contaminant exchange, and together with results from previous publications that demonstrate water flowing through oiled gravel accumulates toxic hydrocarbons, explains how pink salmon eggs in Prince William Sound became contaminated by *Exxon Valdez* oil. The stream substrate characteristics selected by spawning adult pink salmon and required for successful incubation of eggs (including high porosity and water exchange) are the same characteristics that permitted contaminated water ready access to these eggs.

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Table 1.1. Sediment characteristics.

	Sleepy Creek	Junction Creek
organic content (%)	1.5 ± 0.1	0.7 ± 0.1
porosity		
unconsolidated (%)	40 ± 0.3	46 ± 0.7
consolidated (%)	32 ± 0.3	35 ± 0.3
permeability (cm^2)	1.6×10^{-5}	2.4×10^{-6}
geometric mean grain size (mm)	57	25
cobbles and boulders (≥63 mm) (%)	46 ± 5	22 ± 4
fine sediment (≤0.25 mm) (%)	0.3 ± 0.1	0.6 ± 0.1
hydraulic conductivity (cm sec^{-1})		
in situ estimate	0.4 ± 0.3	1.6 ± 0.4
estimated from permeability	1.1 ± 0.4	0.2 ± 0.02

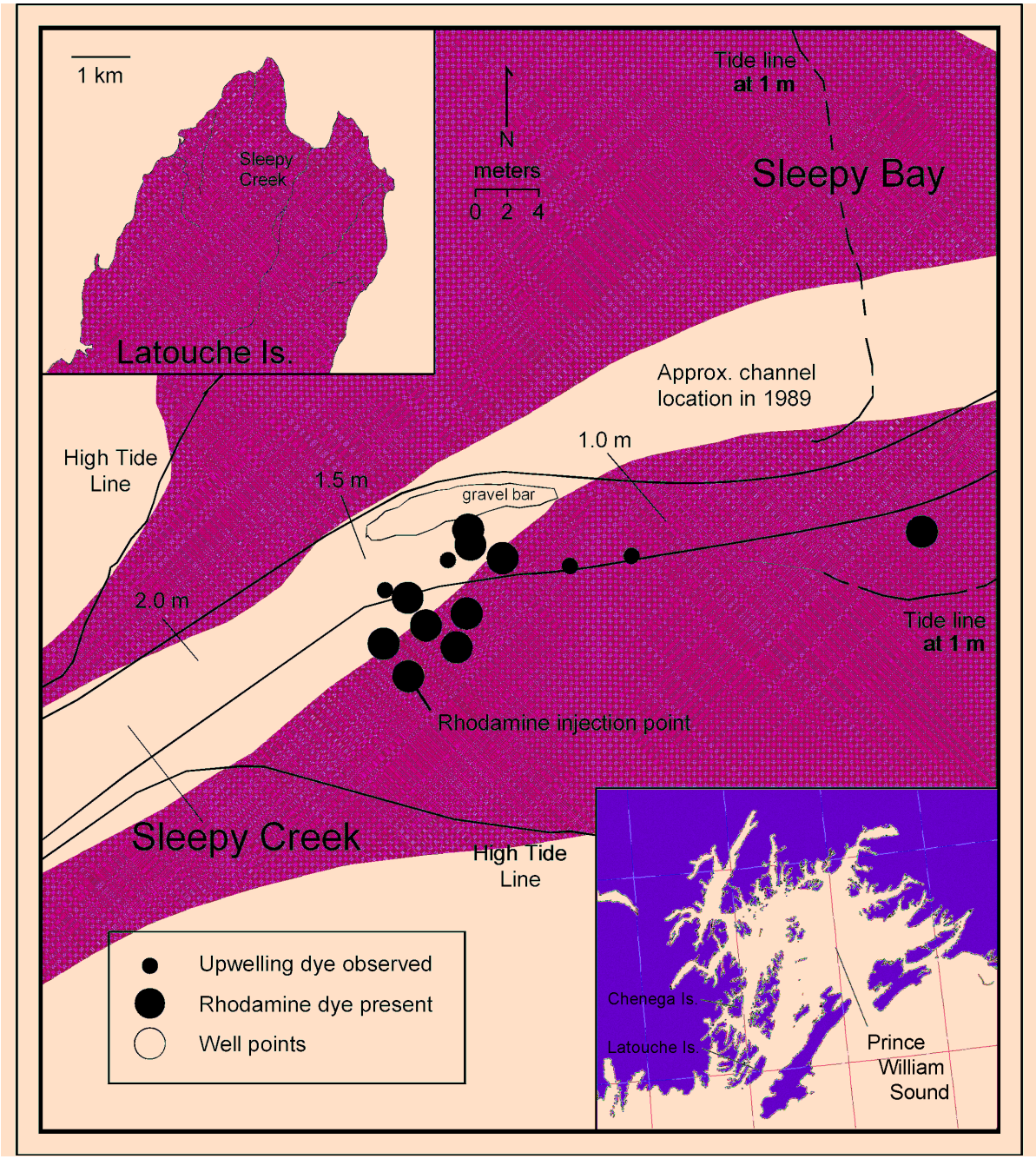

Fig. 1.1. Sleepy Creek, Latouche Island, Prince William Sound, Alaska: tracer dye detection area, wellpoint installations, and dye injection point. Large solid circles summarize dye detection across all observation times. Small dots indicate locations where dye upwelled from sediment into stream water (not all such observations were mapped). Vertical stream bed elevations are indicated (meters above mean lower low water). Shaded areas approximate oil distribution in 1989 (ADFG 1989).

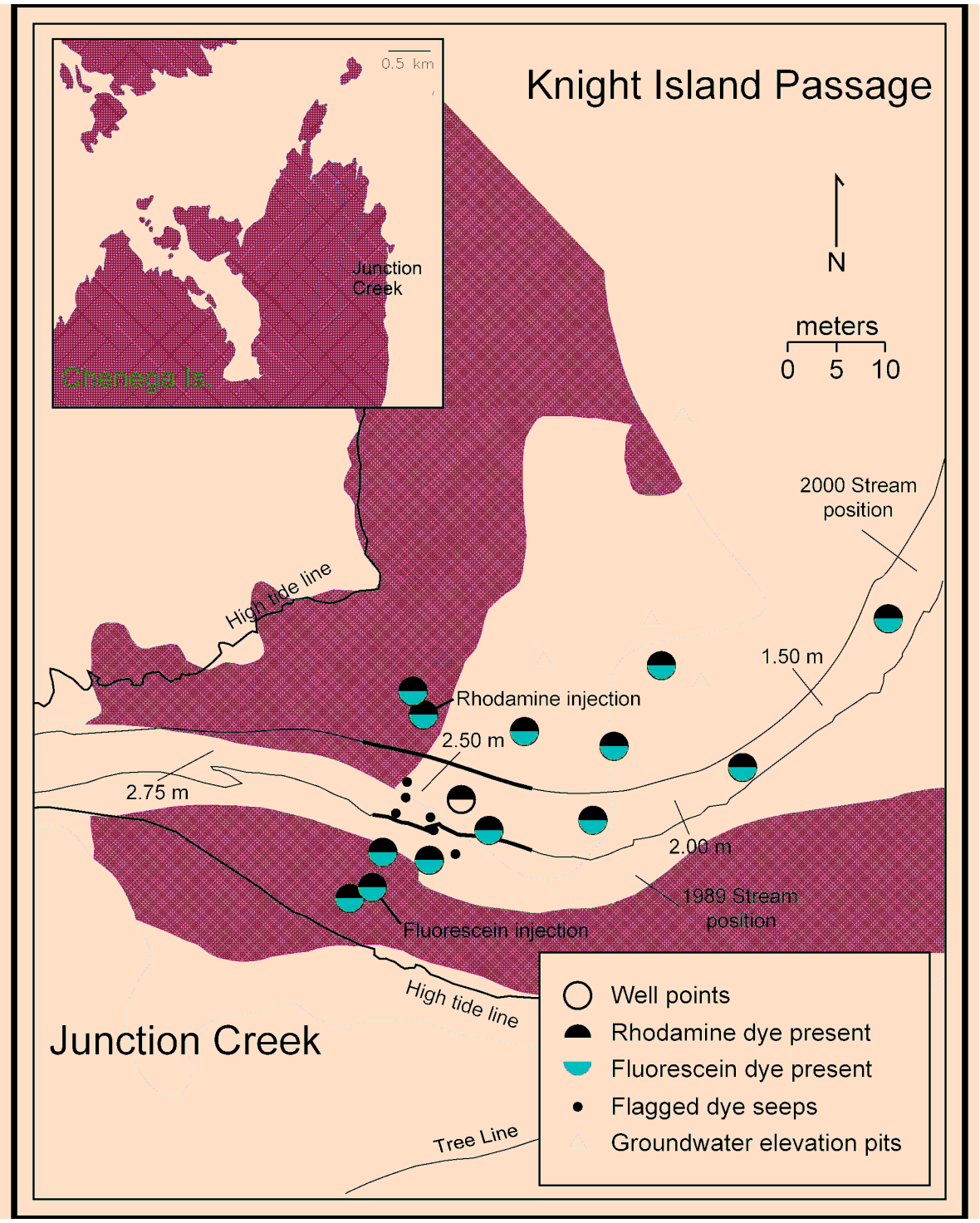

Fig. 1.2. Junction Creek, Chenega Island, Prince William Sound, Alaska: tracer dye detection area, wellpoint installations, dye injection points, and groundwater elevation pits. Rhodamine and fluorescein presence is summarized across all observation times. Small dots indicate locations where dye was observed upwelling from sediment into stream water (a minority of such observations were mapped). Vertical stream bed elevations are indicated (meters above mean lower low water). Shaded areas approximate oil distribution in 1989 (ADFG 1989).

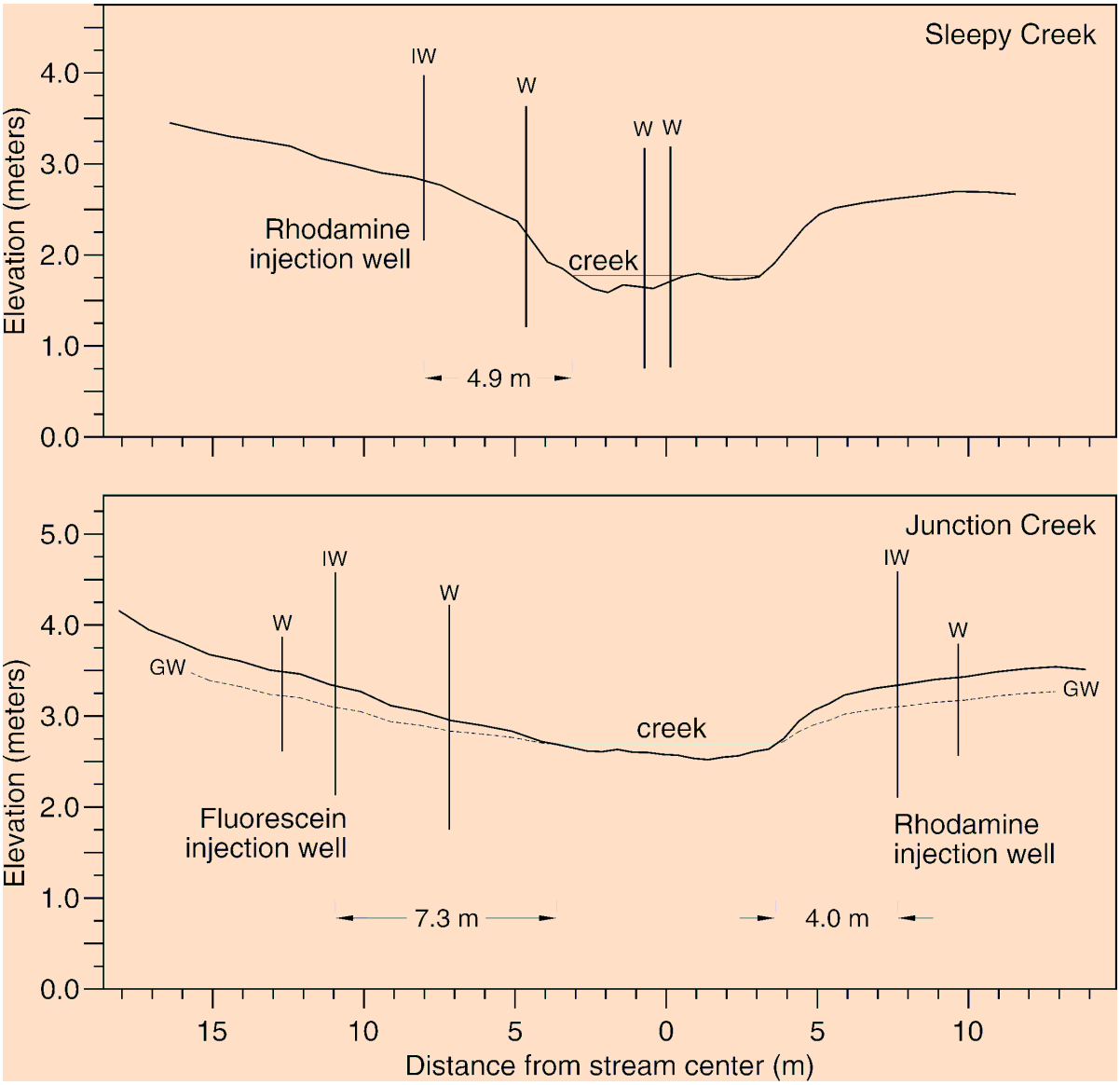

Fig. 1.3. Example cross-section profiles at Sleepy Creek (on Latouche Island) and Junction Creek (on Chenega Island), including injection wells (IW) and sample wells (W) (facing upstream). Distances from injection wells to stream margins are noted. An estimation of groundwater elevation (GW) is included for Junction Creek, calculated using equations relating beach surface and groundwater elevations (see text). Depicted stream surface elevations are at ebb tide.

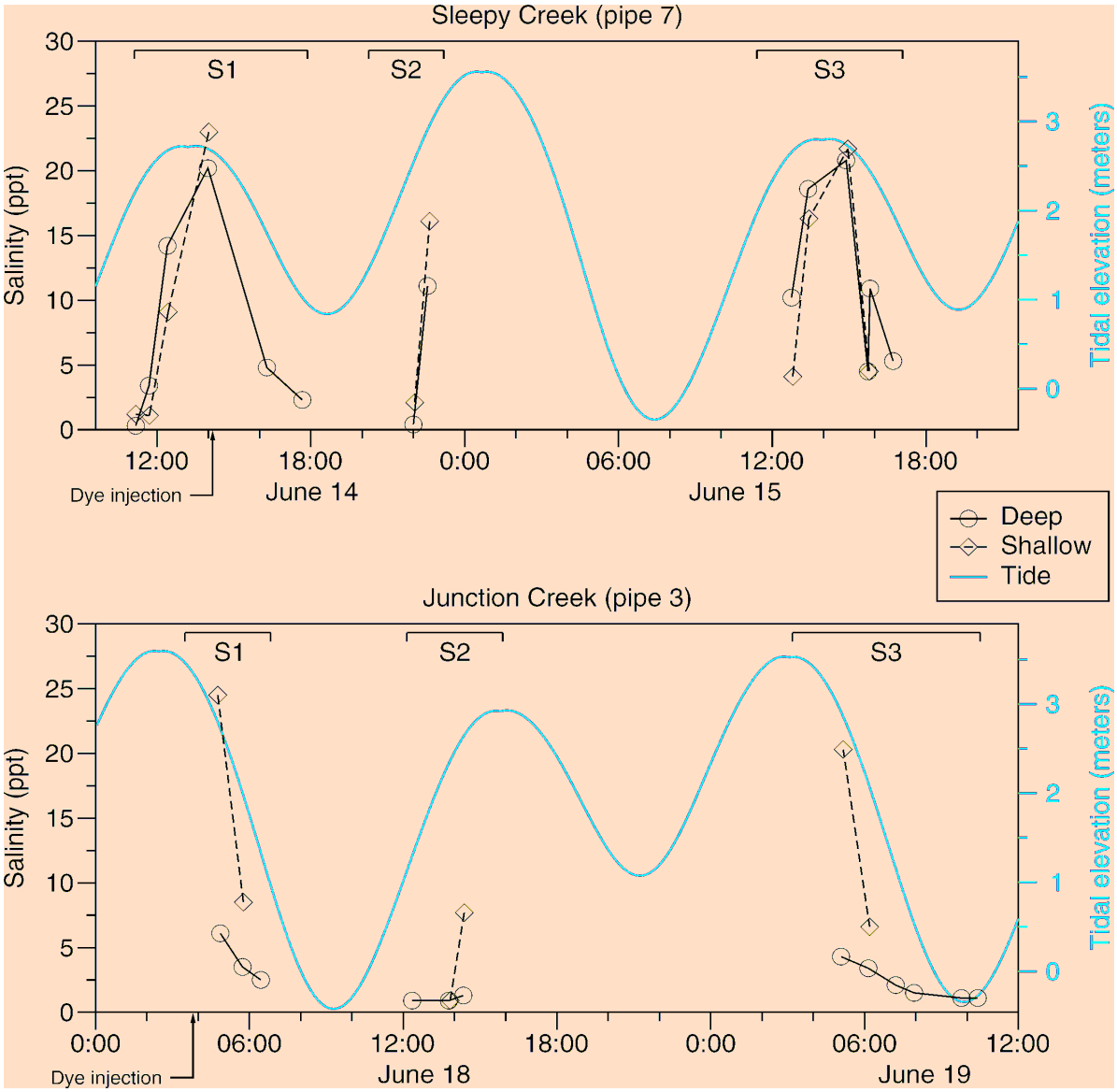

Fig. 1.4. Salinity in deep and shallow groundwater at Sleepy Creek and Junction Creek, Prince William Sound, Alaska, compared to the tidal cycle. Data were collected within three discrete time intervals (S1-S3) at each site.

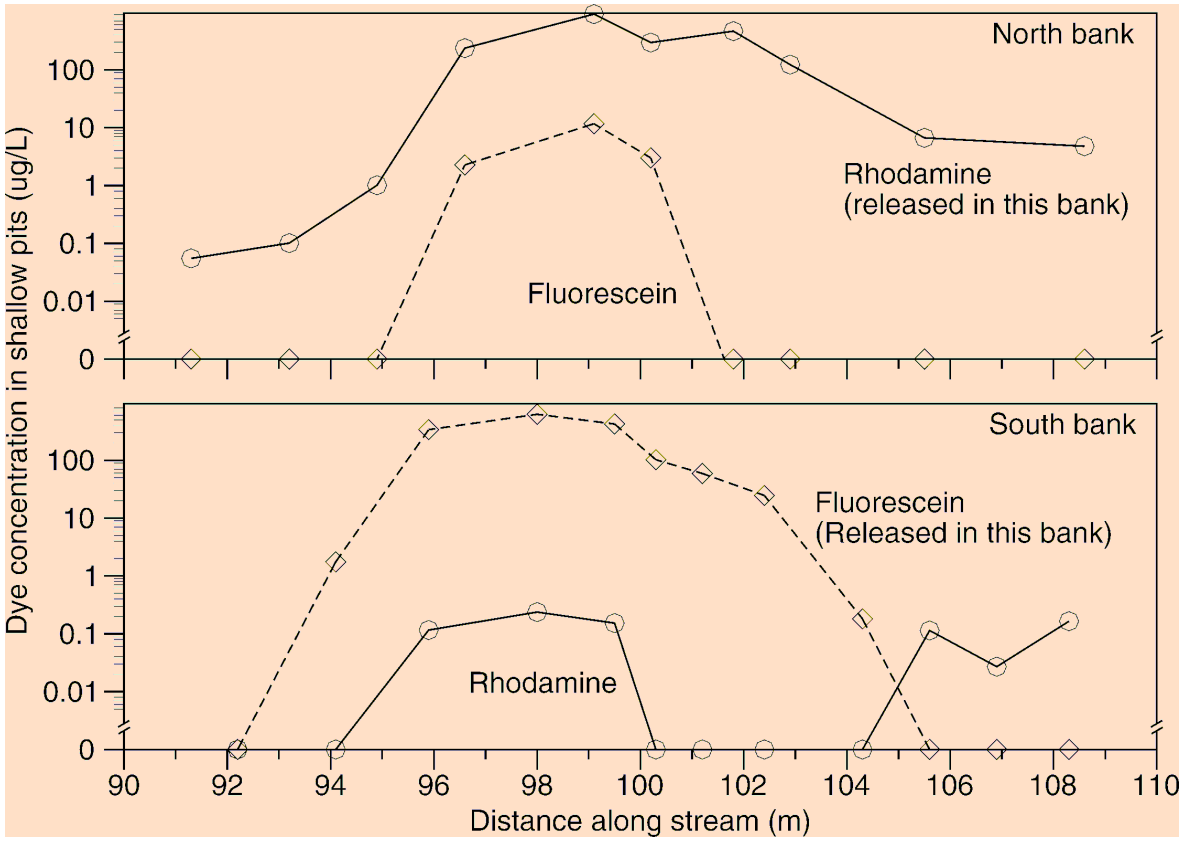

Fig. 1.5. Tracer dye concentrations in shallow pits located on north and south stream banks immediately adjacent to stream water at Junction Creek. Rhodamine dye was released in the north bank, fluorescein in the south; pit samples were collected 3 h later. Pits were positioned linearly in the vicinity of 2.50 m MLLW (along thickened stream margin illustrated in Fig. 1.2).

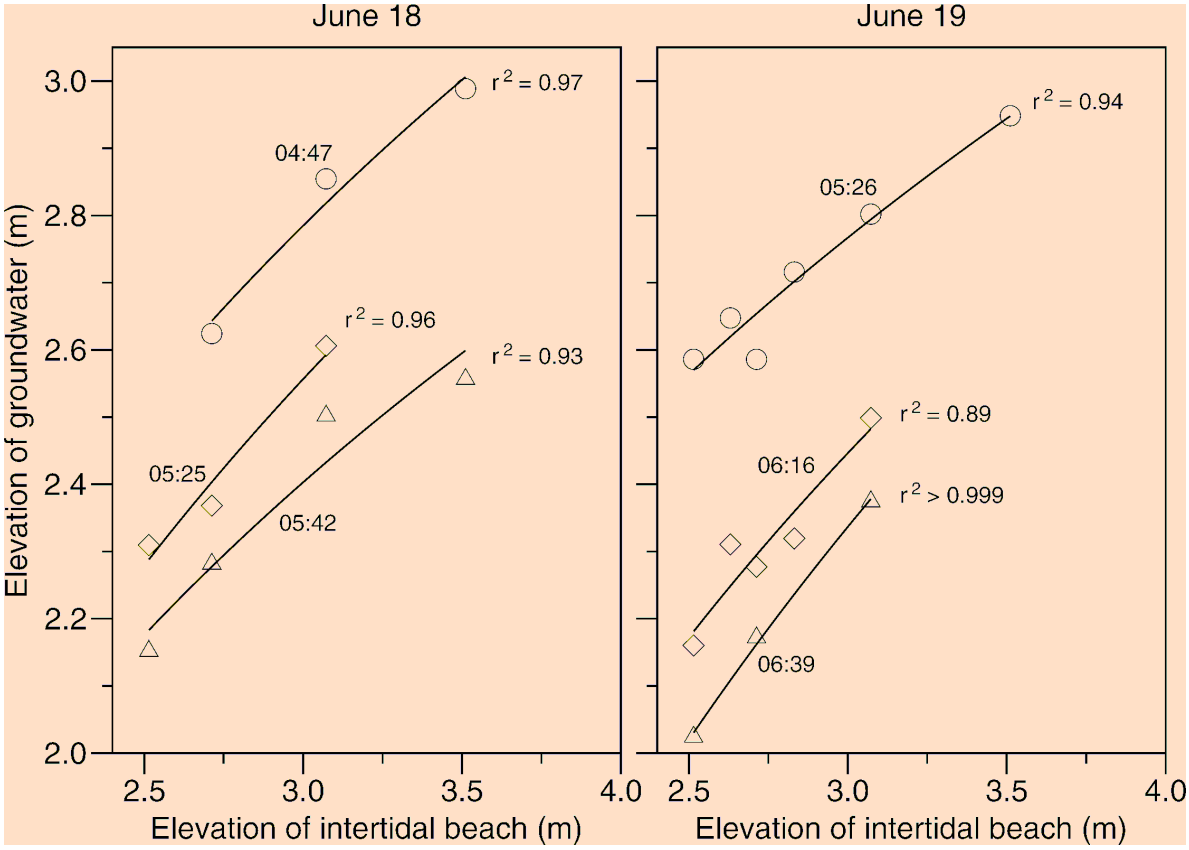

Fig. 1.6. Relationship between beach elevation and groundwater elevation during ebb tides at Junction Creek. Time of observation is printed by each regression; independent data (beach elevation) were log-transformed. Circles are first daily observation, diamonds second, and triangles third. High tide was at 02:28 h on June 18, 2000 and 03:03 h on June 19.

Chapter 2
Pink salmon spawning habitat is recovering a decade after the *Exxon Valdez* oil spill

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Abstract

Intertidal sediment surrounding many pink salmon (*Oncorhynchus gorbuscha*) spawning streams in western Prince William Sound was contaminated by the *Exxon Valdez* oil spill. Biochemical and egg-dig evidence suggested that oil reduced the survival of pink salmon embryos for several years. Previous research also demonstrated that dissolved oil can be transferred to developing embryos from surrounding oiled sediment via drainage of interstitial water as a result of tidal cycling and hydraulic gradients. In this study, completed a decade after the spill, we sampled stream water for the presence of oil using passive membrane sampling devices, collected sediment and pink salmon eggs for hydrocarbon analysis, and examined alevins for induction of cytochrome P4501A (CYP1A). Polynuclear aromatic hydrocarbons (PAH) consistent with *Exxon Valdez* oil were verified in the water of one of six heavily impacted streams; total PAH concentrations were greatest in the lower intertidal. Similarly distributed total PAH in a second stream suggested possible contamination. Oil was not detected in the remaining four streams. Induction of CYP1A in alevins from the two streams with oil was lowest in water above mean high tide and increased downslope. Because our samples were all selected from heavily oiled streams, we infer that most pink salmon spawning habitat either has recovered or is recovering.

Introduction

Extensive intertidal areas in Prince William Sound (PWS), Alaska, were coated with oil in 1989 from the *Exxon Valdez* spill. Approximately 31% of PWS salmon streams were surrounded by oiled beaches (Brannon and Maki 1996; Geiger et al. 1996; Murphy et al. 1999). Oil often penetrated deep into the gravel, creating the potential for years of chronic contamination (Michel and Hayes 1993). Flowing freshwater precluded direct oil deposition in stream channels, but despite this apparent protection of intertidal pink salmon (*Oncorhynchus gorbuscha*) spawning habitat, exposure to oil was indicated by elevated mixed-function oxidase (CYP1A) activity in alevins from streams in oiled areas but not in those from reference streams (Wiedmer et al. 1996). Furthermore, Bue et al. (1996, 1998) and Craig et al. (2002) reported that embryo mortality in these streams was elevated through 1993 and again in 1997, and that the key difference was oil. Although Brannon et al. (1995) and Brannon and Maki (1996) have argued that oil concentrations in stream sediment were too low to damage pink salmon, citing acute exposure bioassays for support, they did detect higher mean total polycyclic aromatic hydrocarbon (TPAH) concentrations in the sediment of oiled streams (0.5-2818 ng/g) than in non-oiled reference streams (0.2-64 ng/g). Later long-term chronic bioassays demonstrated the vulnerability of pink salmon eggs to dissolved oil, by transfer of polynuclear aromatic hydrocarbons (PAH) from rock to embryo via water; lowest-observed effective aqueous concentrations ranged from 1 to 18 µg/L TPAH (Marty et al. 1997; Heintz et al. 1999, 2000).

At the time of the oil spill, no one suspected that pink salmon spawning habitat was damaged, and no effort was made to monitor hydrocarbon concentrations in these streams. Only

when Bue et al. (1996) continued to find elevated embryo mortality in the 1990s was attention refocused on spawning habitat (Rice et al. 2001). Eventually a hypothesis was formulated that the flow of interstitial water from surrounding oil-contaminated beaches transports toxic levels of PAH to pink salmon redds, and that concentrations increase toward the lower intertidal zone (Heintz et al. 1999). Existence of this transport mechanism was subsequently confirmed by Carls et al. (2003) and is consistent with other evidence of intertidal groundwater drainage into intertidal streams (e.g., Harvey et al. 1987; Chow et al. 1988; Nuttle and Hemond 1988; Li et al. 1999). Evidence that toxic concentrations of PAH can move from oil-coated rock into water was provided by several studies, e.g., Marty et al. (1997), Carls et al. (1999), and Heintz et al. (1999, 2000). Murphy et al. (1999) detected high molecular weight PAH in sediment surrounding streams in PWS in 1995 and concluded that concentrations at some streams were likely toxic through 1993. The presence of PAH in pink salmon redds allowed eggs to accumulate PAH, which explains why Wiedmer et al. (1996) observed elevated cytochrome P4501A (CYP1A) levels in eggs from oiled streams. Because these eggs are lipophilic, they accumulate much higher hydrocarbon concentrations than are present in surrounding water (e.g., Heintz et al. 2000). In contrast, stream gravel–which is not lipophilic–is not likely to accumulate dissolved hydrocarbons, explaining why Brannon and Maki (1996) found relatively few contaminant hydrocarbons in gravel from these streams. Still, no one made an effort to directly monitor stream water and embryo tissue for hydrocarbons because contaminant concentrations were not known to be acutely toxic until Marty et al. (1997) published their findings.

The purpose of our study was to examine pink salmon habitat oiled a decade earlier by the *Exxon Valdez* spill either for evidence of continued oil contamination or recovery. Six pink salmon streams were chosen for study based on their history of observation (Brannon et al. 1995; Bue et al. 1996, 1998; Craig et al. 2002), oil retention (Murphy et al. 1999), and long-term embryo mortality (Craig et al. 2002). Specific objectives were 1) to sample stream water for the presence of residual *Exxon Valdez* oil with low-density polyethylene membrane sampling devices (PEMDs), 2) to collect sediment and pink salmon eggs from the same locations for hydrocarbon analysis, 3) to examine alevins for induction of CYP1A, and 4) to relate contemporary data to site contamination history. Sampling was partitioned among four elevation zones, with the uppermost located above mean high tide. Increased aqueous hydrocarbon concentrations were anticipated at low intertidal elevations because water contaminated by contact with intertidal sediment drains downward. Oil-derived hydrocarbons were not expected in stream water at elevations above mean high tide.

This study, implemented a decade after the *Exxon Valdez* spill, is the first and only effort to directly measure oil in water and embryos from pink salmon streams. Analysis was two-tiered. The PEMDs were first deployed in all six streams. On the basis of PEMD results, sediment and tissue were analyzed from two streams with evidence of lingering oil and one stream without evidence of aqueous hydrocarbons. The latter stream was included to test the hypothesis that hydrocarbons from unknown sources in intruding marine water were responsible for elevation-related hydrocarbon levels and CYP1A induction in pink salmon eggs.

Study Area

The intertidal reaches of the six pink salmon streams selected for study were all oiled as a result of the *Exxon Valdez* spill. Streams sampled were Herring Creek and Snug Creek on Knight Island, Junction Creek and Chenega Northwest on Chenega Island, Bjorne Creek on Evans Island, and Sleepy Creek on Latouche Island. Bue et al. (1996), extended by Craig et al. (2002), observed elevated embryo mortality in these streams plus four other oiled streams in western Prince William Sound (compared to mortality in 15 reference streams) from 1989 to

1993 and again in 1997. Average ranking for post-spill years 1989-1993 placed half of the streams in this study among the worst 20% of streams for embryo survival, and all were ranked in the upper half of streams with poor survival. (All 25 streams observed by Bue et al. (1998) were ranked, regardless of oil designation.) Water, sediment, and eggs were sampled for hydrocarbon analysis within four tidal elevation intervals in or adjacent to each of the six streams. Additional egg and alevin samples were collected for CYP1A analysis. Elevations corresponded to those of Bue et al. (1998) and ranged from 1.8 to >3.7 m above mean lower low water. The uppermost elevations were above mean high tide at each stream and served as site-specific controls. A full spectrum of samples was collected from each stream but complete analysis was restricted to two streams with evidence of hydrocarbons in stream water (Sleepy Creek and Junction Creek) plus a third stream (Herring Creek) for comparison.

Methods

Sample collection

To sample stream water, PEMDs (98 μm × 4.9 cm × 50 cm) were buried approximately 10-20 cm below each stream channel in August 1999. The PEMDs were suspended centrally in perforated aluminum tubes (3 mm holes spaced 4.8 mm on center) on stainless steel clips fastened to nylon line. Tube ends were capped with aluminum window screen. Tubes, screens, associated hardware, and tools were washed with soap and water, dried, and rinsed with methylene chloride prior to use. Blank PEMD samples were collected by first rigging membranes in a sampler tube, then retrieving them without placement in stream water. Four PEMDs were placed within each of four elevation zones, 1.8 to 2.4 m above mean lower low water, 2.4 to 3.0 m, 3.0 to 3.7 m, and >3.7 m. The PEMDs were recovered 45-56 days after placement and frozen pending analysis.

Surface sediments were collected with hydrocarbon-free tools from both stream banks and the stream channel at each tidal elevation and were frozen until analysis. Typically, one randomly selected bank sample plus the stream channel sample from each elevation were later analyzed.

Pink salmon eggs and alevins were collected by hydraulic pumping (per Bue et al. 1996) and frozen for hydrocarbon analysis. For histopathological analysis, a subset of these eggs and alevins was preserved in 10% neutral buffered formalin and transferred to physiological saline after 24 h in the fixative.

Hydrocarbon analyses

The PAH and alkanes were extracted, separated, and analyzed by a gas chromatograph equipped with a mass selective detector (Short et al. 1996a). The PEMDs were extracted according to Carls et al. (in press). Tissue was extracted as described by Short et al. (1996a). Experimentally determined method detection limits depended on sample weights, and generally were 0.18-3.94 ng/g in PEMDs, 1 ng/g in tissue, and < 2 ng/g in sediment. Concentrations of individual PAH below method detection limits were treated as zero. Tissue concentrations are reported in μg/g wet weight, but wet to dry weight ratios were measured by dehydrating 1 g wet samples for 24 h at 60°C and weighing the remaining mass. The accuracy of the hydrocarbon measurements was about ±15% based on comparison with National Institute of Standards and Technology values, and precision expressed as coefficient of variation was usually less than approximately 20%, depending on the PAH. Concentrations of TPAH were calculated by summing concentrations of 39 PAH analytes (see Fig. 2.1 legend). Relative PAH concentrations were calculated as the ratio of PAH to TPAH. Concentrations observed at control elevations (>3.7 m) were considered to represent background concentrations.

Possible naphthalene and C1-naphthalene contamination was encountered in egg tissue from one creek, and some data are presented both with and without these compounds for the purpose of discussion. Accordingly, ΣPAH = TPAH minus naphthalene and c1-naphthalene concentrations.

Historical hydrocarbon data

Additional historical data (TPAH in sediment and mussels) were obtained for each study stream from the Natural Resource Damage Assessment database (Short et al. 1996b). Data within 500 m of each stream were accepted unless there were clear reasons for exclusion (e.g., targeted asphalt patches known to be outside stream drainage basins). Data included those reported by Murphy et al. (1999) and are extended by results from this study.

CYP1A analysis

Each alevin was coded for blind study, processed routinely into paraffin and embedded in lateral recumbency; at least six 5-µm-thick sections were then cut from each alevin as previously described (Marty et al. 1997). Immunohistochemistry was done using a standard streptavidin-biotin-horseradish peroxidase (HRP) detection system slightly modified from Olivry et al (1997). After hydrating to buffer (PBS, pH 7.4), slides were pretreated with steam heat while immersed in a citrate buffer (pH 6.1, Dako Corp.). The steamer (#HS800 Black & Decker, Shelton, CT.) was preheated for 5 minutes, and the slides in their buffer were added and steamed for 30 minutes (97-98°C), followed by cooling at room temperature for 20 minutes. The primary antibody was a mouse anti-fish CYPlA (clone C10-7, a synthetic peptide corresponding to an amino acid sequence in rainbow trout CYP1A, Biosense Laboratories, Bergen, Norway). The antibody was applied at a dilution of 1:1000 and incubated on the slides for 2 hours at room temperature. Negative controls were prepared by omitting the primary antibody and substituting a mouse myeloma IgG correlate. Additional positive and negative control sections were from pink salmon larvae exposed to a high-oil dose and control groups from a laboratory experiment.

Staining intensity and occurrence of CYP1A expression were evaluated microscopically for each major tissue known to respond to oil in every larva (Marty et al. 1997 and unpublished results). Staining intensity and occurrence were each scored on a 5-point scale: none (0), very light (1), light (2), moderate (3), or strong/widespread (4) and their product was used for statistical analysis (Marty et al. 1997). Tissues included in the statistical analysis were present in ≥ 90% of all records and included gill epithelium, gill arch/filament endothelium, pharyngeal epithelium, atrial endothelium, ventricular endothelium, liver sinusoidal endothelium, larger liver veins, kidney sinusoidal endothelium, kidney tubular epithelium, renal vein endothelium, anterior intestinal epithelium, yolk-sac endothelial or epithelial cells, skin epithelium, and nasal epithelial cells.

Tissues from Herring Creek were examined as a separate random group (completed after other analyses) to test the hypothesis that hydrocarbons from unknown sources in intruding marine water caused elevation-related induction patterns in pink salmon eggs. Staining of CYP1A is highly consistent within randomized groups but because staining levels between randomized groups can vary by ± 1 point, scores in the Herring Creek set may not be directly comparable to those in other streams. Rather, examination of Herring Creek eggs allowed comparison of CYP1A induction to intertidal elevation at a stream without contemporary indications of oiling in its water and sediment.

Statistical analyses

An oil-weathering model developed by Short and Heintz (1997) was used to determine if PAH composition in each matrix was consistent with *Exxon Valdez* oil. Definitions used are:

unweathered ($w = 0$), slightly weathered ($0 < w \leq 2$), moderately weathered ($2 < w \leq 8$), and highly weathered ($w > 8$) (Carls et al. 2001).

Concentration and staining data were related to independent variables with regressions. Linear regression was used to relate ∑PAH in PEMDs, sediment, and pink salmon eggs to intertidal elevation, where x was the mean elevation in each zone. Similarly, CYP1A staining products (staining score • intensity score) were regressed against elevation. Mean historical TPAH concentrations in mussels and sediment were regressed against collection year (1989 to 1999) to describe oiling and oil retention; models accepted were exponential and power. To ensure that regressions were meaningful, we adopted the approach that the F-ratio of a regression (F_o) should exceed the usual significance ratio (F_c) by a multiple of at least 4 times [20]. To summarize relationships among PEMDs, sediment, and pink salmon eggs across all streams, correlation analysis was applied to mean ∑PAH and CYP1A products.

Concentration and staining data from the three lower elevations were compared to stream-specific controls (located above mean high tide) with two-factor (elevation, stream) analysis of variance (ANOVA). 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 less than 0.95 for all comparisons.

Results

Detection of oil in stream water

Subsurface aqueous TPAH concentrations in previously oiled streams, determined with PEMDs, were either inversely related to sample elevation or were low and unrelated to elevation a decade after the *Exxon Valdez* spill (August-October 1999; Fig. 2.2). Aqueous ∑PAH concentrations were clearly related to elevation at Sleepy Creek and Junction Creek ($0.84 \leq r^2 \leq 0.87$, $P_{regression} < 0.001$, $12.0 \leq F_o/F_c \leq 16.2$). In both these streams, ∑PAH concentrations in PEMDs deployed at the lowest two elevations were significantly greater than in controls ($P_{ANOVA} < 0.001$). Concentrations also declined with elevation in three other streams (Snug Harbor, Bjorne Creek, and Chenega Northeast) but were almost always less than the greatest mean background estimate (49 ± 10 ng/g at Junction Creek) and no ∑PAH concentrations were significantly elevated ($0.28 \leq r^2 \leq 0.52$, $0.002 \leq P_{regression} \leq 0.033$, $1.2 \leq F_o/F_c \leq 3.2$). There was no relationship between intertidal elevation and ∑PAH at Herring Creek where ∑PAH ≤ 23 ng/g. The ∑PAH concentration in the PEMD field blank was 9 ng/g. A background signal (anthracene through benzo[a]anthracene) was apparent in PEMDs (Fig. 2.1).

Composition of PAH in PEMDs was consistent with moderately weathered *Exxon Valdez* oil in the lower two elevations at Sleepy Creek only ($4.8 \leq w \leq 5.8$; Short and Heintz 1997; Fig. 2.1a-2.1b). Composition of PAH was not consistent with two alternative sources, Katalla seep oil and a Constantine Harbor background signal (Short and Heintz, 1997). Junction Creek was the only other stream where all PAH required to estimate weathering were present ($5.0 \leq w \leq 6.8$), but *Exxon Valdez* oil could not be verified as the source. Residual *Exxon Valdez* oil could not be verified in water of the remaining four streams.

Aqueous ΣPAH concentrations at Chenega Creek and Herring Creek were at background levels throughout these watersheds, suggesting recovery. Because more alevins were collected at Herring Creek, sediment and tissue samples from this stream were analyzed for comparison. Remaining samples (sediment and tissue) from other streams were not analyzed.

PAH in sediment

In 1999, ΣPAH concentrations were elevated or slightly elevated in sediment at the two streams with evidence of PAH in water (Sleepy Creek and Junction Creek) but not at a third stream (Fig. 2.3). Sediment from the channel and bank of Sleepy Creek contained plausible

evidence of residual *Exxon Valdez* oil, and although too weathered to be positively identified as *Exxon Valdez* oil, dibenzothiophenes, phenanthrenes, and chrysenes were present, consistent with a crude oil source (Fig. 2.1). Mean ΣPAH concentrations in sediment at Sleepy Creek was significantly greater at the lowest elevation than in the control (33 ng/g dry weight, P_{ANOVA} = 0.002) and decreased as elevation increased ($r^2 = 0.61$, $P = 0.022$, $F_o/F_c = 1.6$). Concentrations of ΣPAH in the Junction Creek channel and bank sediment also declined with elevation, but maximum values were lower than at Sleepy Creek, no concentrations were significantly elevated, and the regression was not significant (Fig. 2.3). Phenanthrenes and chrysenes were observed in Junction Creek banks at the lower two elevations, suggesting the possibility of highly weathered oil, but elsewhere there was no evidence of residual oil. Concentrations of ΣPAH in channel sediment were less than or equal to those in corresponding bank sediment. There was no evidence of oil in Herring Creek sediment (∑TPAH = 0 in all samples).

PAH in pink salmon eggs

In pink salmon eggs, ΣPAH concentration was significantly elevated in the lowest intertidal zone at Sleepy Creek ($P_{ANOVA} = 0.002$) and tended to decline as elevation increased ($r^2 = 0.48$, $P = 0.058$, $F_o/F_c = 0.9$): ∑PAH was not correlated with elevation in other streams ($0.00 \leq r^2 \leq 0.01$; Fig. 2.3). These eggs were potentially exposed to residual oil for roughly 1 month before sampling. Although ∑PAH was significantly elevated in one instance, *Exxon Valdez* oil was not identifiable in any pink salmon eggs in 1999-2000. In general, no PAH larger than acenaphthylene were observed in tissues; the only exceptions were at lower elevations in Sleepy Creek where some fluorenes and phenanthrenes were detected in eggs (e.g., Fig. 2.1).

Naphthalene concentrations were exceptionally high in egg tissue from Herring Creek, a location that otherwise appears to have returned to normal (based on PEMD, sediment, and CYP1A data; Figs. 2.2 and 2.3). We suspect contamination from sources other than *Exxon Valdez* oil. Reanalysis of two Herring Creek tissue samples confirmed the presence of naphthalene and C1-naphthalenes but could not eliminate the possibility of contamination earlier in the collection process. Exclusion of the PAH in question from all TPAH values made little difference in sediment and PEMD data and did not change relationships across tidal elevations but had a somewhat more pronounced effect in tissue (Figs. 2.2 and 2.3). Although deposits of *Exxon Valdez* oil were still present near the mouth of Herring Creek in 2001 (asphalt and mousse; Mandy Lindeberg, Auke Bay Laboratory, personal communication), high naphthalene and C1-naphthalene concentrations in stream water above mean high tide suggests these deposits were probably not responsible for the anomalous values.

CYP1A induction in alevins

A mixed function oxidase enzyme, CYP1A, was induced in alevins from streams with evidence of PAH in water (Sleepy Creek and Junction Creek) and was inversely correlated with elevation ($0.30 \leq r^2 \leq 0.52$, $P_{regression} < 0.001$, $3.6 \leq F_o/F_c \leq 7.5$; Fig. 2.3). Tissues with CYP1A induction included choroidal tissue (eye), gill epithelium, pharyngeal epithelium, atrial and ventricular endothelium, vascular sinus (vena cava), hepatocytes, bile ducts, larger veins (liver), and sinusoidal endothelium (liver and kidney). In alevins from Herring Creek, CYP1A induction was not related to tidal elevation ($r^2 = 0.00$, $P = 0.877$, $F_o/F_c = 0.0$)

Relationships among matrices across all streams

Summarized across all three completely analyzed streams, data that were correlated among matrices were consistent with stream-specific observations (Table 2.1). The strongest relationship was between ∑PAH in PEMDs and in sediment ($r^2 = 0.80$, $P < 0.001$, $n = 12$). The CYP1A induction product was correlated with ∑PAH in PEMDs and in sediment when the

independently analyzed Herring Creek CYP1A data set was not included ($0.57 \leq r^2 \leq 0.70$; $0.037 \leq P \leq 0.083$, $n = 6$). In eggs, ∑PAH was not correlated with ∑PAH in PEMDs or sediment, nor with the CYP1A product in alevins ($0.00 \leq r^2 \leq 0.44$, $0.149 \leq P \leq 0.952$), probably because PAH were eliminated from tissue by metabolic activity.

Oiling, oil retention, and biological availability of oil at study streams, 1989-1999

Concentrations of TPAH in sediment surrounding study streams declined throughout the decade following the *Exxon Valdez* oil spill (Fig. 2.4). After the *Exxon Valdez* oil spill, TPAH concentrations in intertidal sediment around oiled pink salmon streams exceeded 10,000 ng/g dry weight at four of six study streams; observed concentrations were typically highest immediately after the spill. In general, TPAH concentrations in sediment declined as might be expected after a one-time oiling event. Hydrocarbons from oil became progressively less biologically available to mussels near Sleepy Creek (Fig. 2.4). In 1999-2000, TPAH concentrations ranged from 0-120 ng/g dry weight.

Discussion

Positive identification of *Exxon Valdez* oil in Sleepy Creek water >10 years after the spill, together with evidence that PAH levels in sediment remain elevated and that pink salmon eggs were exposed to PAH is evidence of long-term habitat damage. These results are consistent with prior research that 1) sediment surrounding about 1/3 of the pink salmon streams in western PWS was oiled (ADFG 1989; Brannon and Maki 1996; Bue et al. 1996, 1998; Geiger et al. 1996; Murphy et al. 1999; Craig et al. 2002), 2) that a mechanism exists to transport toxic PAH in solution from oiled intertidal rock to pink salmon eggs (Carls et al. 2003), 3) that pink salmon embryos were exposed to PAH from *Exxon Valdez* oil (CYP1A induction; Weidmer et al. 1996), and 3) that exposure to oil caused reduced survival of exposed embryos (Rice et al. 2001). However, our results also clearly suggest that most pink salmon habitat in PWS has either recovered or is recovering because oil was evident in only one or two of six previously heavily oiled streams and contemporary hydrocarbon concentrations in sediment surrounding these streams is low compared to concentrations in the years immediately after the spill.

Combined CYP1A, PEMD, and sediment PAH data from this study clearly indicate that the contemporary source of PAH responsible for CYP1A induction in pink salmon eggs is residual oil in surrounding intertidal sediment. CYP1A was induced in streams with indications of residual oil in water and sediment but not in the stream where oil was not detected these matrices (Herring Creek). Although the mean CYP1A product was relatively higher in Herring Creek than in upstream zones in the other streams, we suspect that staining differences in these independently analyzed eggs were responsible, not differences in hydrocarbon exposure. Product levels less than one are not considered high because mean products reached sixteen under laboratory conditions where initial aqueous TPAH = 17 μg/L (unpublished data). The CYP1A data at Herring Creek demonstrate that induction was not caused by contaminants in intruding saltwater because induction was unrelated to intertidal elevation, and we infer that this is the general case for all streams. At the time of this study, there were no known contemporary sources of planar hydrocarbons in seawater capable of inducing CYP1A in salmon eggs incubating in intertidal streams in PWS. Rather, the case for lingering oil constituents leaching from contaminated intertidal sediment in PWS is well supported (Marty et al. 1997; Heintz et al 1999; Carls et al. 2001; Carls et al. 2003) and explain past (Wiedmer et al. 1996) and present egg exposure.

Recovery of pink salmon habitat is nearly complete. Concentrations of TPAH in sediment surrounding study streams declined throughout the decade following the *Exxon Valdez* oil spill. Lingering hydrocarbon contamination was verifiable only in the water of Sleepy Creek,

where TPAH concentrations were consistently highest in spawning substrate shortly after the spill (Brannon et al. 1995), and where TPAH concentrations in intertidal sediment remained as high as 1125 ng/g dry weight in 1995 (Murphy et al. 1999). Continued contamination was also plausible in a second (of six) study stream. Contamination indicated in eggs and alevins from Herring Creek was inconsistent with hypothesized oiling mechanisms (there was no relationship between response and intertidal elevation), suggesting this contamination was not related to the *Exxon Valdez* spill. Because selection of study streams was based on history of observation, oil retention, and long-term embryo mortality (Brannon et al. 1995; Bue et al. 1996, 1998; Craig et al. 2002; Murphy et al. 1999), extrapolation to all oiled PWS streams is not possible. However, these data can be used to set a lower bound on the number of recovered streams (67 to 83%) because the streams sampled represent worst-case conditions. Recovery is also likely in progress at the two streams where oil was either verified or plausible in 1999; TPAH concentrations had declined by 91 to 96% in 1995 and just 0 to 1% remained in 1999-2000.

Studies that provided initial evidence of habitat damage also corroborate evidence that pink salmon habitat has gradually recovered with time, including improved pink salmon embryo survival, declining hydrocarbon concentration in intertidal sediment, and declines in CYP1A induction (Bue et al. 1998; Craig et al. 2002; Murphy et al. 1999; Wiedmer et al. 1996). Evidence that *Exxon Valdez* oil was biologically available in the vicinity of these streams is also substantiated by initially high but declining accumulations in mussel tissue (Short et al. 1996b; Murphy et al. 1999; Fig. 2.4). Spawning habitat recovery begins as oil is depleted from intertidal sediment, reducing the transfer of hydrocarbons to living inhabitants.

The sensitive new passive sampler technology utilized in this study allowed detection of oil in worst-case stream water at concentrations lower than previously possible, but it has also verified that the majority of pink salmon streams in Prince William Sound have likely fully recovered from oiling.

Acknowledgments

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Table 2.1. Relationships among matrices, summarized across three streams in Prince William Sound (Sleepy Creek, Junction Creek, and Herring Creek). The independently analyzed CYP1A data set at Herring Creek was not included in this analysis.

	∑PAH in sediment	∑PAH in eggs	CYP1A product
Correlation (r^2)			
∑PAH in PEMD	0.80	0.00	0.57
∑PAH in sediment		0.06	0.70
∑PAH in eggs			0.44
Probability			
∑PAH in PEMD	0.000	0.952	0.083
∑PAH in sediment		0.457	0.037
∑PAH in eggs			0.149

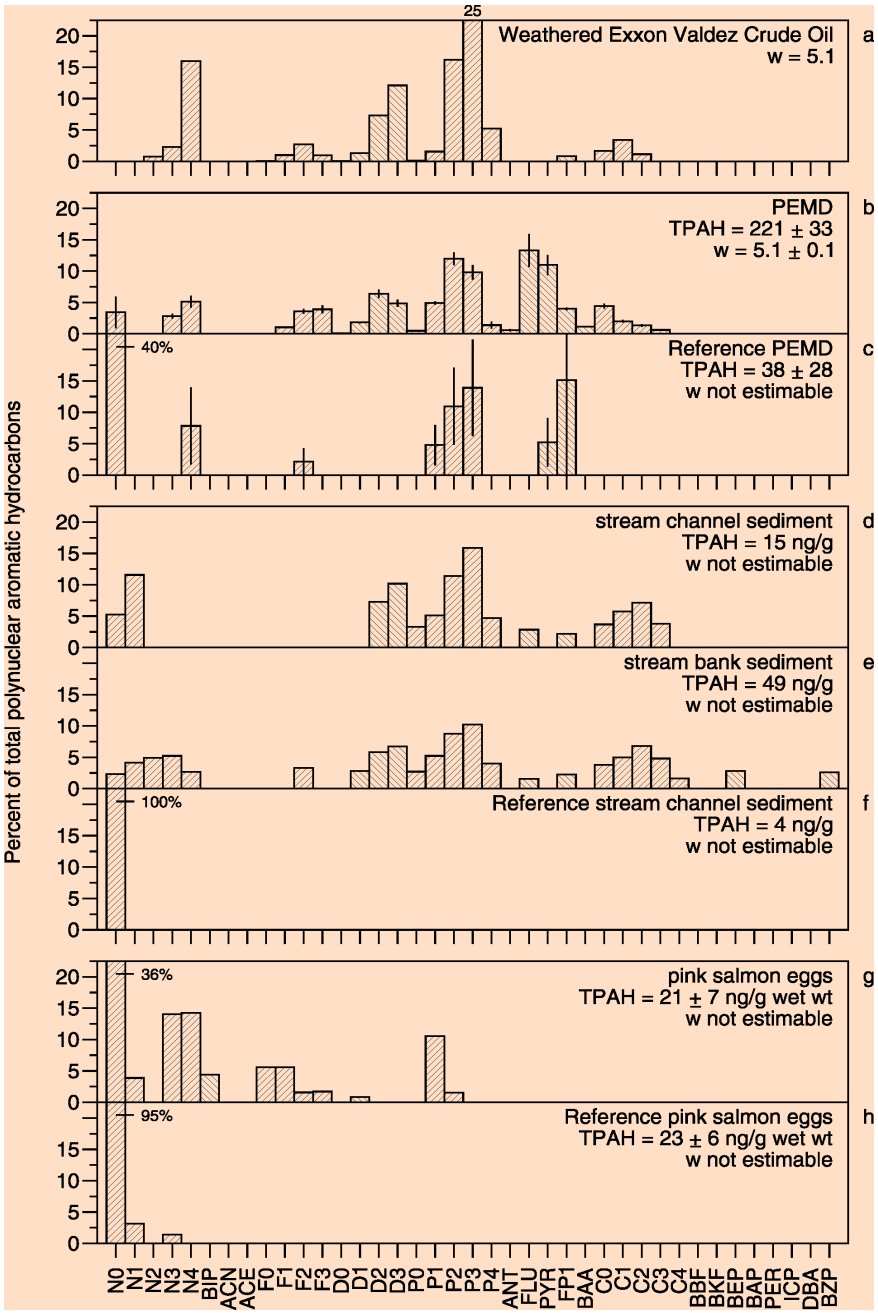

Fig. 2.1. Polynuclear aromatic hydrocarbon (PAH) composition in PEMDs, sediment, and pink salmon eggs at Sleepy Creek (1.8-2.4 m above mean lower low water) compared to similarly weathered *Exxon Valdez* crude oil. Control samples were collected upstream (> 3.7 m above mean lower low water). Total PAH (TPAH) concentrations (± SE) are listed for each sample. Weathering indices (*w*) and the crude oil profile were generated using an oil-weathering model (Short and Heintz 1997). The PAH analyzed were naphthalenes (N0 to N4, where the numeral indicates the number of alkyl carbon atoms), fluorenes (F0 to F3), dibenzothiophenes (D0 to D3), phenanthrenes (P0 to P4), chrysenes (C0 to C4), biphenyl (BIP), acenaphthene (ACE), acenaphthylene (ACN), anthracene (ANT), fluoranthene (FLU), pyrene (PYR), methyl-fluoranthene/pyrene (FP1), benzo-a-anthracene (BAA), benzo-b-fluoranthene (BBF), benzo-k-fluoranthene (BKF), benzo-e-pyrene (BEP), benzo-a-pyrene (BAP), perylene (PER), indeno-1,2,3-c,d-pyrene (ICP), dibenzo-a,h-anthracene (DBA), and benzo(ghi)perylene (BZP).

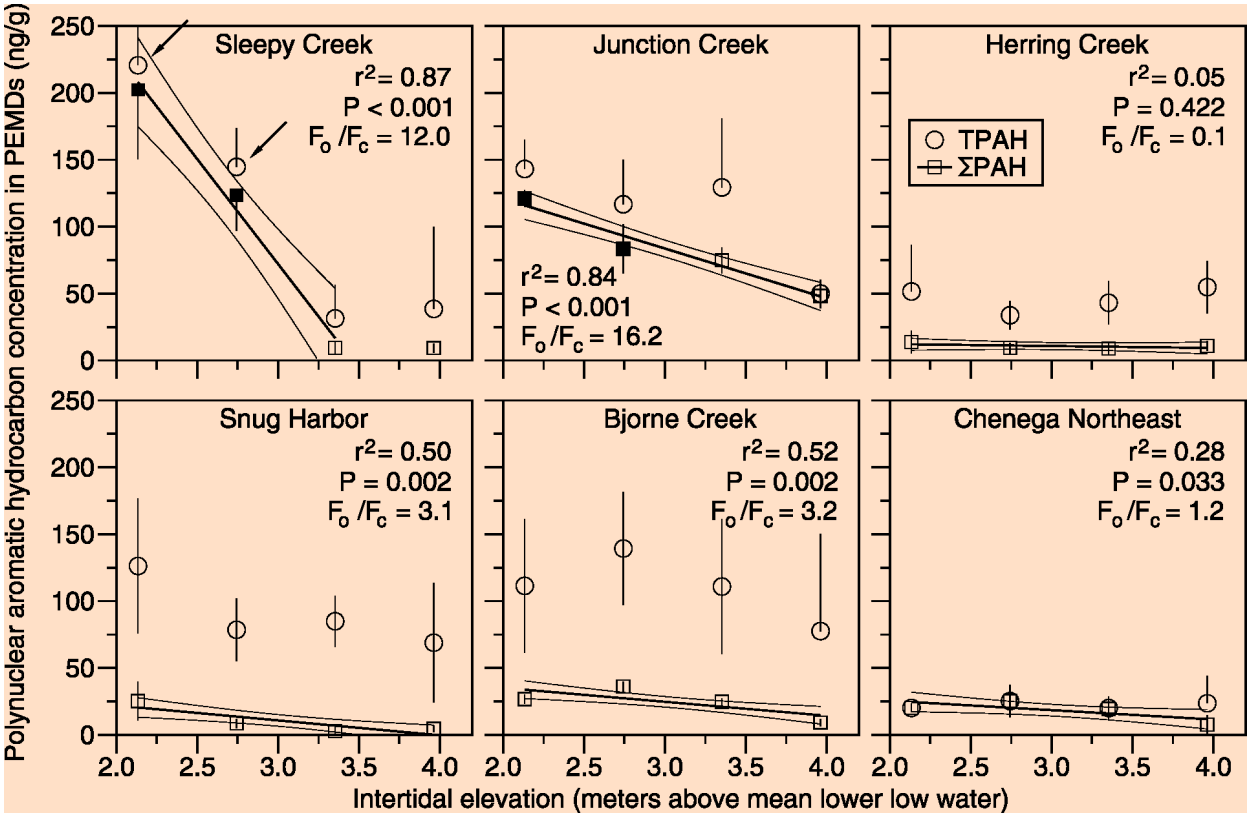

Fig. 2.2. Total polynuclear aromatic hydrocarbon (TPAH) concentrations and sum polynuclear aromatic hydrocarbon (ΣPAH = TPAH minus naphthalene and c1-naphthalene concentrations) in low-density polyethylene membrane devices (PEMDs) as a function of elevation in six streams in Prince William Sound, Alaska. Fitted lines are linear regressions with 95% confidence bands. The regression at Sleepy Creek was restricted to the lower three elevations. Solid symbols indicate significant differences from the uppermost elevations, located above mean high tide and considered stream-specific controls. F_o/F_c is the ratio between the observed F value and the critical F value. Arrows indicate elevations where *Exxon Valdez* oil was present in one or more contributing samples (Short and Heintz 1997).

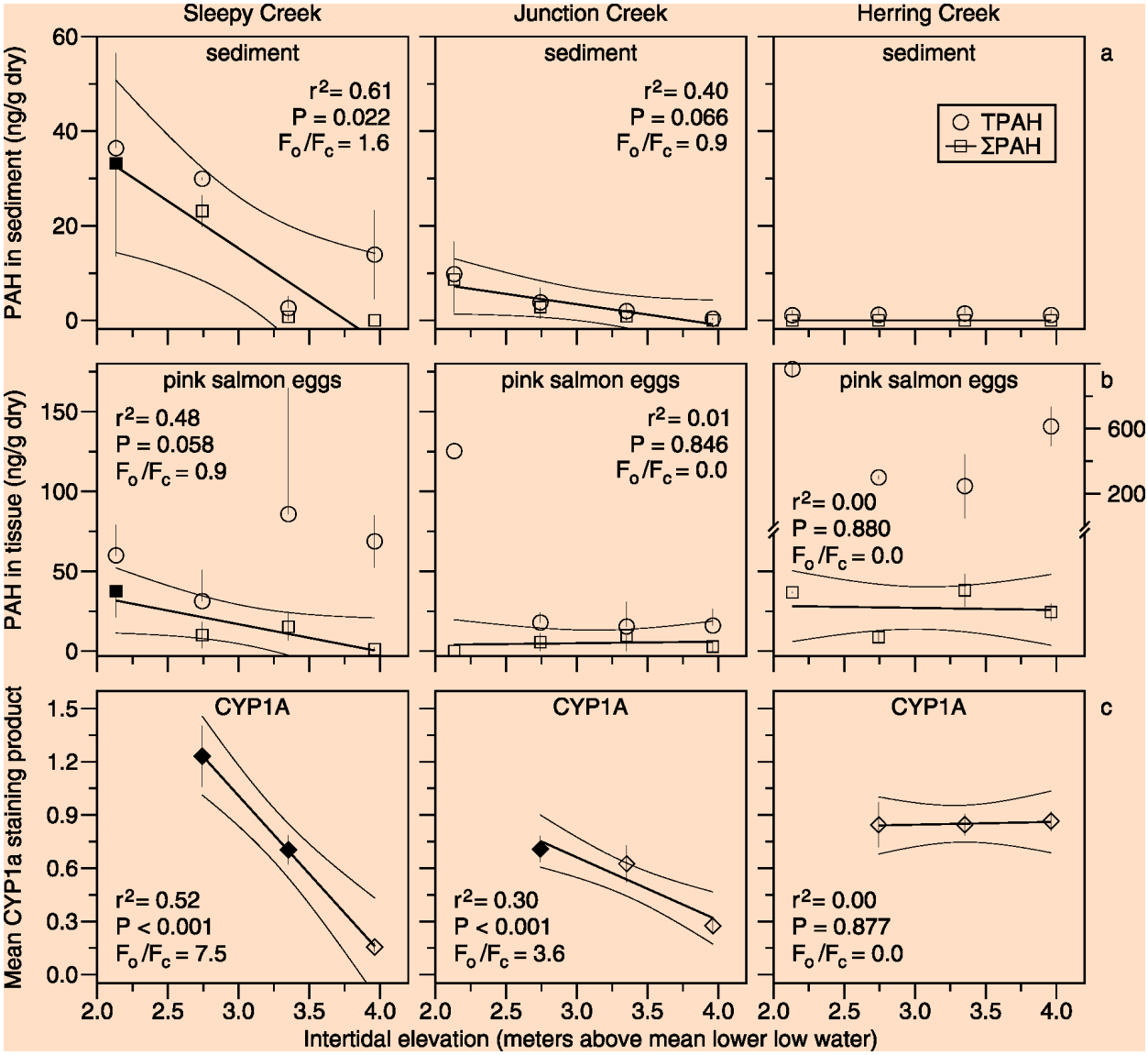

Fig. 2.3. Total polynuclear aromatic hydrocarbon (TPAH) concentrations and sum polynuclear aromatic hydrocarbon (ΣPAH = TPAH minus naphthalene and c1-naphthalene concentrations) in sediment (a) and pink salmon eggs (b) and cytochrome P4501a (CYP1A) staining product (c) in alevins as functions of elevation at three streams in Prince William Sound, Alaska. Fitted lines are linear regressions with 95% confidence bands. Solid symbols indicate significant differences from the uppermost elevations, located above mean high tide and considered stream-specific controls. F_o/F_c is the ratio between the observed F value and the critical F value.

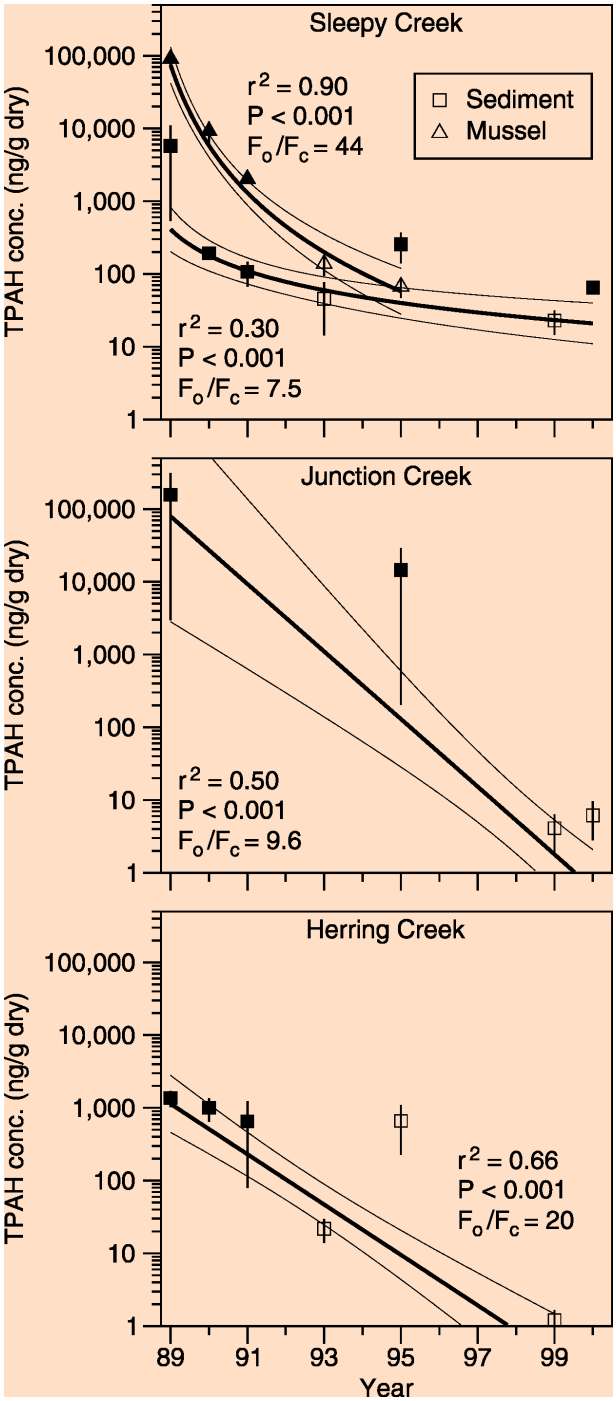

Fig. 2.4. Total polynuclear aromatic hydrocarbon (TPAH) concentrations in sediment and mussels at three previously oiled streams in Prince William Sound, Alaska. Statistics for regressions are printed adjacent to each; bounding curves are 95% confidence bounds. Solid symbols indicate the presence of *Exxon Valdez* oil was verified in one or more contributing samples (Short and Heintz 1997). F_o/F_c is the ratio between the observed F value and the critical F value.

Chapter 3
Cytochrome P4501A induction in oil-exposed pink salmon embryos predicts reduced survival potential

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Abstract

Cytochrome P4501A (CYP1A) induction in pink salmon (*Oncorhynchus gorbuscha*) embryos exposed to crude oil is causally linked to adverse effects at cellular, organismal, and population levels in pink salmon and can be used to predict these responses. When combined with the results of this experiment, designed to examine CYP1A induction during embryonic stages and growth after emergence, results from a series of experiments spanning four other brood years demonstrate that CYP1A induction is related to a variety of lethal and sublethal effects, including poorer marine survival, reduced growth, poorer predator avoidance, and abnormalities. The lowest observed effective total polynuclear aromatic hydrocarbon (TPAH) concentration in water causing significant physiological responses, including reduced size, was the same as that causing CYP1A induction (< 0.94 µg/L). Thus, CYP1A induction can be considered a bioindicator; observation of induction in early life stages at similar exposure levels implies long-term negative consequences for the individual and the population.

Introduction

Induction of mixed function oxidase enzymes, such as cytochrome P4501A (CYP1A), in fish tissue as a result of exposure to axenic hydrocarbons has been used as evidence of oil exposure (e.g., Wiedmer et al. 1996; Mondon et al. 2001; Hodson et al. 2002) and is useful as a biomarker. How CYP1A induction relates to more serious physiological responses has not been completely resolved. In order for CYP1A to be considered a bioindicator, this biochemical change must be correlated or linked to biological effects at organism, population, community, or ecosystem levels of biological organization (McCarty and Munkittric 1996). Such linkage (or lack thereof) will help resolve a controversy involving oil impacts on pink salmon (*Oncorhynchus gorbuscha*) embryos in Prince William Sound after the *Exxon Valdez* oil spill. Wiedmer et al. (1996) found elevated CYP1A in pink salmon alevins from oiled Prince William Sound streams, and Bue et al. (1996; 1998) and Craig et al. (2002) reported elevated embryo mortality, suggesting exposure to oil reduced survival (Rice et al. 2001). However, others dismiss this mortality as a non-oil artifact (Brannon and Maki 1996; Brannon et al. 2001) .

The first steps linking CYP1A induction to short-term adverse physiological responses of pink salmon embryos to oil have been completed but induction had not previously been linked to long-term effects. CYP1A was induced at low total polynuclear aromatic hydrocarbon (TPAH) concentrations, e.g., 4 µg/L (Marty et al. 1997a) and detrimental effects in fish embryos at similar levels has been demonstrated (Marty et al. 1997a,b; Carls et al. 1999; Heintz et al. 1999; 2000; Barron et al. 2003). However, none of these experiments linked induction to long-term physiological consequences. Our goal, therefore, was to examine the usefulness of CYP1A induction as a predictor of short, intermediate, and long-term biological consequences of embryonic exposure to oil. To accomplish this, pink salmon eggs were exposed to a series of low and rapidly declining oil doses that began immediately after fertilization and ended at fry emergence. Samples were collected periodically to determine CYP1A activity. Survival was

assessed at eyeing, emergence, and periodically for the next 5 months. Emergent fry were examined for gross and cellular abnormalities and cultured 5 months for growth measurements. Growth was identified as a integrator of intermediate- and long-term physiological response and because failure to grow rapidly reduces marine survival (Parker 1971; Hargreaves and LeBrasseur 1985). In the discussion, our results are linked to responses to similar oil exposures in previous brood years, including reduced growth and long-term marine survival. To aid data interpretation, we measured polynuclear aromatic hydrocarbon (PAH) exposure, tissue accumulation, and composition, and determined the onset of CYP1A activity, tissue-specific induction levels, and persistence after transfer to clean water.

By causally linking CYP1A to oil exposure effects at cellular, organismal, and population levels, we elevate this biomarker to bioindicator status. Our results support the hypothesis that oil damaged pink salmon embryos spawned intertidally in Prince William Sound. Wiedmer et al. (1996) demonstrated that embryos were exposed to oil, and in another paper we demonstrate how and why oil entered these drainages (Carls et al. 2003). Demonstration of exposure (Wiedmer et al. 1996), field observation of embryo mortality (Bue et al. 1998), laboratory demonstration of short-term damage (Marty et al. 1997a; Heintz et al. 1999) and long-term damage (Heintz et al. 2000), linked to CYP1A induction with this study support field evidence that the *Exxon Valdez* oil spill damaged pink salmon.

Methods

Pink salmon eggs were exposed to a graded dose series (five treatments including control) of weathered Alaska North Slope crude oil. The oil was artificially weathered by heating until its initial mass was reduced by approximately 20% (12 h at 70° C), then sprayed onto tumbling rock (mid diameter 5.4 mm) at different dose levels (0 to 3.8 g oil per kg rock). The resultant PAH composition was dominated by the naphthalenes followed by phenanthrenes, dibenzothiophenes, fluorenes and chrysenes. Homologs with 2-methyl substitutions (C2) generally had the highest concentrations followed by C1 or C3; C4 homologs were least for phenanthrenes and chrysenes, parent compounds were least for all others. Oiled rock was dried 12 to 24 h (at ambient air temperature), then placed in 60 cm tall × 15 cm diameter polyvinyl chloride incubators (27 kg rock per incubator) where it remained dry an additional 13 d. Water flowed 2 d prior to the start of the experiment to further weather the oil and remove any particulates. Flow was 1.6 L min^{-1} and alternated between fresh water (8 h) and saltwater (4 h) to simulate an intertidal environment representative of typical pink salmon spawning habitat. About 2700 pink salmon eggs were added to each incubator circa 2.6 h after fertilization (September 16, 1999). There were 8 replicate incubators per treatment except 2 at the upper extreme. Eggs were removed for approximately 1 h from incubators at eyeing (between days 54 and 58) and mechanically shocked. Survival was recorded and live eggs were returned to their incubators. Alevins hatched between November 25, 1999 and December 9, 1999 and exposure continued until emergence (March 16, 2000 to April 18, 2000). Water temperature declined from 10° C in September to 2° C in late January, then increased to 4° C in late April.

Water and tissue samples were collected periodically (Fig. 3.1) for hydrocarbon analysis. Water samples, 3.8 L, randomly composited from 3 incubators at each treatment level, were extracted immediately with dichloromethane. Tissue samples, about 10 g, were also randomly composited from 3 replicate incubators. Additional replicate tissue samples were also collected at eyeing (day 53) to estimate variance. Tissue and extracted water samples were frozen pending analysis.

At emergence, fish were scored for macroscopic abnormalities (presence or absence). Potential abnormalities included external hemorrhaging, short opercular plate(s), mouth or jaw malformations, deformed caudal fin, pigmentation, bulging eyes, malformed head, pigmentation,

tumors, short or malformed body, starvation, herniated yolk, damaged eyes, twinning, and scoliosis. Abnormalities that correlated positively with initial aqueous total PAH (TPAH) concentration were summarized as total percent with abnormalities; abnormalities that correlated negatively were summarized likewise. Summary data were analyzed with ANOVA (arc-sin transform).

For histopathological and CYP1A analysis, tissue samples (8-16 per treatment) were collected on days 27, 53 (eyeing), 76 (hatching), 146, and 201 d (emergence). Additional fish were sampled weekly for 4 weeks thereafter to determine organ-specific declines in CYP1A induction (n = 12 per treatment, controls and high treatment only). Samples were preserved in 10% phosphate-buffered formalin and transferred to physiological saline after 24 h. Beginning at emergence, larval abdomens were slit open and flushed with preservative to ensure rapid fixation of internal organs. Eggs were manually dechorionated; embryos or larvae were mounted in paraffin. Before final embedment, larger larvae were transected at the level of the anus and again 3 mm caudal to the anus; the caudal fin was discarded. The piece that included head and trunk was embedded in lateral recumbency; the postanal cylinder was embedded transversely. Sections (5 μm) were cut in a parasagittal plane through the body piece and in a transverse plane through the postanal piece. At least six step-sections were saved through each larva and stained with hematoxylin and eosin for histopathology. Six other sections, each from the same level, were saved for immunohistochemical staining.

Immunohistochemistry was performed using a standard streptavidin-biotin-HRP detection system (Olivry 1997) with minor modifications. Sections were deparaffinized, hydrated to 70% ethanol, and then endogenous peroxidase was blocked with 0.3% hydrogen peroxide in methaol for 30 min. After rehydrating with buffer (PBS, pH 7.4) slides were pretreated with steam heat while immersed in a citrate buffer pH 6.1 (Dako Corp.) The steamer (#HS800 Black & Decker, Shelton CT) was preheated for 5 min, the slides in their buffer were added and steamed for 30 min (97-98°C) followed by cooling at room temperature for 20 min. The primary antibody was a mouse anti-fish CYP1A clone (C10-7, a synthetic peptide corresponding to an amino acid sequence in rainbow trout CYP1A, Biosense Laboratories, Bergen, Norway). The antibody was applied at a dilution of 1:1000 and was incubated on the slides for 2 h at room temperature. Negative controls were prepared by omitting the primary antibody and substituting a mouse myeloma IgG correlate. Additional positive and negative control sections were pink salmon larvae from known high dose and control groups from the experiment.

Tissues were analyzed blind for CYP1A expression. Staining intensity and occurrence of CYP1A were evaluated microscopically for each major tissue in every fish, and were each scored on a 5-point scale: none (0), very light (1), light (2), moderate (3), or strong/widespread (4) and their product was used for statistical analysis (Marty et al. 1997). Histopathological examination was also completed blind; samples from each replicate incubator were subdivided into two portions and independently assessed. Fish from the extreme treatment were not examined.

Post-emergence culturing

To evaluate growth, the fish from randomly selected incubators representing each dose were cultured between emergence and early September 2000. From emergence to late July, 22 populations were cultured, 5 each for control, low-, mid-, and high-oil treatments and 2 for the extreme treatment. Each population corresponded to one randomly selected treatment incubator. Populations were merged into one net per dose in late July and cultured until September. Fish were fed a commercially available diet *ad libitum* with automatic feeders during daylight hours.

Lengths (tip of snout to fork of tail) and whole wet weights of fish from each population were measured in late March, April, June and September.

The first 500 emergent fish from randomly selected incubators from each treatment were transferred to 1.2 m × 1.2 m nets suspended in the saltwater estuary at Little Port Walter, Alaska. Lengths (± 0.1 mm) and weights (± 1 mg) of approximately 250 newly emergent fish were measured at random from each incubator after blotting each dry with paper towel. Fish from each treatment except the extreme were transferred daily between March 21-29, 2000; extreme treatment fish were transferred March 22-23, 2000. Salinity averaged 20 ‰ and water temperatures averaged __°C during the transfer period.

All populations in net pens were inventoried and fish were measured between April 1-10. Fish were starved for 24 h before counting; approximately every seventh fish was anaesthetized, measured and returned to the unmeasured population (65 individuals from each population except the extreme). Salinity averaged __‰ and water temperatures averaged __°C (March 21-June 8).

Populations were transferred to micro-raceways (Heintz and Joyce 1992) between June 5-8 and supplied with estuarine saltwater pumped from a depth of approximately 10 m. Raceway volumes (__L) were smaller than net volumes (__L), so populations were reduced to approximately 250 individuals. Every third and fourth fish were transferred from net pens to the micro-raceways; every fourth fish was anaesthetized and measured before transfer. Salinity averaged 28 ‰ and temperatures __°C (June 5-July ??). This allowed the fish to grow in water conditions more similar to those of migrating pink salmon.

In late July, fish were transferred into five 3.5 × 3.5 × 3.5 m nets, to yield a single population per dose. The depth of the nets allowed fish to continue rearing at salinities (__ ‰) and temperatures (__°C) consistent with those of wild migrants. Between August 30 and September 5, populations were inventoried, and approximately 100 fish from each were measured and weighed at random.

Hydrocarbon analysis

Samples were extracted with dichloromethane after addition of six internal standards (Short et al. 1996). Isolation and purification of calibrated and uncalibrated compounds was completed by silica gel/alumina column chromatography followed by size-exclusion high-pressure liquid chromatography and fractionation; seawater samples were not fractionated. Extracts of PAH were separated and analyzed by gas chromatography equipped with a mass selective detector. Calibrated PAH were identified by retention time and two mass fragment ions characteristic of each PAH and quantified using a five point calibration curve. Uncalibrated PAH homologs (which included alkyl-substituted isomers of naphthalene, fluorene, dibenzothiophene, phenanthrene, and chrysene) were identified by retention time and the presence of a single characteristic mass fragment ion. Uncalibrated PAH were quantified by using calibration curves of their respective parent homologs. Experimentally determined method detection limits (MDL) depended on sample weights, and generally were 1 ppb in tissue, and 1 to 8 pptr (ng/L) in water. Concentrations below MDL were treated as 0. The accuracy of the hydrocarbon analyses was about ±15% based on comparison with National Institute of Standards and Technology values, and precision expressed as coefficient of variation was usually less than about 20%, depending on the PAH. TPAH concentrations were calculated by summing concentrations of individual PAH. Reported doses are based on initial TPAH concentrations, thus are preceded by a '<' because exposure concentrations decreased exponentially. Relative PAH concentrations were calculated as the ratio of PAH concentration to the TPAH concentration. Bioconcentration factors (BCF) were calculated by dividing TPAH concentrations in tissue (ng/g dry weight) by initial aqueous TPAH concentration (µg/L) at the time of maximal accumulation; these estimates were

conservative because of the rapidly declining aqueous TPAH concentrations. Consequently, BCF calculations with geometric mean aqueous concentrations as the divisor are also reported.

Alkane extracts were analyzed with a gas chromatograph equipped with a flame ionization detector (Short et al. 1996). Calibrated alkanes (C10-C34 plus pristane and phytane) were summed and are reported as total alkanes.

Statistical analysis

Total PAH concentrations in water and tissue were modeled to describe change over time. Change in aqueous TPAH was modeled with two-compartment exponential decay (TPAH = $ae^{(-bt)} + ce^{(-dt)}$, where a, b ,c and d are estimated constants and t is time in days. A first-order kinetic model that accounted uptake from water and declining aqueous TPAH concentrations was used to predict change in TPAH concentration in tissue (Heintz et al. 1999).

Evaluation of biological responses through emergence

Analysis of variance (ANOVA) was applied to continuous variables, Kruskal-Wallis and Mann-Whitney tests were used to analyze ranked data, and correlation was determined by regression. Percentage data were arc-sine transformed, and corrected for small *n* as necessary before ANOVA (Snedecor and Cochran 1980). 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 less than 0.95 for all comparisons. An exceptionally large error term was frequently evident in the extreme treatment; this was likely due to much smaller sample sizes (2 replicate incubators versus 8 incubators in all other treatments). Where variance was heterogeneous due to the extreme treatment, ANOVA were repeated without this group; conclusions reached were unaltered. Histopathological data were summarized as means by incubator before further analysis to avoid pseudoreplication. For each tissue, CYP1A product scores in the high-oil treatment were compared to controls using the Mann-Whitney rank test, again applying the Bonferroni inequality.

Biological responses were regressed against initial TPAH concentration: models tested were ladder of powers (x-transformations from linear through $-1/x^3$), $y = ae^{bx}$ and $y = a^{bx}$. Reported correlations are based on best fit models. To ensure that correlations were meaningful, we adopted the approach suggested by Draper and Smith (1981) that the F-ratio of a regression (F_o) should exceed the usual significance ratio (F_c) by a multiple of at least 4 times.

The mean of CYP1A product scores was computed for responsive tissues to provide an overview of induction. Tissues with little or no induction were excluded. Tissues with > 3% missing data were also excluded [bulbus endothelium (heart), hepatocytes, cecal epithelium, and spleen endothelium]. Included were gill epithelium and endothelium, pharyngeal epithelium, atrial and ventricular endothelium, vena cava, bile ducts, liver and kidney sinusoidal endothelium, tubular and renal vein endothelium (kidney), anterior intestinal epithelium, yolk-sac endothelial or epithelial cells, skin epithelium and nasal epithelial cells. Products were summed across included tissues and divided by the number present to determine summary means. These means were analyzed by Kruskal-Wallis one-way analysis of variance on ranks with Dunnett's multiple comparison test at hatch and at emergence, the two times for which all treatment groups were present (controls through high treatment).

Evaluation of responses after emergence

Survival in each interval between start of culture and July was evaluated by ANOVA with exposure level as the only factor and cultured populations represented replicate observations. Survival in a given population over a specific interval was calculated as the number alive at the

end of a sampling interval divided by the number alive at the beginning. The absence of replicate populations after July precluded the final observations (September) from analysis.

Overall survival (5.4 months, March to September) was estimated as the product of survival over each of the sampling intervals (start of culture to April, April to June, June to July, and July to September) because populations were randomly reduced in June and July. Variance was estimated; $(\triangle z_j/z_j)^2 = \sum (\triangle a_{ij}/a_{ij})^2$, where a_{ij} is the survival in each interval (i) of the j^{th} oil treatment, $\triangle a_{ij}$ is the standard error of a_{ij}, z_j is overall survival ($z_j = a_{1j} \bullet a_{2j} \bullet a_{3j} \bullet a_{4j}$), and $\triangle z_j$ is the standard error of z_j. Standard errors in the July to September interval ($\triangle a_{4j}$) were assumed to be equal to the maximum variance previously observed in the corresponding treatment. Treatments where 95% confidence intervals ($z_j \pm t_{(0.05,n-1)} \bullet \triangle z_j / \sqrt{n}$) did not overlap those of controls were considered significant. Overall survival was regressed against initial aqueous TPAH, TPAH concentration in tissue at emergence, and CYP1A induction at hatch and emergence: models tested were x-transformations from linear through $-1/x^3$), $y = ae^{bx}$, $y = a^{bx}$, and logistic.

Sizes (length and weight) of fish representing each of the treatments were compared by ANOVA and regressions. Size at emergence, in April, and in June was evaluated by ANOVA, with exposure level as the main effect and population nested in treatments. Because populations were combined in late July, sizes in September were compared by one-way ANOVA with oil treatment as the only factor. Sizes in each treatment were compared to controls with Dunnett's multiple comparison with an overall $\alpha = 0.05$. Weights were converted to natural logs prior to analysis, and the assumption of homogeneity of variance was tested prior to analysis using Levene's test. Lengths and weights were regressed against initial aqueous TPAH, TPAH concentration in tissue at emergence, and CYP1A induction at hatch and emergence: models tested were x-transformations from linear through $-1/x^3$), $y = ae^{bx}$, and $y = a^{bx}$.

Results

Hydrocarbon exposure, accumulation, and loss

Eggs and larvae were exposed to exponentially declining oil concentrations for about 200 d. Initial aqueous TPAH concentrations ranged from 0.9 µg/L (low treatment) to 45 µg/L (extreme treatment) and declined exponentially with time (Fig. 3.1a; Table 3.1). By eyeing (day 54), TPAH concentrations were 1-8% of initial levels and after 184 d (near emergence), aqueous concentrations were <1% of initial values (0.3-0.7%). As a result, arithmetic and geometric mean TPAH concentrations through hatch were considerably lower than initial aqueous concentrations (Table 3.1). Background (control) concentration was 0.015 ± 0.004, $n = 6$.

Tissues rapidly accumulated PAH (Fig 3.1b). The maximum observed TPAH concentration in the high-oil treatment occurred 15 d after exposure began. Estimated times of maximum concentration (dry weight), based on first-order kinetic modeling of all treatments, ranged from 19 to 24 d. Total PAH concentrations in tissue were highly correlated with initial aqueous TPAH concentration at eyeing, hatch, and emergence ($0.991 \le r^2 < 1.000$; $32 \le F_o/F_c \le 2823$; $P < 0.001$).

The relative abundances of PAH in water changed with time, consistent with normal weathering processes (Fig 3.2a-b). Naphthalenes were initially dominant, followed by phenanthrenes, then fluorenes and dibenzothiophenes. By day 54 phenanthrenes were either dominant or approached dominance. Within homologous families, unsubstituted compounds were lost first. Increasingly substituted compounds were typically retained progressively better in the upper two doses. An apparent increase in percent naphthalenes in some treatments at the endpoint (183 d) was likely due to interference as aqueous TPAH concentrations approached detection limits (e.g., Fig. 3.2b).

PAH composition in tissue was increasingly naphthalene dominated, a trend opposite that in water, possibly because phenanthrenes and larger PAH were metabolized more rapidly than

smaller PAH such as naphthalenes (Fig 3.2c-d). Phenanthrenes were the second-most abundant group (typically 10-21%) and chrysenes were the least represented (≤0.7%). Relative phenanthrene concentrations in tissue consistently decreased throughout exposure, despite relative increases in water, suggesting active removal by metabolic action.

Evidence that aqueous alkane and UCM concentrations were related to dose was inconsistent and sporadic. Aqueous total alkane concentrations were initially related to TPAH concentrations ($0.97 \le r^2 \le 0.98$, $9 \le F_o/F_c \le 15$, $0.001 < P \le 0.002$), but there was no correlation at eyeing and hatch ($r^2 < 0.01$), and a trend at emergence was not significant ($r^2 = 0.68$, $F_o/F_c = 0.6$, $P = 0.084$). Aqueous UCM concentrations were never significantly correlated with initial aqueous TPAH concentration and typically no trends were evident ($0.01 < r^2 \le 0.55$, $0.0 < F_o/F_c \le 0.1$, $0.256 < P \le 0.964$). Alkane concentrations in tissue were not correlated with treatment ($0.06 \le r^2 \le 0.11$, $F_o/F_c \le 0.1$, $0.572 < P \le 0.685$) and the UCM was below detection in tissue at eyeing, hatch, and emergence.

Biological response through emergence

Embryo survival was reduced by exposure to oil (Fig. 3.3a). Survival at emergence was significantly depressed (6-35%) in the upper two treatments (TPAH < 16.5 µg/L; $0.001 < P_{ANOVA} \le 0.006$). Earlier survival (at eyeing) trended slightly downward as dose increased but was not significantly reduced ($r^2 = 0.16$, $P = 0.020$, $F_o/F_c = 1.4$; $P_{ANOVA} = 0.146$).

Incidences of several macroscopic abnormalities increased and were positively correlated with exposure to oil (Fig. 3.3b); incidences of other abnormalities were either negatively correlated with exposure or were uncorrelated. Positively correlated abnormalities were ascities ($r^2 = 0.78$), bulging eyes (0.77), malformed head (0.54), short opercular plate(s) (0.50), external hemorrhaging (0.49), mouth or jaw malformations (0.42), and deformed caudal fin (0.39). In each of these cases, $F_o/F_c \ge 5$ and $P < 0.001$, strong support for a significant relationship. Unusual pigmentation ($r^2 = 0.33$) and tumors (0.13) were negatively correlated with exposure ($1 \le F_o/F_c \le 4$; $0.001 < P \le 0.037$), probably because embryos with these developmental problems were less likely to survive oil exposure. No other abnormalities were correlated with oil exposure ($0.01 \le r^2 \le 0.10$, $0.1 \le F_o/F_c \le 0.9$; $0.064 \le P \le 0.508$), but the incidence of body malformation, herniated yolk, and twinning fell to zero in the extreme dose, suggesting a possible negative relationship at high oil concentrations (< 45 µg/L TPAH). The combined incidence of all positively correlated abnormalities was significantly elevated in the upper two treatments (TPAH < 16.5 µg/L; $0.001 < P_{ANOVA} \le 0.011$; Fig. 3.3b) and the combined incidence of negatively correlated abnormalities was depressed in these treatments ($0.001 < P_{ANOVA} \le 0.013$).

Some histological changes were positively correlated with TPAH; others were negatively correlated or uncorrelated. Extreme-treatment tissues were not examined. Positively correlated with TPAH concentration were proteinaceous ascites ($r^2 = 0.27$), hepatocellular necrosis (0.21), peritonitis (0.20), epidermal cell apoptosis (0.18), and gastric epithelial apoptosis (0.14) ($1 \le F_o/F_c \le 3$). Proteinaceous ascites was significantly elevated in the high-oil treatment ($P_{Kruskal\text{-}Wallis} = 0.047$; Fig. 3.4). Negative correlates were digestive enzyme activity ($r^2 = 0.43$, $F_o/F_c = 5$), fat (0.35, $F_o/F_c = 4$), and epidermal thickness ($1 \le F_o/F_c \le 3$; $0.17 \le r^2 \le 0.26$). Ventral epidermal thickness was significantly depressed in the high-oil treatment (TPAH < 16.5 µg/L; $P_{Kruskal\text{-}Wallis} = 0.030$) and digestive enzyme activity was significantly depressed in all oil treatments (TPAH < 0.94 µg/L; $P_{Kruskal\text{-}Wallis} = 0.002$) (Fig. 3.4). Trends were positive for renal tubular necrosis and hepatocellular lipidosis and negative for yolk stores, olfactory organ apoptosis, and hepatic glycogen storage.

CYP1A induction

CYP1A was induced by exposure to oil and typically remained at elevated levels until after emergence. Induction was correlated with initial aqueous TPAH concentration at hatching and emergence ($0.66 \leq r^2 \leq 0.82$, $P < 0.001$, $30 \leq F_o/F_c \leq 71$) (Fig. 3.3c). The lowest observed effective concentration (LOEC) was < 0.94 μg/L (at hatch) and CYP1A was consistently induced at < 3.7 ug/L. At hatch, CYP1A was significantly correlated with TPAH in 13 of 26 tissues analyzed ($0.26 \leq r^2 \leq 0.77$, $5 \leq F_o/F_c \leq 51$; $P < 0.001$). At emergence, CYP1A was significantly correlated with TPAH in 16 of 27 tissues analyzed ($0.27 \leq r^2 \leq 0.89$, $6 \leq F_o/F_c \leq 123$; $P < 0.001$).

A variety of CYP1A induction patterns were evident, including those that peaked early, then declined (11 of 34 tissues; choroidal rete, ventricular endothelium, bile ducts, sinusoidal endothelium (kidney), tubular and glomerular epithelium (kidney), renal vein endothelium, gastric epithelium, anterior and distal intestinal epithelium, and yolk-sac endothelial or epithelial cells), those that peaked about midway (150 d; 9 tissues, gill arch endothelium, pharyngeal epithelium, atrial and bulbus endothelium, vena cava, sinusoidal endothelium (liver), larger liver veins, spleen endothelium, and skin epithelium), and a minority that increased until emergence (2 tissues, gill epithelium and nasal epithelial cells) (e.g., Fig. 3.5). Little or no induction was evident in 10 of 34 tissues (eye lens, maxillary, periocular, pectoral, and vetebral perichondrial cells, pseudobranch vessels and glandular cells, archinephric duct epithelium, pancreatic acinar cells, and gonads).

The least mature embryos examined (day 27) had high CYP1A induction (CYP1A product > 5 units above control product). On day 27, shortly after the estimated onset of eye pigmentation (Smirnov 1975), induction was high in 11 of 34 tissues; gill arch endothelium, pharyngeal epithelium, ventricular endothelium, hepatocytes, sinusoidal endothelium (kidney), tubular epithelium, renal vein endothelium, gastric epithelium, anterior intestinal epithelium, yolk-sac endothelial or epithelial cells, and skin epithelium.

CYP1A induction in controls tended to increase towards emergence and often increased abruptly after emergence (e.g., Fig 3.5b-c). While these changes in baseline did not obscure dose-induced relationships, they clearly indicate that unknown endogenous or exogenous inducers were present, particularly after emergence. Increased induction in controls indicates that suitable reference eggs are necessary for interpretation of induction in field studies. After emergence, induction suddenly increased in 10 of 26 control tissues (gill epithelium and endothelium, atrial endothelium, vena cava, sinusoidal endothelium (liver), larger liver veins, gastric epithelium, cecal epithelium, and anterior and distal intestinal epithelium).

CYP1A induction in high-treatment fish merged with control induction after emergence (transfer to clean water), a process that ranged from < 1 week to > 3 weeks (e.g., Fig. 3.5). Of the 16 tissues with significantly elevated CYP1A at emergence, 5 returned to baseline within 1 week (bulbous endothelium, tubular epithelium (kidney), cecal epithelium, anterior intestinal epithelium, and spleen endothelium), 7 within 2 weeks (gill epithelium and endothelium, pharyngeal epithelium, sinusoidal endothelium (liver), larger liver veins, skin epithelium, and nasal epithelial cells), 3 within 3 weeks (ventricular endothelium, sinusoidal endothelium (kidney), and renal vein endothelium), and 1 within 4 weeks (atrial endothelium).

Post-exposure survival, size, and growth

Overall survival of fish exposed as embryos and cultured in clean water from March to September was correlated with initial aqueous TPAH ($r^2 = 0.95$, $P = 0.005$, $F_o/F_c = 5.3$), TPAH in tissue at emergence ($r^2 = 0.88$, $P = 0.018$, $F_o/F_c = 2.2$), and CYP1A induction at hatch and emergence ($0.87 \leq r^2 \leq 0.93$, $0.008 \leq P \leq 0.021$, $1.9 \leq F_o/F_c \leq 4.1$). Overall survival was significantly less in the extreme oil treatment (TPAH < 44.7 μg/L) than in controls (Fig. 3.6). Fish from the extreme-oil treatment consistently had the lowest average survival rates in each

observation interval. Survival in the highest treatment in earlier time intervals (e.g., July to September) was consistently less than in controls but did not differ significantly.

Fish from all oil treatments were consistently shorter than controls and declined in a dose-dependent fashion (Fig. 3.6). High-treatment fish were significantly shorter than controls at all times; mid-treatment fish were significantly shorter at emergence and in September. Extreme-treatment fish were also significantly shorter at emergence. Because only the most robust extreme-treatment fish apparently survived, length in this treatment did not follow general trends in September. (Fish length in the extreme treatment followed expected patterns in early June, prior to increasingly poor survival in this treatment.) Differences in mean length among treated fish and controls were small at emergence (0.2-1.4 mm) and increased with time (1.8-6.9 mm in September). Excluding the extreme-treatment response, fish length in September was related to initial aqueous TPAH concentration, TPAH concentration in tissue at emergence, and CYP1A induction at hatch and emergence ($P_{\text{regression}} \leq 0.001$, $6.6 \leq F_o/F_c \leq 10.4$) but correlation was consistently very poor because length varied widely in each treatment ($0.08 \leq r^2 \leq 0.09$). Similar dose-dependent length declines were evident at each earlier observation time and depressed lengths in the extreme treatment followed expected patterns. The LOEC for reduced length was < 3.7 μg/L aqueous TPAH in September.

Fish mass also declined in a dose-dependent fashion (Fig. 3.6). Weight at emergence was significantly depressed only in the extreme treatment. As with lengths, differences among treated fish and controls increased with time, and in September weights in low-, mid-, and high-oil treatments decreased systematically with exposure level and were significantly less than in controls ($P_{\text{ANOVA}} < 0.001$). Because only the most robust extreme-treatment fish apparently survived, mass in this treatment did not follow general trends in September. (Fish mass in the extreme treatment followed expected patterns in early June, prior to increasingly poor survival in this treatment.) Excluding the extreme-treatment response, fish mass in September was related to initial aqueous TPAH concentration, TPAH concentration in tissue at emergence, and CYP1A induction at hatch and emergence ($P_{\text{regression}} \leq 0.001$, $3.6 \leq F_o/F_c \leq 6.6$) but correlation was consistently very poor because wet weight varied widely in each treatment ($0.03 \leq r^2 \leq 0.06$). The LOEC for reduced mass was < 0.94 μg/L aqueous TPAH in September.

Discussion

Induction of CYP1A in embryos predicted short- and intermediate-term physiological responses and survival in pink salmon embryos exposed to crude oil during incubation. Later in this discussion we link induction to long-term effects including population survival by drawing parallels between this and previously published work. Abnormalities were caused by 7 months exposure to exponentially declining aqueous TPAH concentrations and size differences became more pronounced in the months after emergence to clean water. Aqueous LOECs for CYP1A induction and organism response were both < 0.94 μg/L TPAH. CYP1A levels typically merged with control levels less than a month after exposure ended, thus observation of elevated levels in fish with unknown histories indicates recent (or current) exposure. Together these results demonstrate that CYP1A induction can be considered a bioindicator, thus observation of induction in early life stages implies long-term negative consequences for the individual and the population.

Depressed size as a result of embryonic exposure to oil not only implies ultimately smaller fish, it predicts reduced marine survival. Size differences between oil-treated fish and controls were small at emergence but became progressively greater in the months that followed as oiled fish failed to catch up. Rapid fry growth after emigration to the marine environment is important to escape mortality from size-selective predation (Parker 1971; Healey 1982; Hargreaves and LeBrasseur 1985), thus placing oil-exposed fish at a disadvantage. Oil-exposed fish were less

capable of avoiding predation in controlled tests with chinook (*O. tshawytscha*) smolts (unpublished data), a typical predator in natural systems. Heintz et al. (2000) reported decreased growth rates in fish exposed to <18.0 μg/L TPAH concentrations. Consistent with reduced growth in experimentally oiled fish, the growth of pink salmon fry in Prince William Sound in 1989 was reduced by marine exposure directly to *Exxon Valdez* oil in the water or in food (Carls et al. 1996; Wertheimer and Celewycz 1996; Willette 1996). In years subsequent to this spill, intertidally spawned pink salmon eggs were exposed to contaminated water that drained from oiled sediment (Carls et al. 2003) surrounding about 31% of Prince William Sound salmon streams (e.g., Brannon et al. 1995; Bue et al. 1996; Geiger et al. 1996; Murphy et al. 1999). Teratogenic effects were observed in coho (*O. kisutch*) salmon eggs exposed 1 d to pore-water and sediment extracts from a heavily oiled site in Prince William Sound (Wolfe et al. 1995), demonstrating continued toxicity of oil weathered under natural conditions. CYP1A was induced in alevins from some of these streams for > 2 y after the spill (Wiedmer et al. 1996), direct evidence of exposure. Our results suggest these chronic exposures likely reduced growth, hence the marine survival of these wild pink salmon populations, a conclusion also reached by Geiger et al. (1996).

Reduced marine survival of pink salmon adults exposed to oil during embryonic development has been directly observed in three different brood years (1993, 1995, and 1998; Heintz et al. 2000). Exposure methods in each year were the same as those employed in this experiment, and the LOEC for reduced marine survival was < 5.2 μg/L (Heintz et al. 2000). Depressed marine survival was consistently correlated with depressed growth rate 4 to 10 mo after emergence and was a more sensitive measure of significant response in 1995 fish than growth rate.

Similarities between the current experiment and previous pink salmon studies provide the link between CYP1A induction and population effects. In this experiment, reduced size was related to aqueous TPAH concentration, TPAH concentration in tissue, and CYP1A induction. In the Heintz et al. (2000) experiment, oil exposure depressed growth and marine survival. CYP1A induction was not examined in that experiment but current results clearly demonstrate that induction must have also occurred in these earlier brood years (1993, 1995, and 1998). Consistent with this hypothesis was observation of CYP1A induction in oil-exposed fish from the 1992 brood year (Marty et al. 1997a). Thus, the meta-experiment, which includes embryos exposed in 1992, 1993, 1995, 1998, and 1999, demonstrates that CYP1A induction is linked to reduced marine survival, ergo population-level effects.

PAHs are clearly implicated as the toxins responsible for embryo damage in this and similar studies; conversely, correspondence with aliphatic exposure and accumulation was poor. The CYP1A subfamily of cytochrome P450 is induced by planar hydrocarbons, including PAH (e.g., Ronis et al. 1992; Stegeman et al. 2001), thus tissue exposure and cellular response to PAH are indicated. The toxicity of PAH to marine organisms has been consistently demonstrated, and progressively higher molecular weight PAH are increasingly toxic (e.g., Anderson et al. 1974; Moore and Dwyer 1974; Rice et al. 1977; Hutchinson et al. 1980; Black et al. 1983; Neff 1985). PAH are oxidized by CYP4501A (Livingstone et al. 1990; Akcha et al. 2000) and the resultant intracellular water-soluble metabolites are often toxic (e.g, Ma 2001). The resultant potential problems, including cell damage, mutagenesis, teratogenesis, genotoxicity, and cancer (e.g., Longwell 1977; Hose et al. 1981; Neff 1985; Schirmer et al. 1998; White et al. 1999), can require prolonged observation to discern.

Alternative toxins (ammonia, sulfides, and bacterial metabolites) suggested by some (Neff et al. 2000; Pearson 2002; Page et al. 2002) for the oiled-rock column method employed in this and similar experiments do not explain toxic responses. High water flow and reasonable loading ensured adequate oxygenation and inconsequential ammonia and sulfide concentrations. Indeed,

Pearson (2002) demonstrated that only when water is static did ammonia accumulate in similar oiled-rock columns, a situation that never occurred in our tests. Metabolites potentially released into the water by oil-degrading microbes likely contributed little to toxicity. Nutrient addition (nitrogen and phosphate) was required to initiate microbial growth in both water-column and oiled sandy-gravel column assays designed to study oil degradation (Gibbs and Davis 1976; Middaugh et al. 1996; 1998; Shelton et al. 1999; Albert Venosa, U.S. EPA, personal communication). Further, the time required for hydrocarbon-utilizing microbes to begin to degrade substantive quantities of hydrocarbons apparently requires 1-3 weeks (Gibbs and Davis 1976; Shelton et al. 1999). Although nutrient levels may have slowly increased in our relatively long pink salmon assays (0.5 yr), significant toxic effects were observed in Pacific herring embryos similarly exposed for 4 days (Carls et al. 1999). Comparable toxic response in these two species [(LOECs were 4-18 µg/L TPAH for pink salmon (Marty et al. 1997a; Heintz et al. 1999) and 9 µg/L for herring (Carls et al. 1999)] at very different time scales argues against microbial metabolites as the critical toxins. LOECs (17 µg/L) were also comparable in a static renewal assay of high-energy water-accommodated fraction of weathered Alaska North Slope crude oil on herring larvae where possible microbial contact with oiled water was limited to 24 h (Barron et al. 2003). We conclude that PAH, and possibly unidentified heterocyclic compounds with similar planar structures, are principally responsible for the observed toxicity. However, we do not discount the possibility that metabolic byproducts could contribute to toxicity both in the laboratory and in intertidal settings. Polar oxidation products were detected in sediment and pore-water collected from a heavily oiled site in Prince William Sound a year after the *Exxon Valdez* spill (Wolfe et al. 1995). Nutrient availability for microbial growth in intertidal sediment is likely better than in our laboratory experiments where seawater was filtered before entering experimental apparatus and bare washed rock was obtained from terrestrial deposits (e.g., Carls et al. 1999). Yet even in Prince William Sound beaches, biodegradation was nutrient limited and the addition of fertilizer (as a bioremediation procedure) increased populations of oil-degrading microbes (e.g., Pritchard et al. 1992; Prince and Bragg 1997). We also suggest that bacterial metabolites are less likely to bioaccumulate in incubating fish eggs than parent PAH because they are more soluble in water (e.g. Wolfe et al. 1995).

The least mature embryos examined (about the time of eye pigmentation) exhibited strong CYP1A induction in oil treatments, evidence that the cellular enzymes responsible for internal metabolite formation are active early in development and that embryonic tissues were exposed to PAH. CYP1A induction has been observed in the early life stages of other fish, including newly hatched cod larvae (*Gadus morhua*; Goksøyr et al. 1988) and *Fundulus* embryos (Binder and Stegeman 1980).

Transfer of PAH from lipid storage to the most metabolically active tissues may explain why CYP1A activity peaked in some tissues about 150 d after exposure began, after aqueous PAH concentrations had declined to roughly 1% of their original levels. Jørgensen et al. (2002) suggest that the toxicological potential of persistent organic pollutants may be enhanced in species that undergo cycles of lipid accumulation and depletion. In developing salmon embryos, PAH sequestered in yolk lipids may transfer to embryonic tissue well after concentrations in surrounding water decline. The highest induction levels were in tissues outside the liver, consistent with the distribution in *Fundulus* embryos (Binder and Stegeman 1980), indicating non-hepatic exposure to transferred PAH and relatively high metabolic activity in these tissues.

Declining internal PAH concentrations likely controlled declines in CYP1A induction after fish emerged into clean water. Similar to results of our study, Gagnon and Holdway (1998) found EROD (ethoxyresorufin O-de-ethylase) activity in juvenile Atlantic salmon (*Salmo salar*) remained elevated for 8 d after exposure to WSF of Bass Strait crude oil ended and Collodi et al. (1984) found AHH (aryl hydrocarbon hydroxylase) activity in coho salmon (*O. kisutch*) smolts

induced by Cook Inlet Crude oil persisted for roughly 7-15 d. Persistence of induction was increased by exposure dose and duration (Collodi et al. 1984). Possibly, EROD activity may persist for longer periods (>2 months; George et al. 1995), but exposures were not controlled in this environmental study and we suspect that undetected PAH may have been present longer than directly observed in tissue after the Braer spill. Elimination of organic xenobiotics following short-term exposure is usually rapid and complete, but slower and less complete after long-term exposure (Kocan and Landolt 1984; Livingstone and Pipe 1992) and elimination of higher molecular weight PAH is slower than elimination of lower molecular weight compounds (e.g., Varanasi and Gmur 1981). Immunoassay procedures to determine CYP1A induction such as those used herein may be less sensitive than the AHH assay, but are less prone to unexplained variability than EROD assays (Collier et al. 1995).

We conclude that induction of CYP1A is causally linked to adverse effects at cellular, organismal, and population levels in pink salmon and can be used to predict these responses. Exposure of pink salmon embryos and larvae to oil caused a variety of lethal and sublethal effects. LOECs causing CYP1A induction (<0.94 μg/L) also caused reduced mass and digestive enzyme activity, a response likely directly associated with poorer fish growth. At aqueous TPAH concentrations < 3.7 μg/L both length and weight were depressed six months after exposure ended. The combined results from a series of embryo-larval exposure experiments spanning four brood years are consistent and demonstrate that CYP1A induction is related to a variety of lethal and sublethal effects, including abnormalities, reduced growth, poorer predator avoidance, and diminished marine survival. Thus, CYP1A induction can be considered a bioindicator; observation of induction in early life stages implies long-term negative consequences for the individual and the population at similar exposure levels.

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Table 3.1. Initial and mean aqueous TPAH concentrations by treatment ± standard error. Each water sample was a random composite of 3 incubators; n = 1 for initial observations and $4 \leq n \leq 5$ for mean observations. Arithmetic and geometric mean concentrations were calculated for the interval between first exposure and hatch.

	control	**low**	**mid**	**high**	**extreme**
initial	0.02	0.94	3.70	16.52	44.67
arithmetic	0.02 ± 0.00	0.22 ± 0.18	0.94 ± 0.69	3.81 ± 3.19	11.88 ± 10.93
geometric	0.02 (0.01-0.02)	0.07 (0.03-0.13)	0.39 (0.21-0.71)	0.75 (0.30-1.91)	2.13 (0.74-6.17)

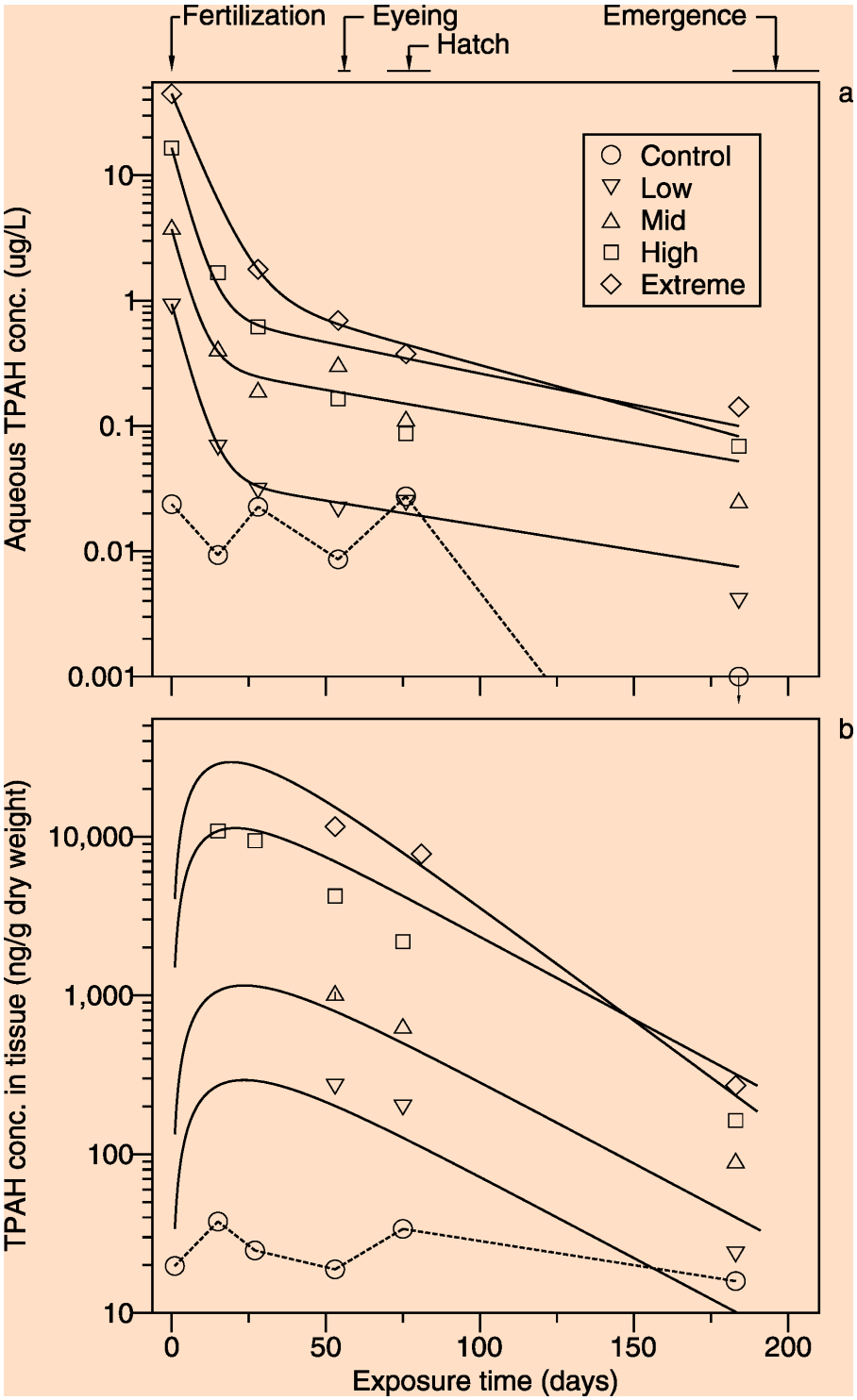

Fig. 3.1. Total polynuclear aromatic hydrocarbon (TPAH) concentrations in water and egg or larval tissue from fertilization until emergence. Aqueous models are two-compartment exponential; tissue models are first-order kinetic that account for uptake from water and declining aqueous TPAH concentrations.

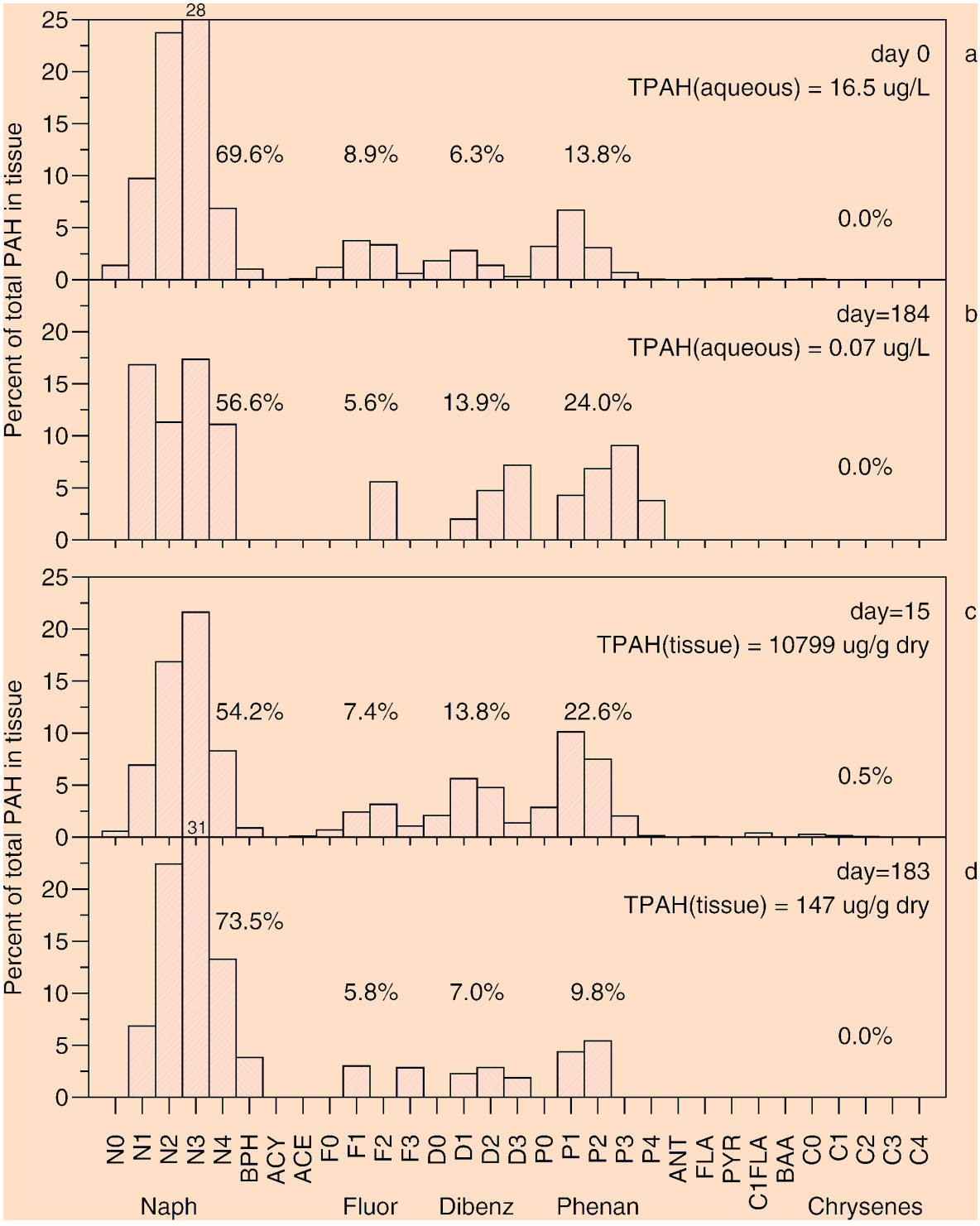

Fig. 3.2. Example polynuclear aromatic hydrocarbon composition in high-oil treatment water (a-b) and in egg or larval tissue (c-d). Printed inside each panel are total percentages of the following homologous groups: naphthalenes (naph), fluorenes (fluor), dibenzothiophenes (dibenz), phenanthrenes (phenan), and chrysenes.

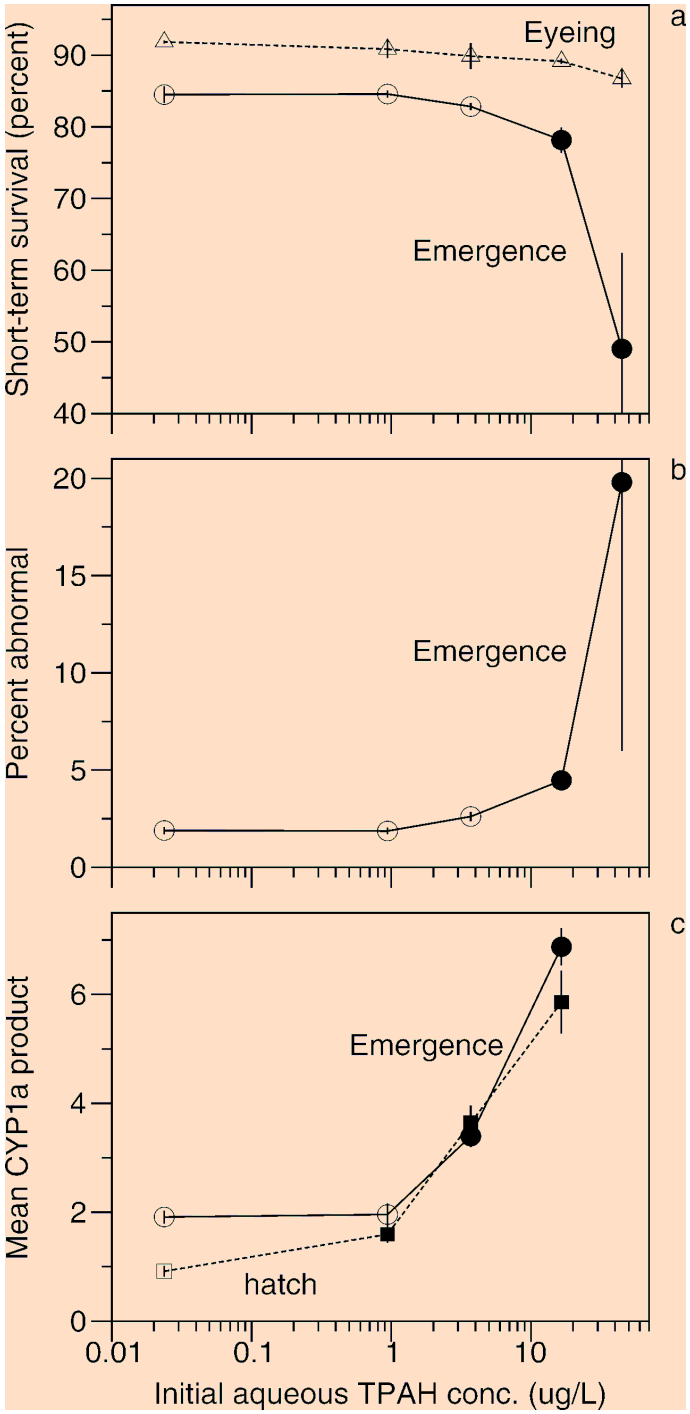

Fig. 3.3. Mean short-term larval survival, incidence of macroscopic abnormalities, and cytochrome P4501A (CYP1A) induction (± SE) as related to initial total polynuclear aromatic hydrocarbon (TPAH) concentration in water. Observation times are indicated for each data set (eyeing, hatch, or emergence). Abnormalities summarized in "b" are those where incidence increased with oil exposure (see text). The mean CYP1A product summarizes 15 responsive tissues (of 35 examined; see text). Solid symbols indicate significant differences from control response.

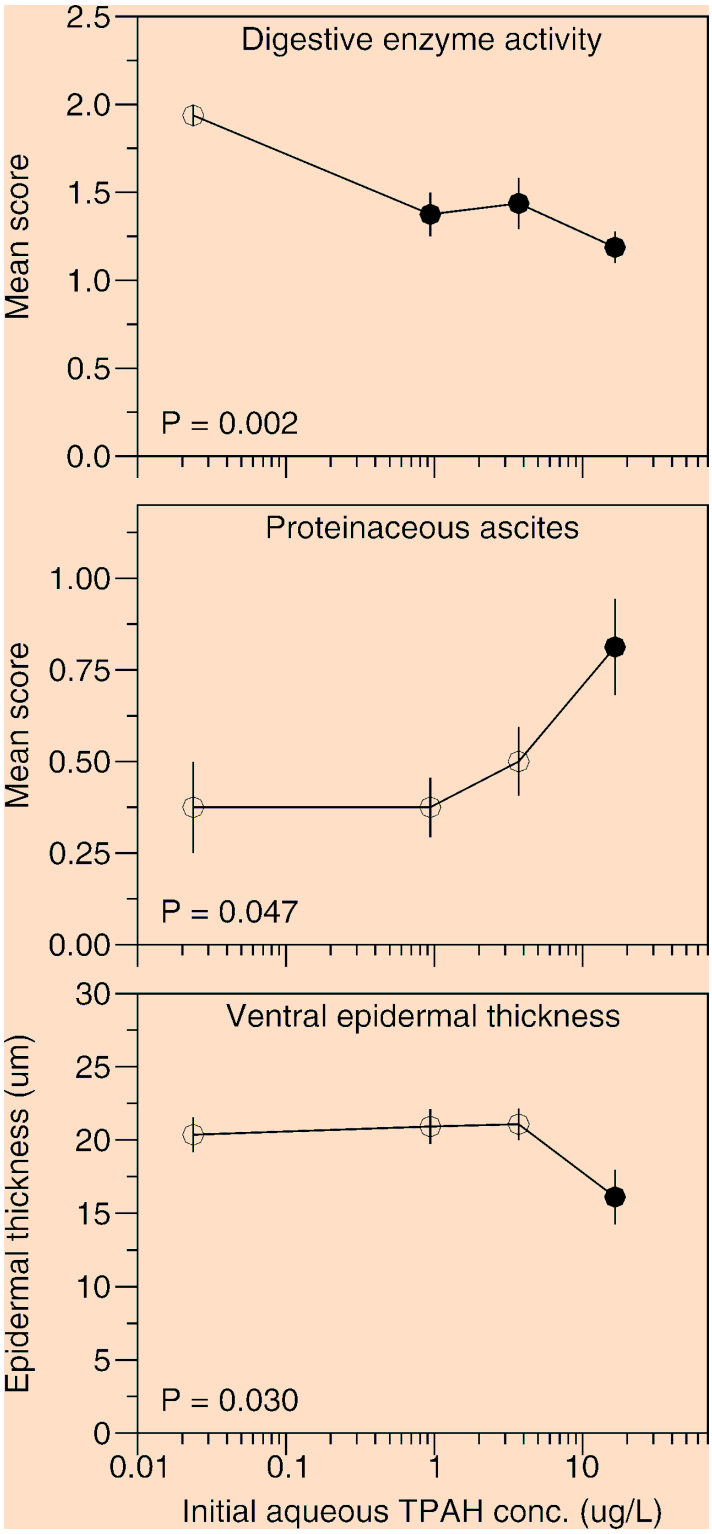

Fig. 3.4. Example mean (± SE) histological changes in emergent fry (of 20 responses monitored) as related to initial total polynuclear aromatic hydrocarbon (TPAH) concentration in water. Solid symbols indicate significant differences from control response.

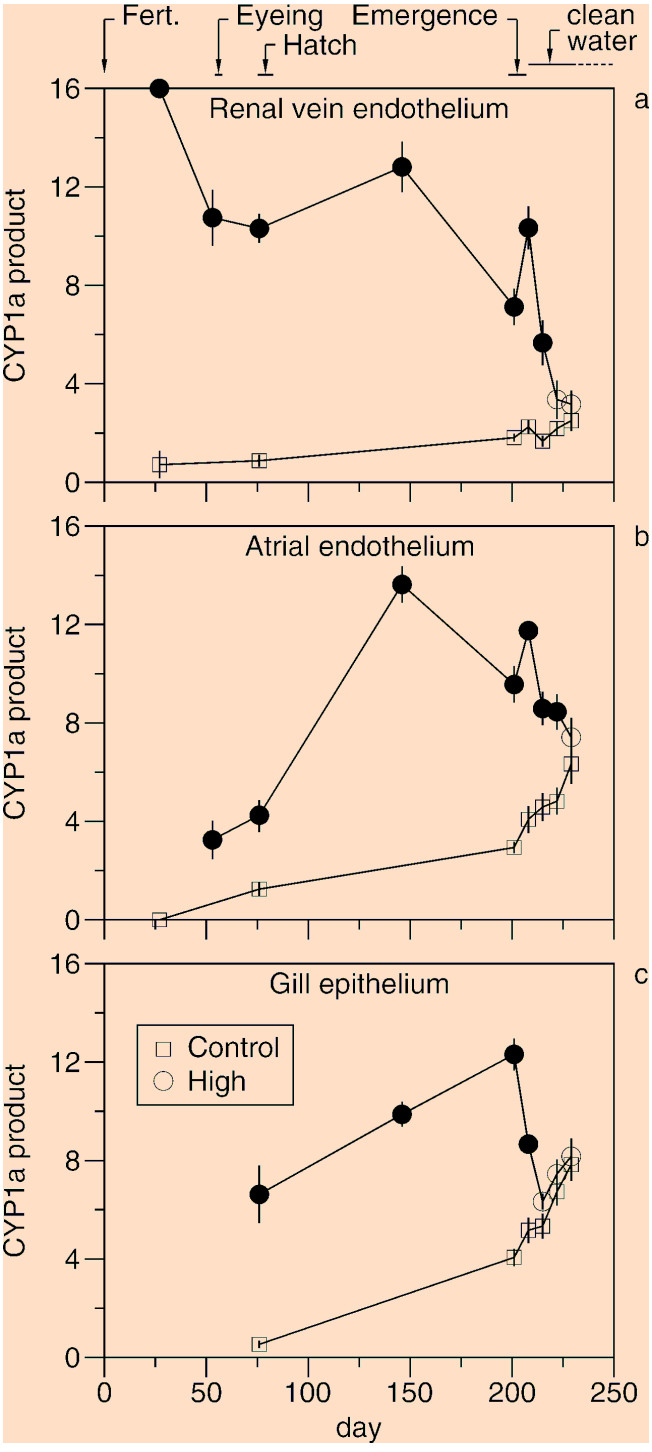

Fig. 3.5. Summary of cytochrome P4501A (CYP1A) induction in three tissues (of 34 examined) across time (mean response ± SE). These patterns typify responses in other tissues: a) rapid induction, followed by decline (11 tissues), b) activity peak about midway through treatment (9 tissues), and c) increasing activity until emergence (2 tissues). Solid symbols indicate significant differences from controls (Mann-Whitney test).

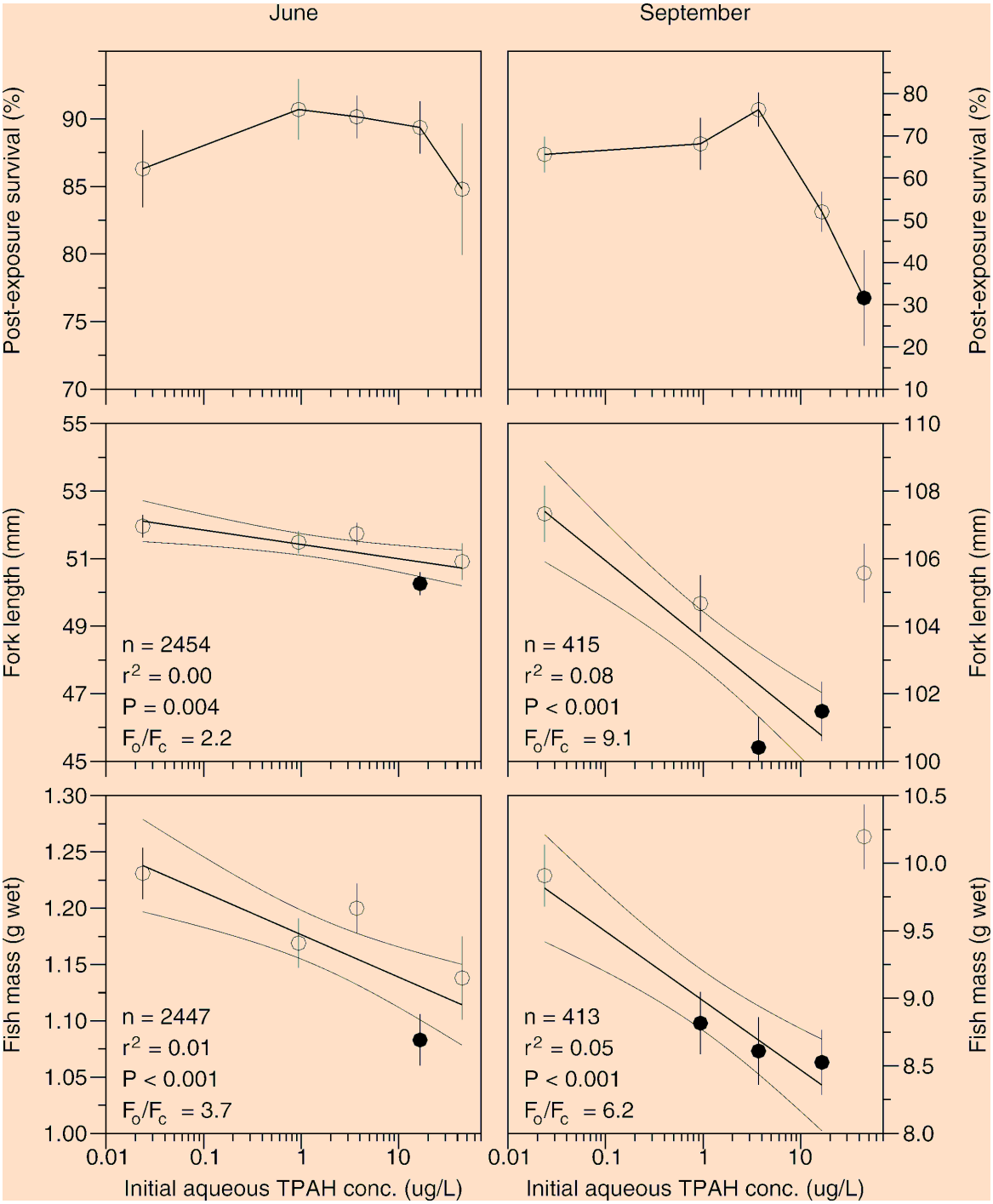

Fig. 3.6. Mean fish survival, length, and weight (± SE) 2.5 months (June) and 5.4 months (September) after emergence as related to initial total polynuclear aromatic hydrocarbon (TPAH) concentration in water. Solid symbols indicate significant differences from control response. By September, length and weight at <45 µg/L were likely biased upward by selective mortality in this treatment, leaving only the most robust fish for measurement. Thus, the <45 µg/L treatment was not included in the regressions.

Chapter 4
Monitoring polynuclear aromatic hydrocarbons in aqueous environments with passive low-density polyethylene membrane devices

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Abstract

Low-density polyethylene membranes, typically filled with triolein, have been previously deployed as passive environmental samplers designed to accumulate nonpolar hydrophobic chemicals from water, sediments, and air. Hydrocarbons in such samplers, known as semi-permeable membrane devices (SPMDs), diffuse through pores in the membranes and are trapped in the central hydrocarbon matrix, mimicking uptake by living organisms. Here we describe laboratory and field verification that low-density polyethylene membrane devices (PEMDs) without triolein provide reliable, relatively inexpensive time-integrated hydrocarbon sampling from water. For comparison, polynuclear aromatic hydrocarbon (PAH) uptake in SPMDs and pink salmon eggs was also studied. Total concentrations of PAH accumulated by PEMDs were highly correlated with concentrations in water ($r^2 \geq 0.99$) and linear over the range tested (0 to 17 µg/L). Higher molecular mass PAH preferentially accumulated in PEMDs and in pink salmon eggs but the source of oil in PEMDs remained identifiable. Accumulations of PAH were highly similar to those in SPMDs. The PEMDs retained about 78% of accumulated total PAH for 40 days in clean water. Thus, a simple plastic membrane can be conveniently used for environmental monitoring, particularly in situations where contaminant concentrations are low (in the parts per billion range), variable, and intermittent.

Introduction

Environmental water quality sampling options for detection of organic hydrocarbons typically include direct extraction of water samples, monitoring of biological tissue, and passive chemical sampling devices. Direct measures of hydrocarbon concentrations in water relate directly to water quality standards but discrete sampling can easily miss intermittent pulses of contaminants, or quantities may be below practical detection limits [1-3]. Mussels or other biological tissue may be used, if present and available, to monitor hydrocarbons but compounds are typically actively or passively exported and often metabolized, reducing the probability of detection [3-5]. In addition, separation of trace-level contaminants from a biological matrix can be problematic. Passive sampling devices have emerged in recent years that have excellent detection sensitivity, integrate aqueous concentrations over time, and are cheaper and easier to analyze than biological tissue [1,6,7].

Low-density polyethylene membranes, typically filled with triolein, have been successfully deployed as passive environmental samplers designed to accumulate nonpolar hydrophobic chemicals from water, sediments, and air [e.g., 1,8-11]. Hydrocarbons in such biomimetic samplers, known as semi-permeable membrane devices (SPMDs), diffuse through pores in the polyethylene and are trapped in the central hydrocarbon matrix, mimicking uptake by living organisms (but without metabolism). For example, uptake of chlorinated hydrocarbon and pesticide congeners was similar in SPMDs, mussels, and fish [6,10,12].

At low ambient concentrations of organic compounds, low-density polyethylene membrane devices (PEMDs) deployed without inclusion of the central hydrocarbon reservoir may

be simpler and less expensive sampling devices than SPMDs, yet provide the same benefits. Although the idea of using PEMDs is not novel, little research concerning them has been published in the primary literature (e.g., [13]). We suspected that PEMDs would not become saturated at total polynuclear aromatic hydrocarbon (TPAH) concentrations of a few μg/L (e.g., 15 μg/L TPAH, the legal water quality limit in Alaska), suggesting the suitability of the simplified sampler design.

Our objective, therefore, was to verify the effectiveness and reliability of simple PEMDs to sample low concentrations of polynuclear aromatic hyrocarbons (PAH), with a series of laboratory and field tests. Motivating factors were the 1989 *Exxon Valdez* oil spill, now more than a decade old, and concerns about petroleum hydrocarbon entry into watersheds proximal to urban areas. In both cases we wished to determine if PAH were biologically available and could pose risks to sensitive organisms at sensitive life stages, such as pink salmon (*Oncorhynchus gorbusha*) embryos, and recognized that such exposures were likely intermittent. Specifically, we compared PAH accumulation (concentration and composition) in PEMDs to that in treatment water and in pink salmon eggs, monitored PAH retention in PEMDs in clean or nearly clean water for up to 40 d, and compared PEMD and SPMD performance in field environments where intermittent low levels of PAH were expected. The influence of molecular mass on the accumulation and retention of PAHs in PEMDs and eggs was explored to further characterize hydrocarbon exchange and explain the differences between them. Size-related differences were expected because hydrophobicity increases with molecular mass, a relationship often characterized by the log of the octanol-water partition coefficient (log K_{ow}; Table 4.1).

Materials and Methods

Laboratory tests

Low-density polyethylene tubing (approximately 98 μm thick) without additives (Brentwood Plastics, St. Louis MO USA) was cut into 0.5 m sections and split longitudinally to form a single layer 4.9 cm wide. A final cleanup was necessary before deployment to ensure that membranes had not previously accumulated hydrocarbons from air, thus PEMDs were immersed in pentane and alternately sonicated 15 min and soaked for 30 min (two cycles). After a final 15 min sonic extraction, each PEMD was rinsed with clean pentane as it was removed from the sonic bath, stored in a hydrocarbon-free glass jar with a dichloromethane-rinsed aluminum foil lid, and frozen (0°C) until deployment. The PEMDs deployed in these experiments were not weighed, but mass was estimated by weighing 10 additional identically sized PEMDs to the nearest milligram (2.210 ± 0.009 g, mean ± standard error).

To measure their capacity to accumulate hydrocarbons, PEMDs were typically exposed to water-accommodated fractions of oil for 26 or 52 days (ranges were 26 to 30 d and 52 to 63 d). The shorter exposure times were within the range typical for SPMD field deployment [e.g., 3,6,7]. To provide a graded dose series (five treatments including control; Table 4.2), 22 kg of oiled or control rock was placed in each of nine 60 cm tall × 15 cm diameter polyvinyl chloride incubators according to the methods of [14]. Alaska North Slope crude oil was artificially weathered by heating for 12 h at 70° C, then sprayed onto rock (mid-diameter 5.4 mm) at different dose levels (0 to 3.8 g oil per kg rock). Water flow was 1.6 L/min and alternated between fresh water (8 h) and salt water (4 h) to simulate an intertidal environment representative of typical pink salmon spawning habitat. Water flowed 2 d prior to the start of the experiment to further weather the oil and remove any particulates. About 2,700 pink salmon eggs were added to each incubator at the start of the study, and one PEMD was placed in the effluent of one randomly selected incubator per treatment a day later. Initial aqueous TPAH concentrations ranged from 0.02 (control) to 16.52 μg/L (Table 4.2). Hydrocarbon-free (dichloromethane-rinsed) tools were used to place and

retrieve PEMDs, which were frozen in jars pending analysis. Water samples (3.8 L) and eggs (about 10 g wet weight) were collected at about the same time for hydrocarbon analysis (Table 4.2). Egg samples were frozen pending analysis. Additional PEMDs were placed in the high-oil treatment every 30 d to ensure reliable concentration factor estimates (Table 4.2). Total experimental duration was 184 d.

To determine hydrocarbon retention, additional PEMDs were similarly exposed to water-accommodated fractions of oil, then transferred to clean water and observed for 40 days. These PEMDs were exposed for 21 d, transferred to clean water, and sampled after 0, 10, 20, or 40 days depuration. One PEMD was sampled at each observation time. Retention of PAH was also examined between 26 and 52 d in the uptake experiment, where aqueous TPAH concentration in two of three independent data pairs was indistinguishable from the control level (in trace- and low-oil treatments) and was 5 to 8% of the initial aqueous concentration in the mid-oil treatment (0.19 to 0.30 μg/L), thus generally approximating depuration conditions.

Field comparison of PEMD and SPMD samplers

To compare performance, PEMDs and SPMDs were placed side by side in freshwater and intertidal areas. The SPMDs were purchased from Environmental Sampling Technologies. Exposed surface area of the 2.6 cm × 100 cm SPMDs was the same as in the PEMDs. In fresh water (Auke Lake and surrounding streams, Juneau, AK USA), two PEMDs paired with one SPMD were periodically deployed at four sites for 21 to 23 d periods (May 1999 to February 2000). Membranes were suspended in 5 to 7.5 cm diameter × 60 cm polyvinyl chloride pipe with open ends and two rows of 1.3 cm holes spaced about 7.5 cm apart. In intertidal streams (Prince William Sound, AK USA), four PEMDs and one SPMD were buried approximately 10 to 20 cm in the hyporheic zone below stream channels in each of four elevation zones [15] in each of six streams. Membranes were suspended centrally in 69 cm long × 8.9 cm diameter perforated aluminum tubes (3 mm holes spaced 4.8 mm on center) on stainless steel clips fastened to nylon line. Tube ends were capped with aluminum window screen. Tubes, screens, associated hardware, and tools were washed with soap and water, dried, and rinsed with methylene chloride prior to use. Blank SPMD and PEMD samples were collected by first rigging membranes in a sampler, then retrieving them without placement in stream water. Membranes were recovered 45 to 56 d after placement and frozen until analysis.

Analyses

All hydrocarbon samples (PEMDs, SPMDs, tissue and water) were extracted with organic solvent. The PEMDs were wiped to remove gross surface contamination, placed in centrifuge tubes and spiked with six deuterated PAH standards (Table 4.3). Spike solvent (hexane) was allowed to evaporate, then the tubes were placed in a sonic bath, and extracted in 80:20 ml pentane/dichloromethane for 130 min. The sonicator was on for the first 20 min of each 50 min period. The PEMDs were rinsed with pentane as they were removed without delay after the final sonication; extracts were concentrated to 20 to 30 ml, dried with 2 to 4 g of sodium sulfate, concentrated to 1 to 2 ml in hexane, and passed through 1.5 g silica gel columns. All SPMD dialyses were completed by Environmental Sampling Technologies (St. Joseph, MO USA). Dialyzate was spiked with six deuterated standards (Table 4.3), reduced in volume with Synder columns on a steam bath, purified by gel permeation HPLC, and reduced in volume with hexane solvent transfer on a steam bath. Water samples were extracted with dichloromethane immediately after collection and spiked with 1 ml of the six deuterated standards in acetone (Table 4.3) according to the methods of [16]. Tissue samples were extracted with dichloromethane after addition of six internal standards (Table 4.3). Isolation and purification of calibrated and uncalibrated compounds in tissue samples was completed by silica gel/alumina

column chromatography followed by size-exclusion high-pressure liquid chromatography and fractionation [16,17]. All extracts were spiked with an internal standard (hexamethyl benzene) and frozen pending analysis.

Extracts were analyzed by gas chromatography equipped with a mass selective detector and PAH concentrations were determined by the internal standard method [16]. Experimentally determined method detection limits (MDL) were generally 1 ng/g for tissue, 1 to 8 ng/L in water, and 0.18 to 3.94 ng/g in PEMDs and SPMDs; concentrations below MDL were considered equivalent to zero and are not reported. The accuracy of the hydrocarbon analyses was about ± 15% based on comparison with National Institute of Standards and Technology values, and precision expressed as coefficient of variation was usually less than about 20%, depending on the PAH. Samples with questionable internal standard recoveries (< 25% or > 150%) were excluded from analysis or accepted only as specifically indicated and where results could be independently corroborated by other data. Internal standard recoveries in PEMDs increased from naphthalenes (54 ± 2%) to phenanthrenes (83 ± 1%) and declined slightly toward higher molecular mass PAH (70 ± 2% for benzo(a)pyrene), except peryelene recovery was 80 ± 2%.

A first order loss-rate kinetic weathering model was used to determine if PAH composition in PEMDs and eggs was consistent with composition in source oil and summarize PAH composition [18]. Oil composition is summarized by a weathering index, w; values range from < 0 (composition dominated by lower molecular weight PAH) to > 8 (composition dominated by higher molecular weight PAH).

Concentrations of TPAH were calculated by summing concentrations of individual PAH (Table 4.1). The time when TPAH concentrations in eggs was at maximum was estimated for each treatment using a first-order kinetic model that accounted for uptake from water and declining aqueous TPAH concentrations [14]. Relative PAH concentrations were calculated as the ratio of PAH/TPAH. Concentration factors in PEMDs were estimated by dividing TPAH concentration (ng/g) by geometric mean aqueous TPAH concentrations (µg/L). Final aqueous TPAH concentrations were estimated by least-squares regression when PEMDs were collected more than one day before measurement of corresponding aqueous TPAH concentration. Concentration factors were similarly estimated for eggs.

Regression models considered when relating TPAH in matrices (water, PEMD, SPMD, and tissue) to each other were ladder of powers (x-transformations, x^3 to $-1/x^3$), exponential, and power. Because ordinary least squares regressions are often not suitable when both x- and y-terms are measured with error, the major-axis method [19] was used to estimate slopes when comparing PEMD and SPMD performance.

Rates of TPAH decline from PEMDs, tissue, and water were regressed (exponential models) and rate constants (k) were compared. To scale them equally, concentrations in each data set were divided by the initial concentration in that set. Initial concentrations began on day 0 for all PEMD and aqueous data and day 53 for pink salmon eggs. Relative declines across oil treatment in tissue and water were similar, thus all oil treatments were used as replicates in this analysis. The TPAH concentrations in tissue were normalized to those on day 53 because this was the first time all treatments were analyzed; normalized results were consistent with those in the high-oil treatment when normalized to TPAH concentration on day 15, the earliest tissue sample. Two-compartment exponential decay was necessary to adequately model TPAH loss from water. To ensure that regressions were meaningful, we adopted the approach that the F-ratio of a regression (F_o) should exceed the usual significance ratio (F_c) by a multiple of at least 4 times [20]. Instantaneous rate estimates, $(\ln(C_t/C_0))/t$, where C_t = concentration at time t and C_0 = initial concentration, were calculated where there were too few data for regression analysis.

Concentration factors of individual PAH by PEMDs and eggs were related to molecular mass by exponential regression. The shortest possible observation period was used for these

calculations, 26 d for PEMDs (all oil treatments) and 15 d for eggs (high-oil treatment only). Concentration factors for each analyte were estimated by dividing the observed concentration (ng/g) by the geometric mean aqueous concentration (μg/L) observed during exposure intervals.

Retention of individual PAH by PEMDs and eggs was examined as a function of molecular mass. Two PEMD data sets were examined, paired 26 and 52 d data (where aqueous TPAH levels had either fallen to control levels or were near them; Table 4.2) and clean-water depuration data. In eggs, paired 53 and 75 d data (all treatments) and 27 and 53 d data (high-oil treatment only) were examined. The concentration of each PAH analyte was divided by that of the corresponding analyte in earlier samples (or mean analyte concentration where replicated) and multiplied by 100 to express the result as a percentage. Data where ratios were not calculable were omitted (initial analyte concentrations < method detection limits). Percent retention was regressed (linear) against molecular mass by treatment or by depuration time. Analysis of PEMDs placed in clean water was restricted to 3 to 5 ring PAH; all PAH were included in other PEMD and egg analyses. To compare slopes, data were further examined with analysis of covariance after removal of outliers.

To compare PAH composition in PEMD and SPMD samplers, differences in relative PAH concentrations in each device were calculated. Comparison of relative PAH composition was restricted to locations with evidence of hydrocarbons (several Auke Lake sites and portions of two Prince William Sound streams). Data where PAH/TPAH were zero in both samplers were not included in estimates of mean differences. Also, C3-phenanthrenes were not included in samples from PWS because a broad competing peak (probably octadecanoic acid or its methyl esters) precluded quantification in SPMDs. Single SPMD concentration estimates at each site and time were subtracted from mean estimates ($1 \le n \le 4$) in paired PEMDs. A more complex multivariate analysis of variance designed to compare composition with normalized PAH vectors [21] failed because the data set was not large enough.

Results

Total PAH uptake by PEMDs and eggs

Concentrations of TPAH in PEMDs and eggs were all closely correlated with initial concentrations in water whenever measured ($0.933 \le r^2 \le 1.000$). Initial aqueous TPAH exposure concentrations ranged from 0.02 μg/L (controls) to 17 μg/L (high treatment) and declined rapidly in oil treatments (Table 4.2). The TPAH concentrations in PEMDs deployed one day after dosing began and soaked for 26 d were linearly correlated with initial aqueous TPAH concentrations ($r^2 > 0.999$, $n = 6$), as were those soaked 52 d ($r^2 > 0.999$, $n = 4$), demonstrating that PEMDs were not saturated at aqueous TPAH concentrations ≤ 17 μg/L. Concentrations of TPAH in eggs were correlated with initial aqueous TPAH concentrations after 53, 75, and 183 d exposures ($0.991 \le r^2 \le 1.000$; $4 \le n \le 8$). Maximum TPAH concentrations in PEMDs (118,000 ng/g) exceeded those in eggs (10,800 ng/g dry) by an order of magnitude.

The capacity of PEMDs to accumulate TPAH from water was large. Estimated concentration factors for PEMDs, which were typically complicated by rapidly changing aqueous TPAH concentrations, ranged from a minimum of 7.1×10^3 to a maximum of 1.6×10^5, depending on the calculation method. Minimal concentration factor estimates were 7,150 ± 160 ($n = 7$), where initial aqueous TPAH was the divisor; this likely underestimates actual concentration factor because aqueous concentrations dropped rapidly. An intermediate estimate of 67,000 ± 10,000 ($n = 7$) was obtained by using geometric mean aqueous TPAH concentrations. The highest, and possibly the most accurate concentration factor estimate, 1.6×10^5, was based on a single 63 d deployment in the high-oil treatment where bounding aqueous TPAH concentrations were nearly constant (0.09 μg/L and 0.07 μg/L on days 75 and 183; Table 4.2).

Total PAH concentration factors in pink salmon eggs were smaller than in PEMDs. Calculation of concentration factors in eggs was complicated by declining TPAH concentrations in both water and tissue and by sample timing. The maximum observed TPAH accumulation in eggs was 10,800 ng/g dry weight on day 15 (high-oil treatment), shortly before the estimated maximum accumulation of 11,000 ng/g on day 18 in this treatment. Most tissue was not analyzed until day 53, thus maximum uptake capacity is probably underestimated. For example, TPAH concentration in high-oil treatment eggs was 4,220 ng/g dry weight on day 53, 39% of that observed on day 15. Under these conditions, the mean concentration factor for eggs was 2,900 ± 340 times (n = 4; day 53; based on geometric mean aqueous concentration), roughly 20 times less than in PEMDs. When expressed per dry weight lipid, the concentration factor for eggs was 9,200 ± 1,100 (about seven times less than in PEMDs), a measure more directly comparable to PEMDs (which are functionally a very large lipid) because most PAH in eggs are likely associated with lipid.

Uptake of specific PAH by PEMDs and eggs

Accumulation of individual PAH in PEMDs and eggs increased exponentially as molecular mass increased ($0.76 \leq r^2 \leq 0.82$, $P < 0.001$, $16 \leq F_o/F_c \leq 103$) and concentration factors consistently increased with alkyl-substitution within each homologous family (Fig. 4.1). However, the slope for PEMDs was significantly greater than for eggs ($P_{ANCOVA} < 0.001$). The slope did not change when uptake in eggs was based on a wet or dry weight basis (instead of lipid) but the position of the curve was displaced downward.

PAH composition in water, PEMDs, and eggs

PAH composition in exposure water, PEMDs, and pink salmon eggs was related to that in the source oil (Alaska North Slope crude) but differed in specific details (Fig. 4.2). Naphthalenes, fluorenes, dibenzothiophenes, and phenanthrenes were consistently present in all three media. Specific PAH composition in each medium differed from that in the source oil for several reasons. In water, enrichment of smaller molecular weight PAH (e.g., naphthalene) was evident because these molecules dissolve more readily from the contaminant oil film (compare Fig. 4.2b to 4.2a). Conversely, larger molecular weight PAH (chrysenes) were under-represented in water and often near or below detection limits, thus verification of source oil was not possible with a first order loss-rate kinetic weathering model [18]. In PEMDs, PAH composition was more characteristic of weathered oil because proportionately fewer lower molecular weight PAH were accumulated or retained (Fig. 4.2d). Percentages of naphthalenes in PEMDs were always less than bounding percentages in water and percentages of phenanthrenes were usually greater (e.g., compare Fig. 4.2d to 4.2b and 4.2c). The PAH were more easily detectable in PEMDs than in water because they were concentrated by the plastic and the source of oil was verifiable in all oil-exposed PEMDs [18]. In pink salmon eggs, naphthalenes were the dominant PAH accumulated and composition was most similar to initial aqueous PAH composition (compare Fig. 4.2e to 4.2b). Percentages of naphthalenes tended to increase with time in eggs and the source oil typically could not be verified [18] because one or more chrysene homologs were below MDL.

Retention of TPAH by PEMDs and eggs

The estimated TPAH retention in PEMDs placed in clean water was 78% in 40 d and the instantaneous loss rate was -0.006. Interpretation was complicated by poor recovery of 2 ring PAH in other depuration samples. However, loss of TPAH from PEMDs after 10 and 20 d was consistent with the 40 d estimate (Fig. 4.3), indicating that mathematical correction for poor recovery in these samples was reasonable. To further assess the accuracy of the clean water depuration data, TPAH loss rates were examined in three paired 26 and 52 d samples where

aqueous concentrations were negligible (trace- and low-oil treatments) or low (mid-oil treatment; Table 4.2). Estimated instantaneous TPAH loss rates in these paired samples ranged from 0.001 to –0.004. That no loss was evident where *w* was greatest (trace-oil treatment; Table 4.2) is consistent with better retention of higher molecular weight PAH than lower molecular weight PAH by PEMDs, as demonstrated in the next section. The estimated rate of loss between 26 and 52 d where initial TPAH concentration was highest and mean *w* was the lowest (mid-oil treatment) was likely slowed by residual aqueous PAH (5-8% remaining between 26 and 52 d).

Loss of TPAH from PEMDs in clean water ($k = -0.006 \pm 0.001$) was slower than loss from pink salmon eggs maintained in declining aqueous TPAH concentrations ($k = -0.021 \pm 0.002$) and much slower than loss from water ($k_1 = -0.216 \pm 0.032$; Fig. 4.3). Although eggs were never removed from treatment water, tissue concentrations peaked between approximately 18 and 22 d [14] and then declined, well before depuration modeling began at day 53 (Fig 4.3).

Retention of individual PAH by PEMDs and eggs

The PEMDs retained higher molecular mass PAH better than lower molecular mass PAH (Fig. 4.4a-b). The relationship between PAH retention and molecular mass was clear in all paired treatments after removal of outliers ($0.61 \le r^2 \le 0.86$, $P < 0.001$, $9.2 \le F_o/F_c \le 41$; Fig 4.4a). Slopes ranged from 0.5 to 1.2 % mole/g and was significantly greater in the mid-oil treatment than in the lower two treatments, possibly because mid-oil aqueous TPAH concentrations were greater than in controls between days 26 and 52 and relatively more high-molecular mass PAH were present in the water than low-molecular mass PAH. However, estimated PAH retention in PEMDs where molecular mass was greater than about 200 g/mole was generally > 100%, suggesting either measurement imprecision or that some larger PAH may have continued to pass through the water undetected and accumulate on all of these PEMDs between days 26 and 52. Some clean-water retention estimates also exceeded 100%, also suggesting measurement imprecision (Fig. 4.4b).

The PEMDs also retained higher molecular mass PAH better in clean-water depuration tests (Fig 4.4b). Only 3 to 5 ring PAH were examined in this analysis; 2 ring PAH were not included due to measurement error. Retention of two analytes, phenanthrene and fluoranthene, was consistently > 200% (theoretically impossible) and these results were also rejected as a measurement error. Retention of PAH in all three observations increased significantly as molecular mass increased ($0.49 \le r^2 \le 0.75$, $0.001 < P \le 0.008$, $2.2 \le F_o/F_c \le 7.6$; Fig 4.4b). Clean water depuration results were consistent with those in paired samples and slopes were indistinguishable from those in paired tests where aqueous TPAH concentrations were at background levels ($P_{ANCOVA} = 0.324$).

In pink salmon eggs, evidence that PAH retention was related to molecular mass was inconsistent (Fig 4.4c). Retention of PAH between day 53 and 75 was unrelated to mass in one treatment (extreme-oil, $r^2 = 0.09$, $P = 0.221$, $F_o/F_c = 0.4$), declined significantly in two treatments ($0.35 \le r^2 \le 0.63$, $0.002 \le P \le 0.021$, $1.5 \le F_o/F_c \le 3.4$), and increased significantly in the remaining treatment (high-oil, $r^2 = 0.72$, $P < 0.001$, $F_o/F_c = 8.4$; Fig 4.4c). Retention of PAH also may have increased with molecular mass in an earlier observation of the high-oil treatment but correlation was poor (days 27 to 53; $r^2 = 0.27$, $P = 0.015$, $F_o/F_c = 1.6$). The inconsistent relationship between retention and molecular mass in eggs was unlike the situation in PEMDs, suggesting that mechanisms influencing PAH loss were different.

PEMD and SPMD comparison

Composition of PAH accumulated by PEMDs and SPMDs in side-by-side field tests was similar (e.g., Fig. 4.5). Differences between relative PAH concentrations in PEMDs and SPMDs (from Prince William Sound and Auke Lake) averaged $-0.1 \pm 0.3\%$ ($n = 336$) and in nearly all

cases were within ± 5%.

The capacity of PEMDs and SPMDs to accumulate PAH was also about the same, as expected because the devices had equal surface areas and probable exposure concentrations were low. The TPAH concentrations in PEMDs and SPMDs (range 2 to 1,430 ng/g) were strongly correlated in samples from a freshwater lake ($r^2 = 0.992$, $P < 0.001$, $n = 12$). Major-axis slope estimates were 0.999 and 1.001, indicating that these devices functioned similarly in the lake. Accumulation in PEMDs and SPMDs (range, 20 to 221 ng/g TPAH) was also similar in intertidal streams (major-axis slope estimates were 0.936 and 1.068). Correlation was poorer within this smaller concentration range ($r^2 = 0.371$, $P = 0.002$, $n = 23$). Clearly PEMDs performed at least as well as SPMDs within observed TPAH ranges, as assessed by accumulation capacity and similarity in PAH composition.

Discussion

These experiments demonstrate that PEMDs are reliable passive sampling devices capable of accumulating PAH from water, a conclusion also reached by [8,9,22]. Concentrations of TPAH accumulated by PEMDs were highly correlated with concentrations in oil-contaminated water, and there was no evidence of saturation at aqueous concentrations < 17 μg/L. Hydrocarbons were highly concentrated in PEMDs, improving the detection of PAH that were only present at low levels in water (e.g., chrysenes). Composition of PAH accumulated in PEMDs was consistent with source oil and although PAH detected in PEMDs were consistently more weathered than in source water (i.e., relatively fewer naphthalenes and more phenanthrenes), composition varied with that in water, and was highly similar to accumulations in commercially available SPMDs. The PEMDs maintained in clean water retained most accumulated PAH (78% in 40 d). The PEMD and SPMD samplers deployed in a field setting clearly provided the same information: TPAH concentrations were correlated and differences in relative PAH composition averaged zero. Thus, a simple plastic membrane can be conveniently used for environmental monitoring, and the lipophilic reservoir present in SPMDs is not always necessary.

Our approach to environmental monitoring has been to simplify the data collection and analysis as much as practical, and we have demonstrated that PEMDs perform well as low-level hydrocarbon monitors in aquatic environments. Unlike directly processed water samples, passive membrane samplers provide time-integrated, concentrated samples, thereby increasing the likelihood of detecting low-level or intermittent contaminants [7]. Sampling costs may also be lowered because frequent collection, processing and extraction of large water samples may be required to achieve the same results [23]. The PEMDs share many of the same attributes of SPMDs, which have been accepted for the monitoring of lipophilic contaminants since their introduction [1] more than a decade ago [e.g., 24,25]. Although the lipophilic central reservoir in SPMDs more closely mimics transfer of hydrocarbons across biological membranes and is capable of storing high hydrocarbon concentrations in the reservoir (theoretically about 3×10^4 for hydrocarbons with log $K_{ow} > 5.5$ [13]), PEMD uptake capacity is also high (roughly 10^4 to 10^5 times aqueous TPAH concentrations). Estimated capacity of PEMDs to accumulate PAH is also similar to pesticide uptake (5.5×10^4 times) by similar devices [13] and in mussels (2×10^5 in *Mytilus edulis* [26]). Our experience indicates that PEMDs provide reliable data at low environmental TPAH concentrations, a conclusion also reached for pesticides and PCBs by [8].

The difference in surface to volume ratios in PEMDs and eggs was likely a primary factor in kinetically controlled uptake differences between them. The surface to volume ratio in PEMDs (227) was much greater than in pink salmon eggs (0.87, with an average egg diameter of 6.9 mm [27]) explaining in part why more PAH accumulated in PEMDs per unit mass than in eggs (about 20 times). The importance of surface-volume relationships is also evident in an experiment by

[9], where PEMDs with twice the surface area of SPMDs accumulated PAH more rapidly.

Differences in rates of PAH accumulation and loss in PEMDs explain why the PAH composition in PEMDs was different than in water. Our results indicate PAH uptake increases exponentially with molecular mass (and with log K_{ow} because it is linearly related to molecular mass; Table 4.1). Furthermore, higher mass PAH are preferentially retained by PEMDs in clean water. This selective sampling causes PAH composition in PEMDs to differ from that in water, yet the source of oil remained identifiable in PEMDs, perhaps in part because smaller PAH preferentially accumulate in water. Size-related, hence lipophilic, differences in PAH uptake and retention are consistent with first-order kinetics [18] and dissolution processes are probably primarily responsible for these differences. The sampling efficiency of PEMDs may decline for organic hydrocarbons where log K_{ow} is greater than about 5.5 [9] but in our uptake study, few PAH with log $K_{ow} \geq 6$ were present (9%) and none were $\geq$6.5, thus we did not observe this decline.

The PEMDs lost TPAH slowly in clean water (22% in 40 d). We conclude the rate of PAH loss is slow enough that PEMD membranes are valuable as passive sampling devices, corroborated by high TPAH retention (90 to 100%) in PEMDs in water where TPAH concentrations had declined to background or near-background levels.

The PAH composition in PEMDs was very different than in pink salmon eggs, indicating that PEMD data cannot simply be substituted for direct measures of biologically accumulated hydrocarbons in all cases. These differences were not surprising because uptake and depuration kinetics likely differ between passive samplers and living organisms; the latter have complex structures and active cellular processes, including the ability to metabolize and actively export hydrocarbons [5]. Greater impedance of larger PAH by the chorion or other intervening membranes may explain differences in accumulation bias between PEMDs and pink salmon eggs (Fig. 4.1). In small planktonic fish larvae (with no chorions), accumulation bias toward larger PAH [28] was about the same as in our PEMDs, substantially greater than in salmon eggs.

The complexity of living systems can make identification of source contamination more difficult than in passive samplers. Uptake and retention of PAH in organisms depends on a variety of factors such as species differences, life stage, physiological condition, lipid composition, temperature, and salinity. Correlation has been reported between uptake of hydrocarbons by SPMDs and biological species of interest, e.g., organochloride pesticides and polychlorinated biphenyls in mussels (*Mytilus edulis*; [6]), organochlorides in freshwater clams (*Corbicula fluminea*; [2]), and PCBs in brown trout (*Salmo trutta*; [29]). However, polychlorinated biphenyl conger distribution in freshwater clams was different than in SPMDs, as were distributions of ionizable chlorinated phenolic compounds in lake mussels (*Anodonta piscinalis*; [2]). Clearly, neither PEMD nor SPMD data can simply replace direct measurement in a particular species without detailed parallel study.

That PAH composition in pink salmon eggs became increasingly naphthalene dominated may have been due to the preferential metabolism of larger PAH, consistent with the conclusion by Echols et al. [3] that differences in contaminant profiles between channel catfish (*Ictalurus punctatus*) and SPMDs were likely due to metabolism and depuration of certain PCB congeners by the fish. For example, cytochrome P4501A is induced by PAH with 3 or more rings but may not be induced by 2 ring PAH [30]. Others have reported that naphthalenes induce cytochrome P4501A and ethoxyresorufin-O-deethylase activity, but less so than benzo[*a*]pyrene [31]. Cytochrome P4501A activity was elevated in oil-exposed embryos throughout the time PAH retention was studied (unpublished data) and these maturing embryos had active circulatory systems, increasing the probability that metabolic activity influenced PAH composition.

The PEMDs reliably accumulate and retain 2 to 5 ring PAH and can be used to assess environmental hot spots, particularly where exposures may be pulsed or intermittent. Because

PEMDs integrate exposure over time, accumulated PAH concentrations are difficult to relate directly to water quality standards. Variables previously identified for SPMD interpretation, including mean exposure temperature, extent of biofouling on samplers, and knowledge of effective daily sampling rates for contaminants of interest [7] are needed for informed back-calculation of aqueous hydrocarbon concentrations. Non-equilibrium conditions further complicates estimation of aqueous hydrocarbon concentrations. However, PEMDs can be used to distinguish areas with relatively higher PAH concentrations from those with lower concentrations. The PEMDs can potentially be used to identify source contamination, but analysis of time integrated samples with multiple exposure possibilities will be challenging.

Conclusions

We conclude that PEMDs reliably sample lipophilic hydrocarbons from aquatic environments and provide a method for monitoring low-level, sporadic hydrocarbon levels. Data provided by PEMDs under these conditions is comparable to SPMD data. Loss of accumulated PAH is slow, thus PEMDs can reliably capture sporadic or fluctuating events. Composition of PAH accumulated by PEMDs can be used to identify hydrocarbon sources in situations not complicated by multiple sources. We recommend PEMDs as cost-effective, simple tools for environmental monitoring.

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Table 4.1. Polynuclear aromatic hydrocarbon (PAH) analytes, abbreviations, deuterated surrogate references, molecular mass, and the log of the octanol-water partition coefficient (K_{ow}). 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).

PAH	Abbreviation	Surrogate	molecular mass (g/mole)	log K_{ow}
naphthalene	N0	1	128.2	3.36
C-1 naphthalenes	N1	1	142.2	3.80
C-2 naphthalenes	N2	2	156.2	4.30
C-3 naphthalenes	N3	2	170.3	4.80
C-4 naphthalenes	N4	2	184.3	5.30
biphenyl	BIP	2	154.2	3.80
acenaphthylene	ACN	2	152.2	3.22
acenaphthene	ACE	2	154.2	4.01
fluorene	F0	2	166.2	4.21
C-1 fluorenes	F1	2	180.3	4.72
C-2 fluorenes	F2	2	194.3	5.20
C-3 fluorenes	F3	2	208.3	5.70
dibenzothiophene	D0	3	184.2	4.53
C-1 dibenzothiophenes	D1	3	198.3	4.96
C-2 dibenzothiophenes	D2	3	212.3	5.42
C-3 dibenzothiophenes	D3	3	226.3	5.89
phenanthrene	P0	3	178.2	4.57
C-1 phenanthrenes/anthracenes	P1	3	192.3	5.04
C-2 phenanthrenes/anthracenes	P2	3	206.3	5.46
C-3 phenanthrenes/anthracenes	P3	3	220.3	5.92
C-4 phenanthrenes/anthracenes	P4	3	234.3	6.32
anthracene	ANT	3	178.2	4.53
fluoranthene	FLU	3	202.3	5.08
pyrene	PYR	3	202.3	4.92
C-1 fluoranthenes/pyrenes	FP1	3	216.3	5.48
benzo(a)anthracene	BAA	4	228.3	5.89
chrysene	C0	4	228.3	5.71
C-1 chrysenes	C1	4	242.3	6.14
C-2 chrysenes	C2	4	256.3	6.43
C-3 chrysenes	C3	4	270.4	6.94
C-4 chrysenes	C4	4	284.4	7.36
benzo(b)fluoranthene	BBF	6	252.3	6.27
benzo(k)fluoranthene	BKF	6	252.3	6.29
Benzo(e)pyrene	BEP	6	252.3	6.44
Benzo(a)pyrene	BAP	6	252.3	6.11
Perylene	PER	5	252.3	6.44
indeno(1,2,3-cd)pyrene	ICP	6	276.3	6.72
dibenzo(a,h)anthracene	DBA	6	278.4	6.71
benzo(ghi)perylene	BZP	6	276.3	6.51

Table 4.2. Dose levels in laboratory experiment 1 and resultant total polynuclear aromatic hydrocarbon (TPAH) concentrations in water, low-density polyethylene membrane devices (PEMD), and pink salmon eggs. Sample times are days after experiment began; mass is dry weight; *na* is not analyzed; *ne* is not estimable; *w* is the weathering coefficient in PEMDs on day 27; *n* = 1 except where standard error is reported: [a]n = 2; [b]n = 4.

	Aqueous TPAH (µg/L)						TPAH in PEMD (ng/g)				TPAH in eggs (ng/g)			
Treatment	Initial	28 d	54 d	75 d	183 d	*w*	1 to 27 d	1 to 53 d	27 to 53 d	83 to 146 d	0 to 27 d	0 to 53 d	0 to 75 d	0 to 183 d
Control	0.02	0.02	0.01	0.03	0.00	*ne*	280 ± 53[a]	355	*na*	*na*	25	19	34	16
Trace	0.66	0.01	*na*	0.02	*na*	5.0	4,560	4,670	*na*	*na*	*na*	*na*	*na*	*na*
Low	0.94	0.03	0.02	0.03	0.00	1.4	7,450	6,680	*na*	*na*	*na*	275	204	24
Mid	3.70	0.19	0.30	0.11	0.02	0.6	26,900	24,200	*na*	*na*	*na*	999 ± 52[b]	620	88
High	16.52	0.62	0.16	0.09	0.07	−1.2	118,000	*na*	29,300	13,000	9,400	4,220	2,170	163

Table 4.3. Deuterated surrogate polynuclear aromatic hydrocarbon (PAH) standards. Listed are concentrations in spike used for water (a) and PEMDs, SPMDs, and eggs (b). Spike volumes were 500 μL for water, SPMD, and eggs and 200 μL for PEMDs. Spike solvent was acetone for water and hexane for PEMDs, SPMDs, and eggs.

(μg/ml)[a]	(μg/ml)[b]	Surrogate
1.0	2.5	naphthalene–d_8
1.0	2.5	acenaphthene–d_{10}
0.8	2.0	phenanthrene–d_{10}
0.8	2.0	chrysene–d_{12}
1.0	2.5	perylene–d_{12}
1.0	2.5	benzo[*a*]pyrene–d_{12}

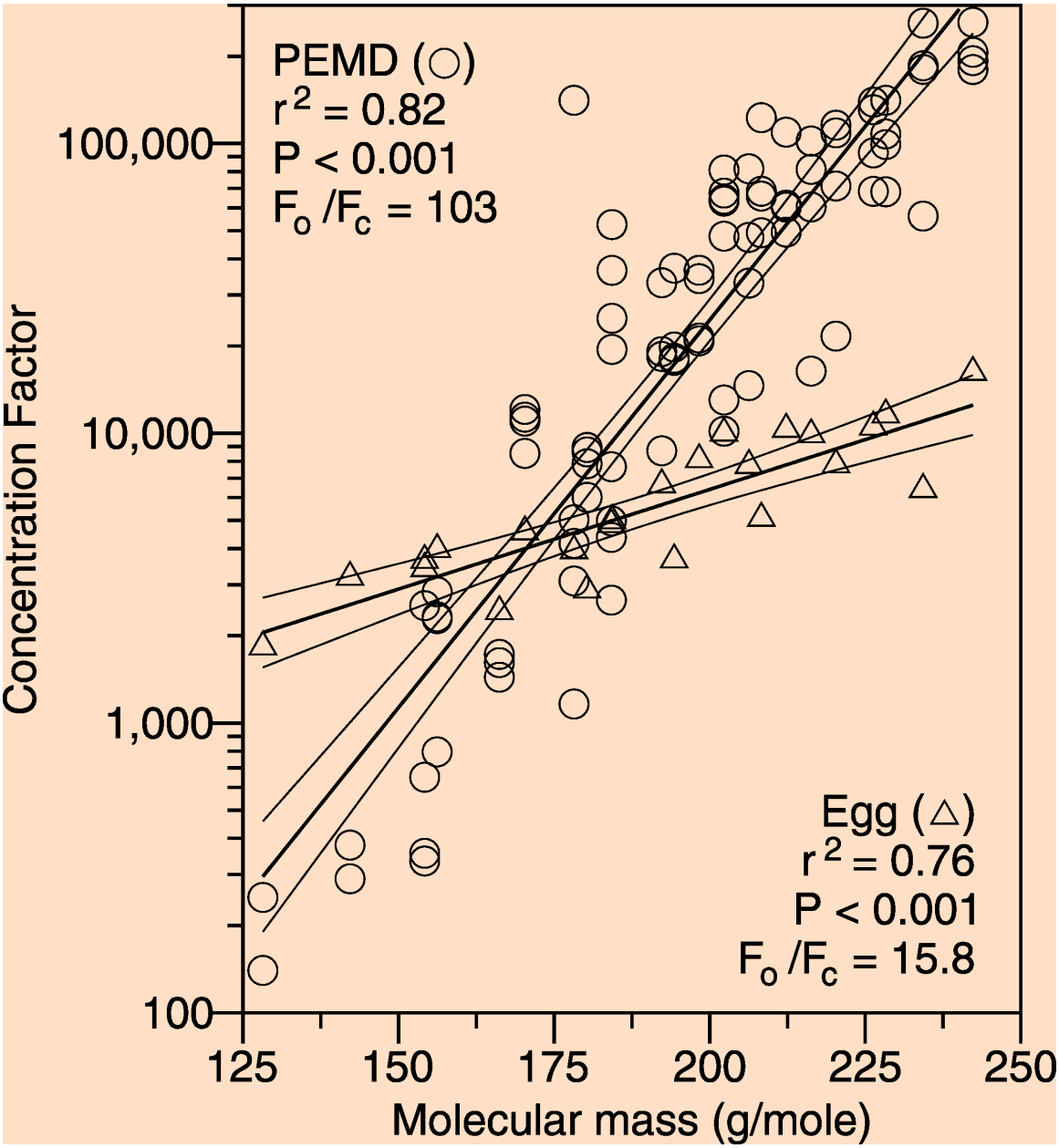

Fig. 4.1. Concentration factors of polynuclear aromatic hydrocarbons (PAH) in low-density polyethylene membrane devices (PEMD) and pink salmon eggs as functions of molecular mass. Illustrated are exponential regressions with 95% confidence bands and associated statistics; F_o/F_c is the ratio between the observed F value and the critical F value.

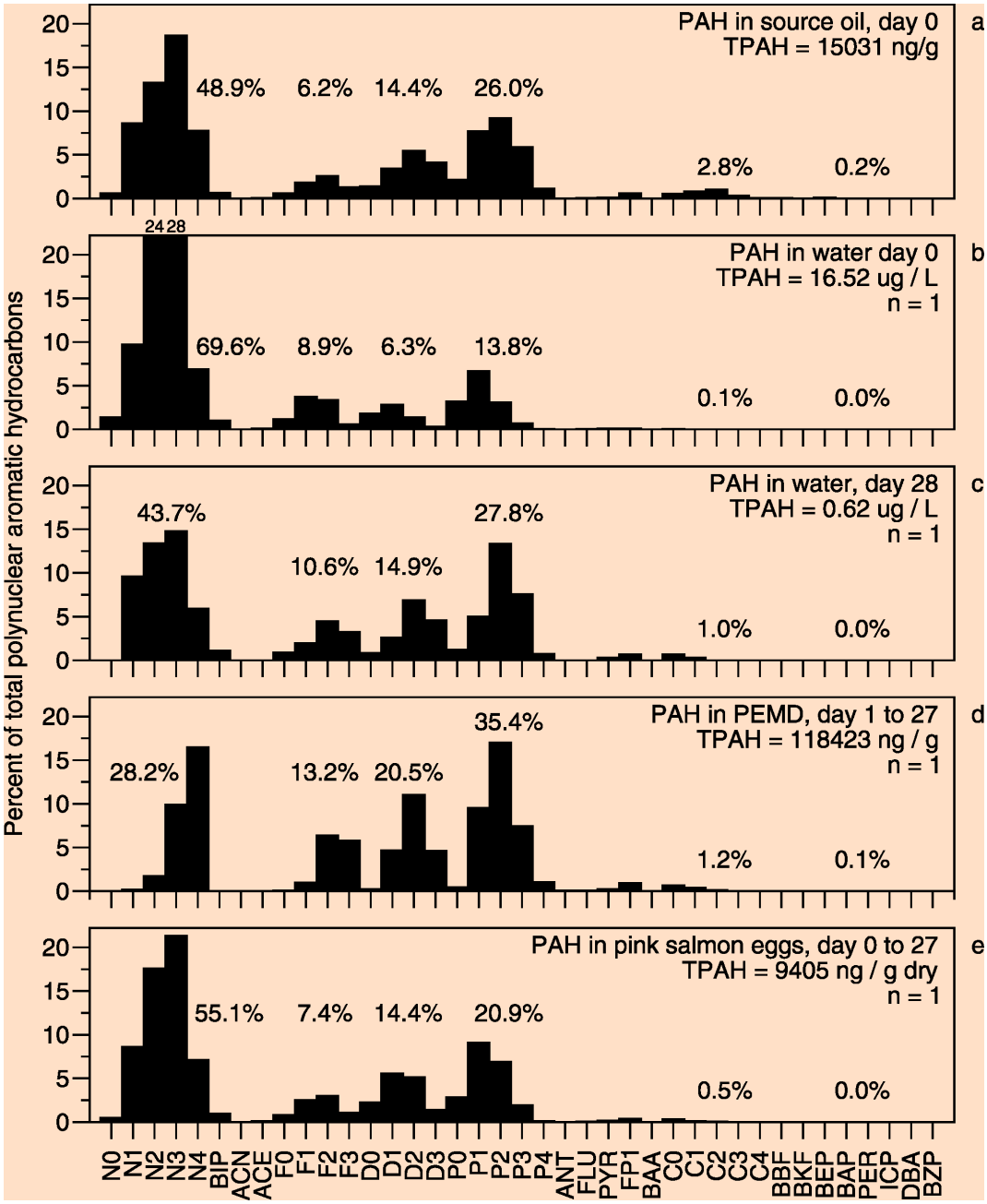

Fig. 4.2. Relative polynuclear aromatic hydrocarbon (PAH) composition in source oil, water, low-density polyethylene devices (PEMDs), and pink salmon eggs. Observation times and total PAH (TPAH) concentration are noted in each panel. See Table 4.1 for explanation of the *x*-axis. Percentages (of TPAH) in each panel indicate relative quantities of naphthalenes (N0 to N4), fluorenes (F0 to F3), dibenzothiophenes (D0 to D3), phenanthrenes (P0 to P4), chrysenes (C0 to C4), and five ring PAHs (BBF to BZP).

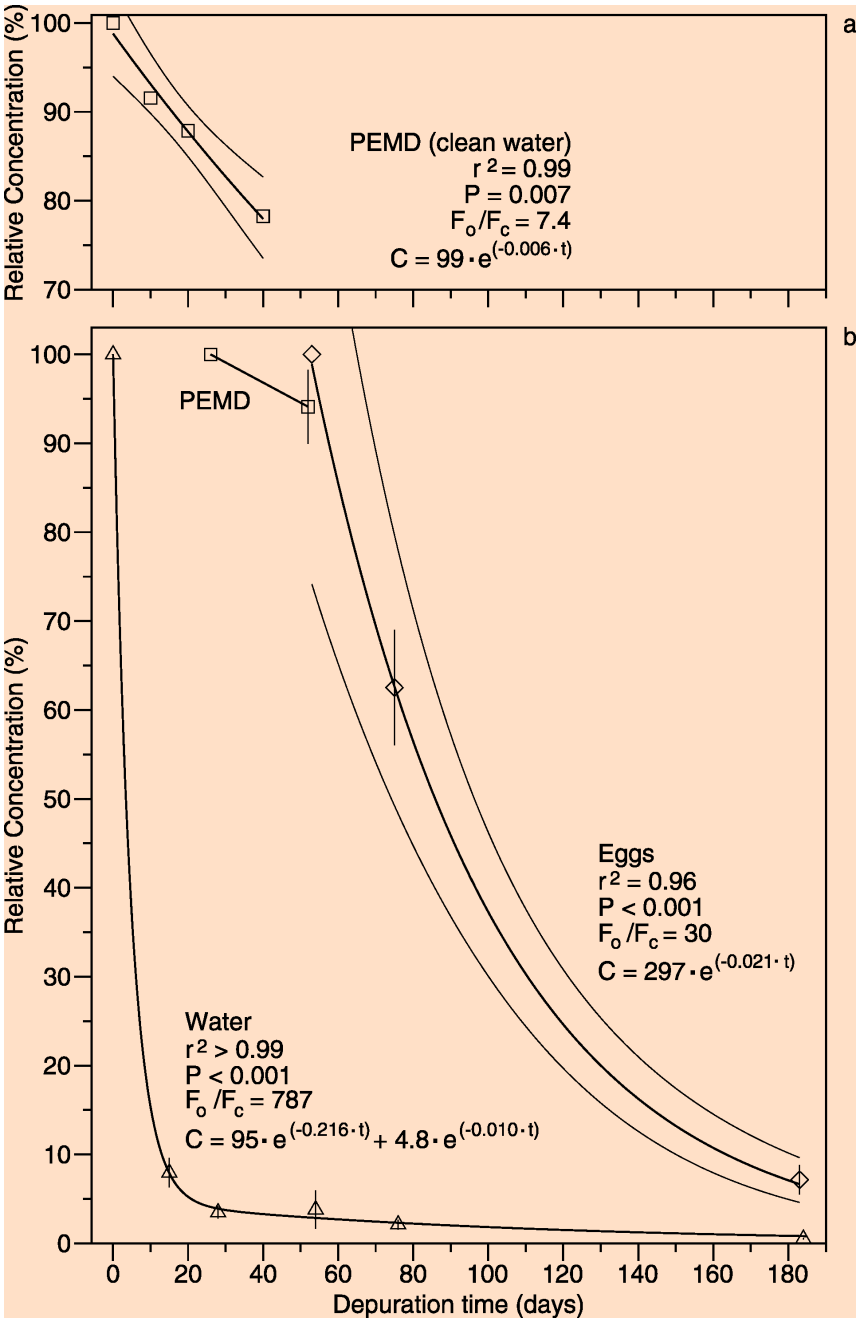

Fig. 4.3. Relative total polynuclear aromatic hydrocarbon (PAH) concentrations (C) in low-density polyethylene membrane devices (PEMDs), pink salmon eggs, and water as exponential functions of depuration time (t). Some PEMDs depurated in clean water (a); $n = 1$ for each plotted point. Other PEMDs and pink salmon eggs (means ± standard error) were maintained in falling aqueous total PAH concentrations (b). Where present, bounding curves are 95% confidence bands; F_o/F_c is the ratio between the observed F value and the critical F value.

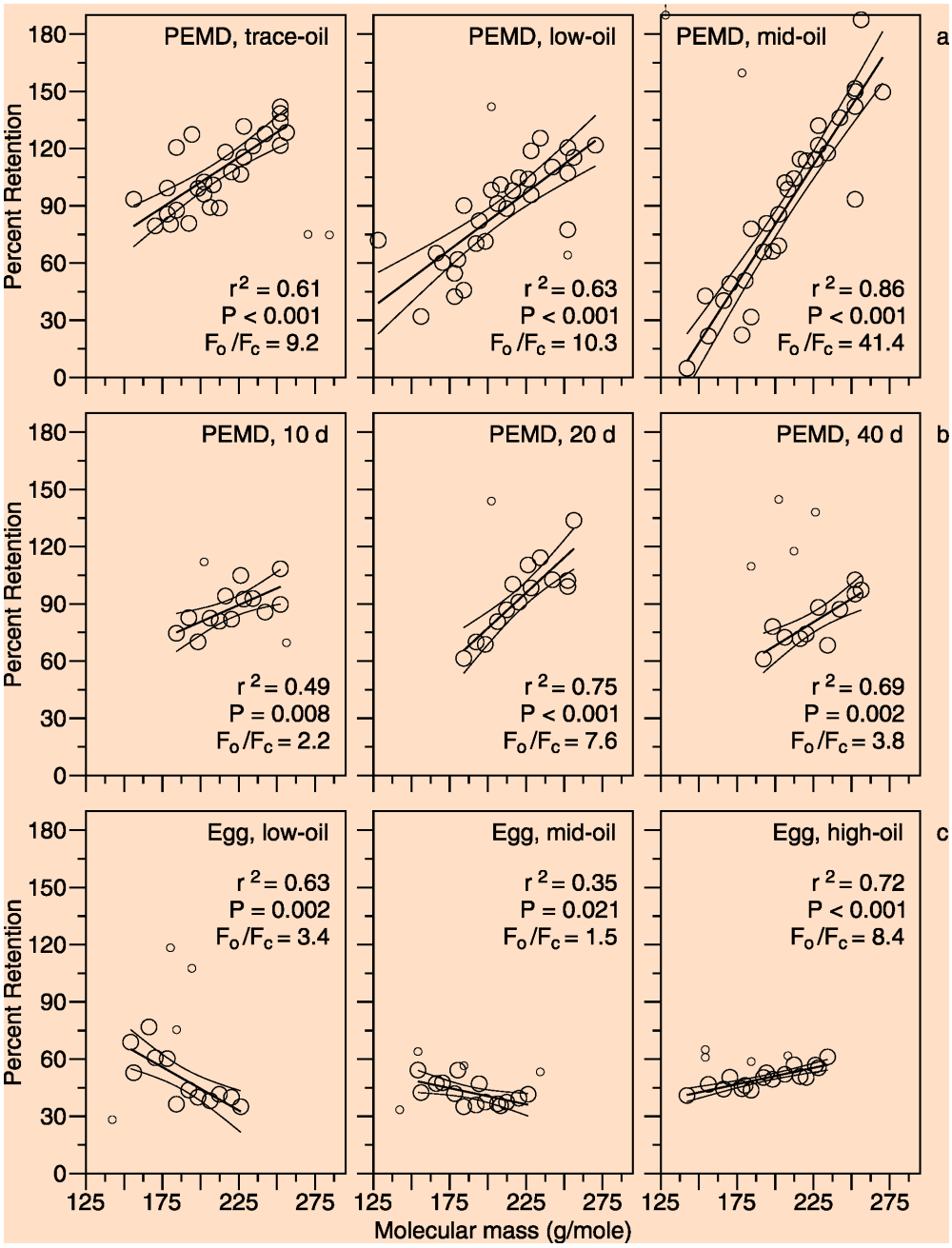

Fig. 4.4. Retention of polynuclear aromatic hydrocarbons (PAH) in low-density polyethylene membrane devices (PEMDs) and pink salmon eggs as functions of molecular mass. Estimates in row (a) compare PEMD retention between days 26 and 52 maintained in water where aqueous total PAH concentrations were at or rapidly approaching background levels. Estimates in row (b) were completed in clean water after 10, 20, and 40 d depuration and include 3 to 5 ring PAH only. Retention estimates in eggs (row c) were completed between days 53 and 75 where aqueous total PAH concentrations were at or near background levels. All illustrated regressions are linear with 95% confidence bands. Small symbols indicate outliers identified by regression analysis and not included in final analyses.

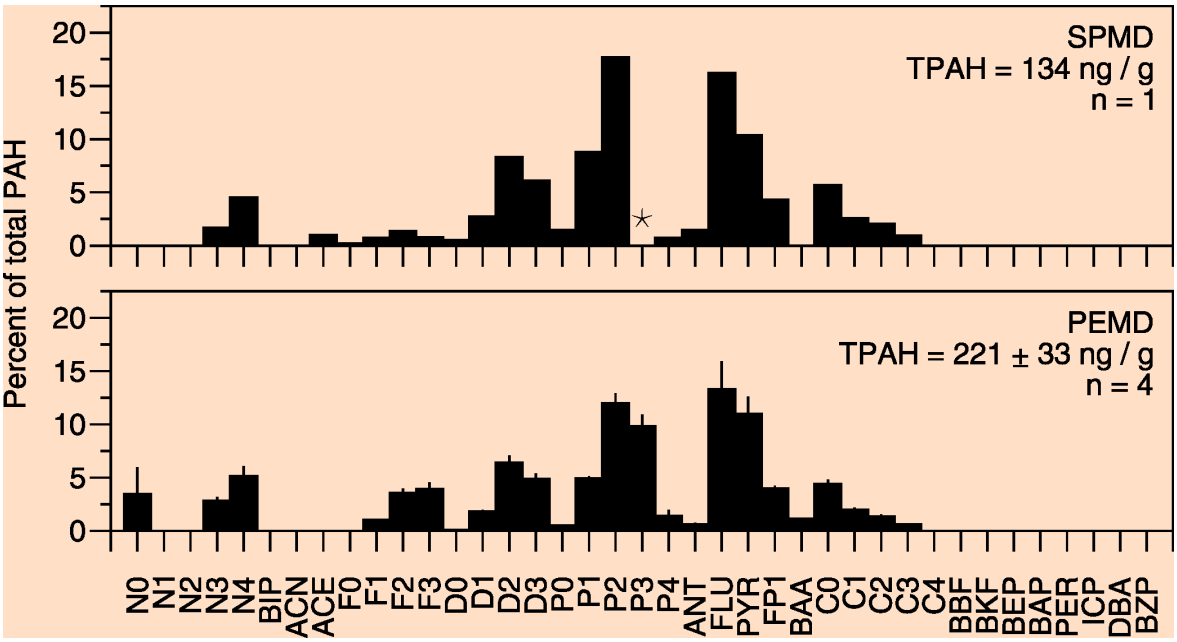

Fig. 4.5. Comparison of polynuclear aromatic hydrocarbon (PAH) composition ± standard error in low-density polyethylene membrane devices (PEMDs) and semi-permeable membrane devices (SPMDs) deployed together at 1.8 to 2.4 m in Sleepy Creek, Prince William Sound, AK USA. Total PAH (TPAH) concentrations are noted in each panel. See Table 4.1 for explanation of the *x*-axis. * C3-naphthalenes were present in the SPMD sample but could not be quantified due to the presence of competing ions associated with triolein or its derivatives.

Appendix 1. Verification of passive sampler method sensitivity by deployment in streams near the Katalla oil seep.

The purpose for deployment of low-density polyethylene membrane devices (PEMDs) in streams near a known oil seep was to verify that polynuclear aromatic hydrocarbons (PAHs) were accumulated by these devices under field conditions. Evidence that PEMDs sample PAHs in field and laboratory conditions is presented in full detail in Chapter 4. However, the Katalla data specified in the original proposal objectives was not included in the paper. Thus, an additional figure (below) is included to fulfill this objective.