Exxon Valdez Oil Spill
Restoration Project Final Report

Synthesis of the toxicological and epidemiological impacts of the *Exxon Valdez* oil spill on Pacific herring in Prince William Sound, Alaska

Restoration Project 99328
Final Report

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Study history: This project was initiated in 1998, and synthesizes published industry and Natural Resource Damage Assessment research concerning the *Exxon Valdez* oil spill and Pacific herring in Prince William Sound.

Abstract: Pacific herring (*Clupea pallasi*) in Prince William Sound (PWS) were affected by two major events in the past decade, the *Exxon Valdez* oil spill in 1989 and a 75% collapse in the adult population in 1993. In this review we compare and reinterpret published data from industry and government sources. Combining site-specific estimates of exposure and recent laboratory effects thresholds, 0.4-0.7 $\mu g \bullet L^{-1}$ total polynuclear aromatic hydrocarbons (PAH), we conclude that 25-32% of the embryos were damaged in PWS in 1989. Significant effects extended beyond those predicted by visual observation of oiling and by toxicity information available in 1989. Oil-induced mortality probably reduced recruitment of the 1989 year class into the fishery, but was impossible to quantify because recruitment was generally low in other Alaskan herring stocks. Significant adult mortality was not observed in 1989; biomass remained high through 1992 but declined precipitously in winter 1992-1993. The collapse was likely caused by high population size, disease, and suboptimal nutrition, but indirect links to the spill cannot be ruled out. These concepts have broad application to future oil spill assessments. For example, safety standards for dissolved aromatics should reflect the previously unrecognized high toxicity of PAH to adequately protect critical life stages.

Key words: Pacific herring, *Clupea pallasi*, oil, petroleum, polynuclear aromatic hydrocarbon, toxicity, abnormality, epidemiology, disease, population, Prince William Sound, *Exxon Valdez*

Project data: *Description of data* - Data are archived in Lotus spreadsheets, graphics files are in AutoCAD format, and text files are in WordPerfect 6.1 format. *Custodian* - Mark G. Carls, NOAA/NMFS, Auke Bay Laboratory, 11305 Glacier Highway, Juneau, AK 99801 (work phone: (907) 789-6019, fax: (907) 789-6094, or email mark.carls@noaa.gov. *Availability* - Copies of all data and related text files are available on CDROM for the cost of duplication.

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EXECUTIVE SUMMARY

The *Exxon Valdez* oil spill occurred in Prince William Sound just before the spawning of Pacific herring, a species of great ecological and commercial importance. Ten years after the spill, controversy continues about the extent and duration of biological effects. Industry investigators consistently concluded that oil effects were smaller in magnitude, spatial, and temporal extent than did Natural Resource Damage Assessment investigators. Industry researchers concluded that herring had recovered from minor spill effects by 1990, but the *Exxon Valdez* Oil Spill Trustee Council only recently (1999) upgraded herring from "not recovering" to "recovering." For these reasons, a synthesis of herring research was initiated to provide an overview of effects and to resolve, as possible, differences between industry and Natural Resource Damage Assessment studies. However, synthesis of government and industry data was complicated by conflicting methodology for detecting oil in the environment, differing estimates of the magnitude of oil exposure, mechanisms of potential toxicity, and difficulties in interpreting population data. By 1990, the preponderance of data from both groups suggested that residual oil impacts were undetectable in herring eggs and larvae. Stock size in Prince William Sound remained strong until 1992, but an unexpected population collapse in the winter of 1992-1993 raised fears that the spill caused long-term damage. A virus was isolated from pooled samples of survivors in 1993, but the extent of its contribution to the population collapse was not known. Because the virus was isolated from other Pacific herring stock in Alaska where population declines were not observed, researchers examined the relation of the spill and other potential contributory factors to population decline.

Available hydrocarbon data from industry and Natural Resource Damage Assessment sources were reexamined to determine the temporal and spatial extent of *Exxon Valdez* oil in areas where Pacific herring spawned in 1989. Data available and appropriate for assessment of site contamination included visual observation, and hydrocarbon concentrations in seawater, sediment, molluscs, and herring eggs. Total polynuclear aromatic hydrocarbon concentrations in seawater, sediment, and molluscs (primarily mussels) from Natural Resource Damage Assessment studies were obtained directly from the *Exxon Valdez* Trustee Council hydrocarbon database. Published data, including text and graphics from industry-sponsored research were included as appropriate, but detailed data from industry studies were generally not available. Sites where herring spawned that were nominally classified as oiled (the Naked Island area and northern Montague Island) were examined most closely.

The short-term consequences of the spill were detrimental to herring in Prince William Sound. We have demonstrated that (1) the nominal classification of the Naked Island and Montague Island areas in Prince William Sound as oiled was corroborated by visual and chemical evidence, (2) that mussels (used as a surrogate index of exposure by NRDA researchers) were exposed to EVO, and (3) that TPAH concentration in mussels was correlated with TPAH in herring eggs ($r^2 = 0.92$, $P = 0.011$). Further, the timing of herring egg incubation roughly coincided with the time of peak hydrocarbon exposure. Recent laboratory observations (Carls et al. 1999) suggest that the amount of hydrocarbons accumulated by Pacific herring eggs in Prince William Sound (Pearson et al. 1995a) was probably detrimental. Field and laboratory observations support this contention because herring larvae collected from oiled areas in Prince William Sound in 1989 were malformed, small in size, and had higher instantaneous mortality rates (Brown et al. 1996a).

The NRDA conclusions that adverse effects in several early life stages of herring from oiled areas in 1989 were caused by exposure to EVO are supported in this review. Industry assertions that EVO exposure was insufficient to have caused these injuries were not consistent with the data from field and laboratory studies. Industry relied on previous WSF literature to conclude that crude oil concentrations in water were 100-1,000 times less than toxic levels (Neff and Stubblefield 1995), but PAH from EVO was about 1,000 times more toxic than previously studied WSF's (Carls et al. 1999; Heintz et al. 1999). Oil was clearly biologically available in most areas within the slick trajectory, including areas where adjacent shoreline oiling was not evident. Thus, reliance of industry on overlap of spawn and visual shoreline oiling to predict risk (Pearson et al. 1995a; 1999; Bienert and Pearson 1995) was too restrictive. Industry argued that risk to herring eggs was limited to direct contact with oil (Pearson et al. 1985; 1995a; 1999; Bienert and Pearson 1995), but herring eggs readily accumulate toxic concentrations of PAH from contaminated water (Carls et al. 1999), as do pink salmon eggs (Heintz et al. 1999). Herring eggs within the slick trajectory were exposed to EVO; both industry and NRDA models detected EVO in tissues of adjacent mussels, and TPAH concentrations in herring eggs were correlated with concentrations in mussels. Industry researchers (Bence and Burns 1995) stated that shifts in PAH composition in internal tissues lead to uncertainties in identification of the source oil, and such composition shifts in herring eggs and tissues were indeed observed (Marty et al. 1998; Carls et al. 1999; 2000). Industry also argued that mussel beds must be tightly coupled with the herring spawn in both space and time to serve as surrogate measures of oil exposure (Bienert and Pearson 1995), and we have demonstrated that mussels met these spatial and temporal requirements in Prince William Sound. The preponderance of evidence demonstrates exposure of Pacific herring eggs to EVO in Prince William Sound resulted in larvae with low survival potential. Recently there has been an acceptance by industry that the 16% ascites prevalence in herring larvae from oiled areas (Marty et al. 1997) was a result of oil exposure (Pearson et al. 1999). We estimate that 25-32% of the entire spawned egg mass in PWS was probably exposed to biologically significant concentrations of EVO, resulting in substantial mortality. Oil-induced mortality probably decreased recruitment of the 1989 year class into the Prince William Sound fishery, but does not entirely explain the poor recruitment because recruitment of the 1989 year class was also generally low in other Alaskan herring stocks (Funk 1995a; Unpublished data, Dave Gordon, Alaska Department of Fish and Game, Sitka, AK).

Possible long-term consequences of the spill are difficult to discern. We conclude that important stressors responsible for the collapse of the adult herring population in 1993 included large population size, poor overwinter fish condition, and possibly environmental conditions, but links between the *Exxon Valdez* oil spill and delayed population response are tenuous. The herring population in Prince William Sound may have approached or exceeded carrying capacity between 1988 and 1992, thus the risk of a disease epizootic was high. Although the major agent in the epizootic, VHSV, can be induced by acute exposure to oil, and can cause rapid mortality in infected adult herring, delayed reaction of herring to oil toxicity in 1993 is unlikely. The estimated additional increase in population in 1989 resulting from the post-spill fishery closure was small, but the precipitous 1993 collapse of the population near its carrying capacity underscores the importance of formulating resource management decisions that integrate knowledge of the historical fishery. Populations at carrying capacity that are exposed to additional stress, such as oil, may be at greater risk than populations below carrying capacity. Environmental conditions unrelated to the oil spill, such as pre-winter prey availability

and winter starvation, may have also been important contributory factors, and probably no single factor can completely explain the population collapse.

Reassessment of response of Pacific herring to oil in Prince William Sound suggests that observed impacts could not have been predicted from routine toxicological monitoring methods in use at the time of the spill. This review emphasizes the need to thoroughly study pollutant exposure, adequately characterize pollutant composition, estimate toxic effects using appropriate laboratory models, critically examine sensitive life stages over appropriate time intervals, and place the findings within broad ecological perspectives. That the Prince William Sound oil spill occurred in a nearly pristine environment (Karinen et al. 1993) yields much information that can be advantageously used in situations complicated by previous habitat degradation. Adverse biological responses were observed orders of magnitude below expected responses in part because the spill chemistry had not been accurately predicted, in part because sensitive early life stages were exposed during critical development periods, and because sufficient funding and controversy ensured intensive study. Because oil typically persists for at least several days after a spill, the research paradigm should shift away from an emphasis on mechanisms of acute toxicity (such as narcosis) to long-term toxicity (e.g., oxidative cellular damage). As a result of this reassessment, we recommend that safety standards for dissolved polynuclear aromatic hydrocarbons should be revised to reflect a new toxicity threshold of $< 1\ \mu g \bullet L^{-1}$ (part per billion) TPAH to adequately protect aquatic organisms and habitat. Assessment of risk should also consider population dynamics (e.g., a population at carrying capacity may be at greater risk than one below capacity), season (e.g., spill effects are more deleterious when they impact critical early life stages), location of the spill (such as spawning grounds), local ecology, hydrographic conditions, and large-scale ecological processes. Results of the many *Exxon Valdez* studies have broad applicability to other situations, such as the combined industrial and non-point source runoff that results in elevated aquatic PAH loads near urban areas (Rice et al. 2001).

INTRODUCTION

The *Exxon Valdez* oil spill occurred in Prince William Sound just before the spawning of Pacific herring, a species of great ecological and commercial importance. In this synthesis, we summarize a decade of spill-related herring research including both Natural Resource Damage Assessment (NRDA) studies and industry-supported studies. Industry and NRDA interpretations of spill effects on Pacific herring were very different (Pearson et al. 1995a; Brown et al. 1996a), and this dichotomy was similarly evident among other studies including pink salmon (e.g., Brannon and Maki 1996; Bue et al. 1996), intertidal biota (e.g., Gilfillan et al. 1995; Stekoll et al. 1996), and sediment chemistry (e.g., Page et al. 1995; Short et al. 1999). Industry investigators consistently concluded that oil effects were smaller in magnitude, spatial, and temporal extent than did NRDA investigators. For these reasons, a synthesis of herring research was prudent to provide an overview of effects and to resolve, as possible, differences between industry and NRDA studies.

The scope of this synthesis was limited primarily to toxicological and epidemiological issues, but underlying ecological conditions are also discussed. Investigative progress in each of these areas has varied. Toxicological questions were addressed beginning immediately after the spill, and most of this research has been published. Disease research was stimulated by a collapse in the Prince William Sound herring population in 1993, and may be nearing completion. Broad-scale ecological research concerning herring is the least complete at present. Thus, toxicological and epidemiological research is emphasized in this synthesis, and a future synthesis of ecological research is anticipated.

We have provided an ancillary background section to more completely describe Prince William Sound, herring biology, the pre-spill hydrocarbon background, characteristics and weathering of *Exxon Valdez* oil, and the general spatial extent of the slick. The background section summarizes important concepts that lead into the main paper, but will not be submitted for peer-reviewed publication.

Several appendices are included which address specific published and anonymous criticisms applicable to NRDA research. Topics include microbial contribution to toxicity in oiled-rock column studies (Appendix 1), comparison of PAH toxicity observed in NRDA tests to short-term, static, acute bioassays (Appendix 2), and estimates of aqueous TPAH concentrations from PAH sequestered in mussel tissue (Appendix 3). While each of these topics is addressed in the primary paper, these appendices provide more detail.

OBJECTIVES

Objectives as proposed in the 1999 detailed study plan

1. Synthesize results of Trustee-sponsored toxicological and epidemiological studies relating to long-term injury and recovery of Pacific herring. All major hypotheses from contributing studies would be examined in the synthesis manuscript.
2. Evaluate and incorporate into the synthesis all of the relevant Exxon-funded research, and attempt to reconcile differences with Trustee-sponsored research where possible.

BACKGROUND

Prince William Sound

Prince William Sound (PWS) is an approximately 23,000 sq km complex fjord-type estuary (Schmidt 1977) with more than 3,200 km of coastline located along the northern Gulf of Alaska (Fig. B-1). As a result of plate tectonics and multiple glacial episodes, the Sound has a complex morphology (Carlson et al. 1991). It is bordered by the Kenai Peninsula on the west and the Chugach Mountains to the north. Islands Montague, Hinchinbrook, and Hawkins form a natural barrier between the Sound and the Gulf of Alaska. Extreme tidal range is about 6 m (-1.2 to 4.8 m mean lower low water), but the average tidal range is about 3 m. Surface water temperature ranges from 0.5 to 12EC (Morris and Loughlin 1994). Annual temperature and salinity cycles are determined by freshwater input, seasonal shifts in large-scale weather patterns, and by interaction with the surface and deep water of the Gulf of Alaska (Niebauer et al. 1994; Brown et al. 1996a). The climate is maritime, and the area is surrounded by a northern temperate spruce-hemlock rainforest. Annual rainfall in PWS communities ranges from 71-221 cm, and snowfall ranges from 203-762 cm (ADCRA 1998).

Prince William Sound is a highly productive ecosystem known for its rich marine life, including sea otters, killer and humpback whales, sea lions, harbor seals, porpoises, seabirds, eagles, salmon, Pacific herring, and groundfish, hence ecotourism is becoming an important industry in the Sound. Herring are fished commercially in PWS for food and bait, sac-roe, and spawn on kelp. Other important fisheries include salmon, Dungeness crab, shrimp, halibut, and other groundfish. Five major salmon hatcheries operate within the Sound

The contemporary human population in PWS is small, and historical human activity has also been limited. The towns of Cordova, Valdez, and Whittier are located along the margins of PWS, and the villages of Chenega Bay and Tatitlek are in the Sound. The total current human population in PWS is about 7,177 (ADCRA 1998). Historically, fish processing plants were located in Drier Bay, McClure Bay, Snug Harbor, Stockdale Harbor, and Thumb Bay.

Pacific herring in Prince William Sound

Few species are of greater combined ecological and economic importance in PWS (and in many other coastal ecosystems) than Pacific herring, *Clupea pallasi*. Herring of all life stages are central to a marine food web that includes humpback whales, harbor seals, a large variety of marine and shore birds, bald eagles, jellyfish and other invertebrates, and an array of other fish (Hart 1993).

Pacific herring spawn once a year in shallow water, typically along the same beaches each year. However, the volume of eggs and shoreline distance spawned varies widely (e.g., Funk and Harris 1992). Female herring produce about 19,000 eggs annually at 19 cm standard length and up to 38,000 eggs (Hart 1973), depending on body weight [about 200 eggs per gram fish weight (Hay 1985)]. Adherent eggs are deposited on eelgrass, kelp, rockweed, other seaweed, and rocks, at depths that range from about -11 m to high tide (Hart 1973), although about 90% of the eggs are deposited between -5 and +2 m mean lower low water (Haegele et al. 1981). Spawning occurs in late March to mid April; timing is apparently related to sea-surface temperature (4.4EC in PWS) and calm seas. [There is a latitudinal cline in spawning time (Stout et al. 2001); information given here pertains solely to PWS.] Intertidally spawned eggs may benefit from fluctuating temperatures and salinity due to tidal action; salinities of 12-17 ppt may yield

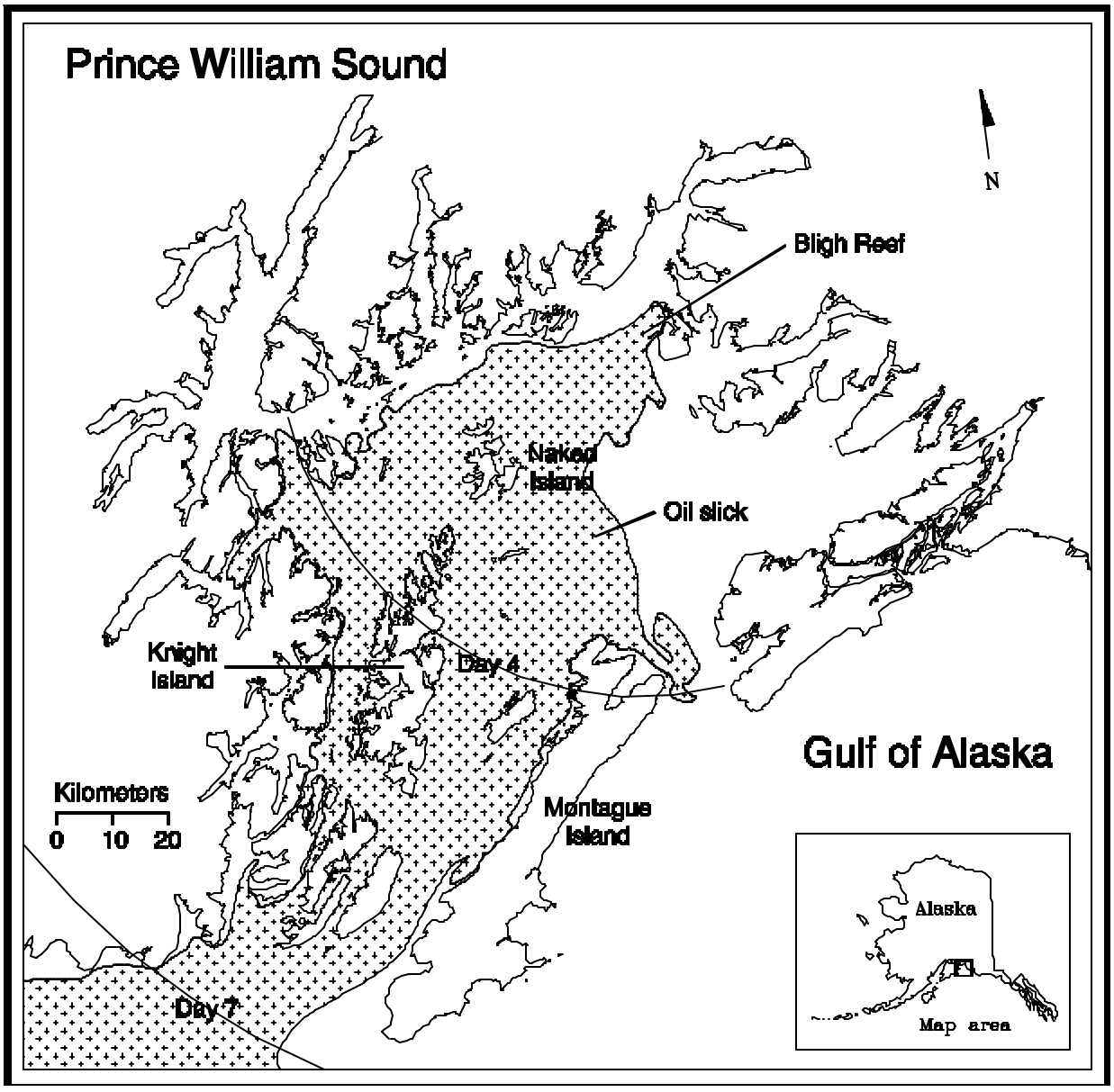

Prince William Sound
N
Bligh Reef
Naked Island
Oil slick
Knight Island
Day 4
Gulf of Alaska
Kilometers
0 10 20
Montague Island
Alaska
Map area
Day 7

Fig. B-1. Prince William Sound and the cumulative extent of the *Exxon Valdez* oil slick (Gundlach et al. 1990).

maximum total hatch and maximum viable larvae (Alderdice and Hourston 1985), and optimal temperatures may range from 5.5-8.3EC (Alderdice and Velsen 1971).

Herring larvae hatch in May in PWS, after incubating circa 24 d (Biggs and Baker 1993). Newly hatched yolk-sac larvae are vulnerable to advection by water currents (McGurk 1989) and predation by jellyfish, other invertebrates, and fish, such as pollock (Hourston and Haegele 1980; Purcell et al. 1990). The yolk-sac reserves are generally exhausted in a week, depending on temperature. Without additional food, starvation is irreversible within about 10 days (McGurk 1984). Larvae eat a variety of zooplankton and are found in mid-waters of PWS throughout the summer (Norcross et al. 1996). Metamorphosis to adult form occurs after about 10 weeks after hatch, when the larvae are about 30 mm in length (Hourston and Haegele 1980).

Juveniles may spend up to two years in nearshore rearing areas (Haegele 1994) before joining the adult population. PWS herring first enter adult schools in the fall of their second year of life and first spawn when 3 years old. (Currently, about 80% of PWS herring recruit at age 3, and most of the rest recruit by the age of 4 but recruitment timing may vary over time.) More than 90% of the juveniles in British Columbia (Haegele 1994) and PWS (Stokesbury et al. 1997) were located within 2 km of shore, often in bays. Overlap in distribution with juvenile pollock, which prey on juvenile herring, is significant (Brown and Carls 1998). Juvenile herring also may be a critical food resource for foraging seabirds and marine mammals because of their high energy lipid content, abundance, and availability in surface waters.

Adult herring disperse into deeper water after spawning, presumably close to the entrances of PWS to feed (Rounsefell and Dahlgren 1931), but aggregate in the fall and overwinter in central and eastern PWS (Brown and Carls 1998). Prey availability is generally low in the winter, and herring survive on stored energy reserves (Paul et al. 1998). Young of the year and age one herring store markedly fewer calories per gram than older fish, and are at greater risk of starvation in winter (Paul et al. 1998).

Pacific herring populations vary tremendously due to natural causes, such as predation, larval drift, disease (Meyers et al. 1994), and starvation (Stocker 1993). Herring also are vulnerable to anthropomorphic activities such as fishing pressure (Rounsefell and Dahlgren 1931; Hourston and Haegele 1980). A strong year-class is recruited in PWS about once every four years; this cohort then dominates the population for the next four years (Funk 1995a). The 1984 year class was dominant in PWS at the time of the *Exxon Valdez* spill and the 1988 year class became dominant by 1992.

Hydrocarbon background in Prince William Sound prior to the *Exxon Valdez* oil spill

Hydrocarbon pollutants were generally negligible in PWS prior to the *Exxon Valdez* oil spill (Short and Babcock 1996), and human impacts have been fairly minimal. There was no indication of petroleum hydrocarbon contamination in 5 of 8 sites in a 1977-1980 baseline survey At the other 3 sites (Constantine Harbor, Rocky Bay, and Mineral flats), aromatic hydrocarbons associated with vessel activity were detected only sporadically in sediment at concentrations near method detection limits (Karinen et al. 1993). All sites sampled by Karinen et al. (1993) were resampled in 1989 before *Exxon Valdez* oil arrived; petroleum hydrocarbons were generally absent from PWS seawater except that naphthalene and methylnaphthalene concentrations were elevated, thus Short and Babcock (1996) concluded that the PWS environment was largely pristine before the EVO spill. Pyrogenic PAH were detected in subtidal sediments at previous fish processing sites in Drier Bay, McClure Bay, Snug

Harbor, Stockdale Harbor, and Thumb Bay sites (Page et al. 1995). However, PAH concentrations in sediments near the head of McClure Bay were essentially at background levels except concentrations of naphthalenes were often elevated (Carls et al. 1994). Traces of California crude oil from tanks ruptured by the 1964 earthquake were reported in intertidal sediments (Kvenvolden et al. 1993). Petrogenic hydrocarbons have also been detected in the deep benthic sediment of PWS (Page et al. 1995; O'Clair et al. 1996), but these natural PAH concentrations are confined mainly to subtidal sediments (below 3 m) (O'Clair et al. 1996; Short et al. 1996a; Page et al. 1995).

Ongoing tanker operations in Valdez, Alaska, terminus of the trans-Alaskan pipeline, are a contemporary source of chronic contamination in Port Valdez, but hydrocarbons discharged from the ballast treatment facility have not been detected along tanker lanes within PWS. Ballast from returning tankers is treated in an onshore facility to remove oil, then discharged through a diffuser pipe into Port Valdez (Lysyj et al. 1979). Primary contaminants in discharged water (about 11 mg carbon$\bullet L^{-1}$) are monoaromatic hydrocarbons (48%), nonvolatile water-soluble organics (36%), and suspended organic matter (16%): on average, roughly 4.5×10^7 liters are discharged daily (Lysyj et al. 1979). The during much of the year when the water of Port Valdez is stratified (April-December), discharged hydrocarbons are trapped in a thin plume that extends 2-3 km from the mixing zone at a depth of 50-65 m (Lysyj et al. 1981). Concentrations of total volatile hydrocarbons at the mixing zone boundary may range up to 120 $\mu g \bullet L^{-1}$ (Lysyj et al. 1981). Although treated ballast discharge is toxic (crustacean larvae ceased swimming within minutes when exposed to 80-90% dilutions; Rice et al. 1981), Shaw et al. (1979) generally found no evidence of petroleum hydrocarbons in Port Valdez biota (*Mytilus edulis, Collisella pelta, Macoma balthica, Nereis vexellosa, and Cancer magister*), nor in intertidal and subtidal sediments 1-2 years after ballast-water discharge began. Petroleum hydrocarbons were detectable in water near the diffuser, but not in water $\geq$ 0.2 km from the discharge (Shaw et al. 1979) and there was no evidence that phytoplankton were negatively affected at this time (Alexander and Chapman 1979). Discharged hydrocarbons may be principally eliminated by microbial decomposition (Robertson et al. 1979).

The source of natural background hydrocarbons in benthic sediments of PWS has been debated by industry and NRDA researchers. Page et al. (1995) concluded the petrogenic background in PWS was derived largely from oil seeps in the eastern Gulf of Alaska. Short et al. (1999) contend that the petrogenic signature is consistent with coal, transported by coastal currents from extensive coal deposits to the east. This debate is important because the seep hypothesis implies biota in PWS may have developed a tolerance to PAH through natural selection, while exposure to coal confers no such adaptive benefit because PAH in coal are not biologically available (Short et al. 1999).

Composition and weathering of *Exxon Valdez* crude oil

Exxon Valdez crude oil (EVO) is a mixture of Alaska North Slope crude oils comprised primarily of aliphatic hydrocarbons and secondarily of aromatic hydrocarbons (8%), plus minor amounts of polar and unresolved high molecular weight material. However, acute toxicity of petroleum is directly correlated to its content of soluble aromatic derivatives (Anderson et al. 1974; Moore and Dwyer 1974; Neff 1979). Although aromatic hydrocarbons are relatively insoluble in water, they are more soluble than aliphatic hydrocarbons, thus partitioning occurs in water, and an enrichment of aromatics

relative to aliphatics is indicative of dissolved oil. Most of the aromatic hydrocarbons in *Exxon Valdez* crude oil were single ring compounds (about 80%) such as benzene, toluene, ethylbenzene, and xylene. However, because volatility of aromatic hydrocarbons is greatest for the monoaromatics, most or all of these compounds were lost to the atmosphere within a few days of the spill (Wolfe et al. 1994; Neff and Stubblefield 1995), and the remaining PAH were the primary potential source of toxicity to biota in PWS. High molecular weight PAHs are more toxic than low molecular weight aromatic hydrocarbons (Rice et al. 1977; Neff 1979; Black et al. 1983), thus the potential toxicity of remaining hydrocarbons per unit mass was higher.

The composition of PAH in EVO is well defined, but changes with weathering. There are five major PAH families, ranging from two to four ring compounds (naphthalenes, fluorenes, dibenzothiophenes, phenanthrenes, and chrysenes, respectively) (Fig. B-2). The majority of PAH within each family have methyl-substitutions. Naphthalenes dominate initially (67%), but composition shifts towards phenanthrenes as the oil weathers (Fig. B-2). Within families, unsubstituted compounds are lost most quickly, while the most substituted compounds are generally retained the longest. The time course of weathering varies primarily as a function of exposed oil surface area, but is greatly, influenced by many factors such as amount and distribution of oil in intertidal substrate, wave action, storm events, etc. Weathering changes have been modeled by Short and Heintz (1997) with first-order loss-rate kinetics and summarized by a weathering index, w, where w summarizes the exposure history of the sample: $w = 0$ in unweathered samples, and increases with weathering (Fig. B-2).

Spatial extent of *Exxon Valdez* oil

The supertanker *Exxon Valdez* went off course and ran aground on Bligh Reef in northeastern PWS on March 24, 1989. The resultant oil spill, about 42 million liters, was the largest in US history. Seas were calm for most of the first 3 d, and the oil meandered southwesterly, forming a 230 sq km slick (Kelso and Kendziorek 1991; Wolfe et al. 1994). In mid-afternoon of the third day (March 26), a 10-12 h northerly gale with 37-46 km/hr winds and gusts of 93-130 km/hr struck, blowing the oil slick to the southwest and beyond any hope of containment (450 sq km) (Kelso and Kendziorek 1991; Wolfe et al. 1994; Spies et al. 1996). By March 29, the slick completely surrounded the Naked Island complex and Smith Island, and extended past the southern tip of Knight Island on the east and nearly to lower Herring Bay on the west (Kelso and Kendziorek 1991) (Fig. B-1). Floating oil began to pass into the Gulf of Alaska on March 30 (Wolfe et al. 1994), and by June 20, the oil was distributed over approximately 28,500 sq km (Gundlach et al. 1990) (Fig. B-1). About 40% of the spilled oil was beached in PWS (Spies et al. 1996). Only about 2% of the original oil mass remained on beaches (Spies et al. 1996) by fall 1992.

Human perceptions

An aspect of the spill in relationship to PWS that merits attention is human emotion. For those living within the Sound, the ecosystem and the fish it produces represent a way of life, a place to respect and be respected. The *Exxon Valdez* spill was by no means a minor incident. It brought grown men to tears as they perceived their special part of the world had been poisoned by a spill so large that it defied belief. These people witnessed firsthand the destruction oil caused - the death of seabirds, marine mammals, and the coating of countless miles of shoreline.

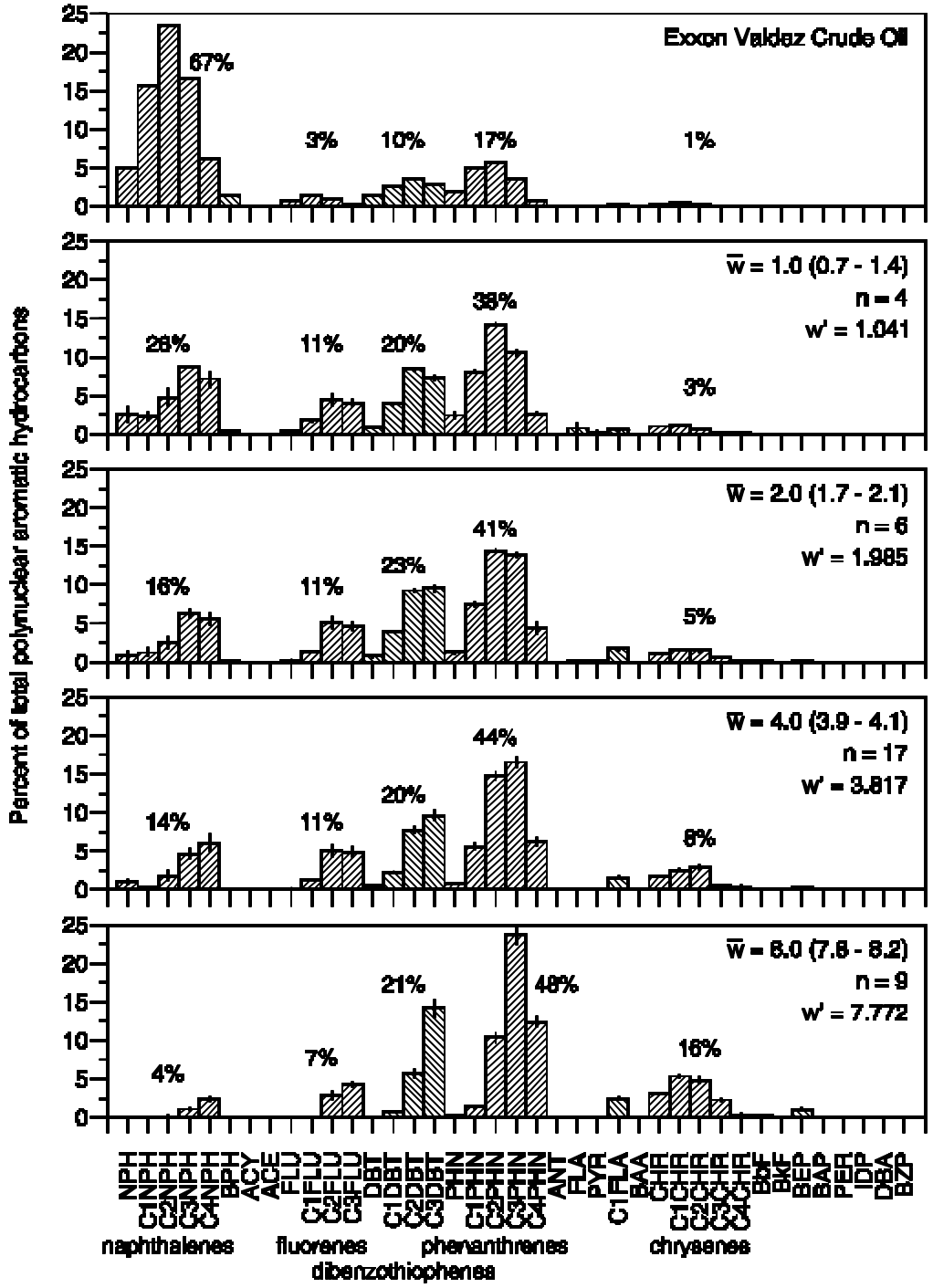


Fig. B-2. Composition of polynuclear aromatic hydrocarbons (PAH) in *Exxon Valdez* crude oil, and typical weathering patterns. Weathering, *w*, is an index of weathering as estimated by a first-order loss-rate model (Short and Heintz 1997). All data were obtained from the *Exxon Valdez* Trustee Council Database (Short et al. 1996b). Data were selected and grouped on the basis of *w* for display purposes; *w'* is recalculated *w* based on mean PAH composition in each group. Percentages written within each panel are total percentages of each homologous group (naphthalenes through chrysenes)

The perception of poisoning extended into the water, fisheries were closed to avoid the harvesting of tainted fish, and the perception was one of irreparable damage. Although the Sound today looks much the same as it did before the spill, the people who live there know that at least some oil remains, that on some beaches rocks coated with remarkably fresh oil can still be collected. Thus, the perception of poisoning persists, and will not go away quickly. Declines in salmon and herring populations, especially rapid events such as the 1993 collapse of the
herring population, and a similar drop in salmon abundance in 1992 and 1993 naturally raise the specter of lingering spill effects in the minds of many. We in science can study, but not solve, the problems created by major oil spills. We can put forward hypotheses, discuss and test them, but we cannot determine with absolute certainty whether a given post-spill event is, or is not, linked to the spill. Perceptions of damage will persist for a long time. However, natural processes will gradually improve the remaining degraded environment and the ecosystem will return to full health. Time alone will heal the emotions evoked by the spill.

Publications concerning Pacific herring and the *Exxon Valdez* oil spill

Some 30 studies and reports about Pacific herring have been published as a result of the *Exxon Valdez* oil spill (Appendix 4). The majority of these studies (24 of 30) were funded by the Natural Resource Damage Assessment process, and involve 12 different first authors. Industry sponsored studies involve three different first authors; foremost is Pearson, who was the primary author on four of the industry papers. Thus, Pearson is heavily cited in the ensuing paper. Nine of the herring publications were either summaries or reviews of primary studies, four written by industry (e.g., Bienert and Pearson 1995) and five written by NRDA-sponsored researchers (e.g., Brown et al. 1996a,b).

Primary herring studies relating to the *Exxon Valdez* oil spill can be separated into two broad categories, field and laboratory (Appendix 4). For the purposes of this discussion, only studies where live eggs, larvae, or fish were maintained in the laboratory after collection are classified as "laboratory." Laboratory studies can be further subdivided as observational (O) or experimental (E). Studies were considered observational if there were no experimental treatments in the laboratory. For example, both McGurk et al. (1990) and Pearson et al. (1995a) collected eggs from PWS, transported them to laboratory settings, and incubated them until hatch. In contrast, experimental studies involved exposure of herring to crude oil or viral hemorrhagic septicemia virus (VHSV). Examples of experimental studies include Carls et al. (1999; oil) and Kocan et al. (1997; VHSV). Experimental studies expanded from oil exposures to include disease research as evidence mounted that exposure to oil influenced parasitic organisms (Moles et al. 1993) and increased susceptibility to disease (Carls et al. 1998). Disease issues became prominent after the collapse of the herring population in PWS in 1993 (e.g. Meyers et al. 1994). Field studies were expanded to include the general health and condition of herring in order to provide baseline observations (e.g., Elston et al. 1997; Marty et al. 1998) against which to assess potential oil and disease effects. Industry sponsored studies included one field study, one laboratory observational study, and no experimental studies. NRDA studies included nine field studies, six observational studies, and nine experimental studies [two related to disease (only) and seven related to oil exposure].

Synthesis of the toxicological and epidemiological impacts of the *Exxon Valdez* oil spill on Pacific herring in Prince William Sound, Alaska

M. G. Carls, G. D. Marty, and J. E. Hose

Abstract

Pacific herring (*Clupea pallasi*) in Prince William Sound (PWS) were affected by two major events in the past decade, the *Exxon Valdez* oil spill in 1989 and a 75% collapse in the adult population in 1993. In this review we compare and reinterpret published data from industry and government sources. Combining site-specific estimates of exposure and recent laboratory effects thresholds, 0.4-0.7 $\mu g \bullet L^{-1}$ total polynuclear aromatic hydrocarbons (PAH), we conclude that 25-32% of the embryos were damaged in PWS in 1989. Significant effects extended beyond those predicted by visual observation of oiling and by toxicity information available in 1989. Oil-induced mortality probably reduced recruitment of the 1989 year class into the fishery, but was impossible to quantify because recruitment was generally low in other Alaskan herring stocks. Significant adult mortality was not observed in 1989; biomass remained high through 1992 but declined precipitously in winter 1992-1993. The collapse was likely caused by high population size, disease, and suboptimal nutrition, but indirect links to the spill cannot be ruled out. These concepts have broad application to future oil spill assessments. For example, safety standards for dissolved aromatics should reflect the previously unrecognized high toxicity of PAH to adequately protect critical life stages.

Introduction

A decade after the *Exxon Valdez* spilled approximately 42 million liters of Alaska North Slope crude oil (ANSCO) into Prince William Sound (PWS), Alaska, controversy continues about the extent and duration of the biological impact. Synthesis of government and industry data has been complicated by conflicting methods for detecting oil in the environment, differing estimates of the magnitude of oil exposure, mechanisms of potential toxicity, and difficulties in interpreting population data. Industry researchers concluded that biological communities in PWS were either not impacted by the spill or recovered within a few years (e.g., early life stages of pink salmon - Brannon and Maki 1996; shoreline ecology - Gilfillan et al. 1995; pink salmon adults - Maki et al. 1995; Pacific herring eggs - Pearson et al. 1995a; seabird overview - Wiens 1995). In contrast, the *Exxon Valdez* Oil Spill Trustee Council (EVOSTC 2001) currently lists populations of 8 species in PWS as not recovering [common loons, cormorants (pelagic, double-crested, and red-faced), harbor seals, harlequin ducks, killer whales (AB pod), and pigeon guillemots]. Pacific herring (*Clupea pallasi*) populations are classified as recovering by the EVOSTC (2001), but were considered never substantially impacted by industry (Pearson et al. 1995a). Only two species are listed as recovered, bald eagles and river otters (EVOSTC 2001).

Pacific herring are central to a food web that includes a large variety of marine and shore birds, invertebrates, fish, humpback whales and harbor seals (Hart 1973). Although the population biomass was at near record levels when the spill occurred, low abundances of adult herring in PWS since 1993

forced either the closure or limitation of this multi-million dollar commercial fishery. Commercial fisheries for Pacific herring were opened in 1997 and 1998, but were again closed in 1999 and remained closed through 2001.

The *Exxon Valdez* oil (EVO) spill may have had both short- and long-term consequences for herring. In the two to three years following the spill, the herring population was closely monitored by both Natural Resource Damage Assessment (NRDA) and industry researchers for evidence of spill effects. Two very different interpretations of results emerged from these studies, divided along industry and NRDA lines. Industry investigators consistently concluded that oil effects in 1989 were of smaller magnitude, spatial, and temporal extent than did NRDA investigators (Pearson et al. 1995a; Brown et al. 1996a). By 1990, the preponderance of data from both groups suggested that residual oil impacts were undetectable in embryos and larvae. Stock size in PWS remained strong until 1992 and did not appear to be affected by the oil spill (John Wilcock, Alaska Department of Fish and Game, Cordova, Alaska; personal communication). By late 1991, monitoring for possible oil impacts was suspended; further study was limited to routine estimates of stock size conducted by the Alaska Department of Fish and Game.

A second major event occurred over the winter of 1992-1993, when the PWS herring population collapsed from about 11×10^7 kg in 1992 to 1.7×10^7 kg in 1994 (Marty et al. 1998). A virus was isolated from pooled samples of survivors in 1993, but the extent of its contribution to the population collapse was not known (Meyers et al. 1994). Because the virus was isolated from other Pacific herring stock in Alaska where disease outbreaks and population declines were not observed (Meyers and Winton 1995), researchers examined the relation of the spill and other potential contributory factors to population decline.

Did the spill impact Pacific herring in PWS in 1989 and 1990? The industry-funded study (Pearson et al. 1995a,b) concluded that impact of the spill on PWS herring was generally minor because their results indicated that few (4-10%) spawned herring eggs were exposed to EVO, and effects, even in oiled areas, were too small to have affected the population. In contrast, NRDA studies drew very different conclusions: the oil trajectory overlapped 40-50% of the total spawn in PWS (Brown et al. 1996a), and exposure of eggs and larvae was sufficient to have substantially reduced the 1989 year class abundance (McGurk and Brown 1996; Hose et al. 1996; Norcross et al. 1996; Marty et al. 1997). Also at issue are differences in the methodologies used to detect EVO in spawning areas, concentration thresholds at which EVO elicits toxicity, and mechanisms of EVO toxicity. In contrast, neither group found oil-related effects on embryolarval herring in 1990 (Pearson et al. 1995a,b; Brown et al. 1996a).

What caused the collapse of the herring fishery in 1993 in PWS? There is little dispute that disease was an important component in the die-off of spawning stock (Marty et al. 1998; Pearson et al. 1999). However, the exact causes and relative contributions of viral hemorrhagic septicemia virus (VHSV) and secondary fungal diseases remain unknown. Elston et al. (1997) argue that poor nutritional condition, coupled with cold winter temperatures, and possibly a cyclical density-dependent downturn in population numbers could have caused the collapse of the herring population. In contrast, two reports (Brown et al. 1996b; Kocan and Hose 1997) contend that a linkage between the previous oil spill and the population collapse cannot be ruled out and suggest that the disease outbreak may have been mediated by immunological damage in the 1989 year class. This concern prompted an evaluation

of recent research on the pathogenesis of VHSV disease in herring, and the most recent population abundance estimates to improve our perspective of recent alterations in the PWS herring stock.

In this review, we examine evidence for short- and long-term consequences of the oil spill on all life stages of Pacific herring in an effort to reconcile divergent NRDA and industry conclusions. Spatial and temporal distributions of oil in PWS in 1989 are considered key to understanding the magnitude and mechanisms of exposure and subsequent short-term effects. We performed a number of new data analyses comparing methods to detect weathering oil in organisms, coupling oil measurements in the environment and in organisms, validating the use of resident mussels as indicators of oil exposure for herring eggs, and refining the estimates of toxic total polynuclear aromatic hydrocarbon (TPAH) concentrations and thus, the extent of biologically relevant exposure in PWS in 1989. The results revealed that the low levels of TPAH accumulated by herring eggs at many areas within the oil trajectory in 1989, even without visible intertidal oil, were sufficient to reduce survival. This finding should trigger a paradigm shift in how oil spill effects need to be evaluated. The second objective is to discuss the possibility of long-term spill consequences, raised by the collapse of the herring population in 1993. Our findings parallel those of industry (Pearson et al. 1999), namely that the oil spill was not directly responsible for the population collapse. Instead, the collapse was likely caused by a combination of factors including high population size, disease, and suboptimal nutrition. However, location and timing of this population collapse suggest a possible relation to the spill, and indirect effects of oil on the ecosystem cannot be entirely ruled out because of the nature of scientific hypothesis testing.

Petroleum hydrocarbon measurement, identification, and weathering

Not only do industry and NRDA researchers reach conflicting conclusions regarding the impacts of the *Exxon Valdez* oil spill, they also developed different analytical tools to reach these conclusions. Consequently, we begin with a brief review of oil identification methods. These different methods have, in part, contributed to the differing conclusions reached by the two groups.

Comparison of oil classification models

Oil identification models independently developed by industry researchers (Bence and Burns 1995) and NRDA researchers (Short and Heintz 1997) successfully recognize pure and weathered EVO in sediment and mussels (Fig. 1), but identification is complicated by changes in PAH composition in water and other tissue, such as herring eggs, as a result of differential hydrocarbon accumulation and (or) metabolism. Both models successfully detected EVO in contaminated mussels, which have little ability to metabolize hydrocarbons (Vandermeulen and Penrose 1978; Stegeman 1985; Livingstone et al. 1989). For example, the Short and Heintz model detected EVO in all analyzable Outside Bay mussel samples in 1989, and the Bence and Burns model found EVO in 36 of the 46 samples (78%) (Fig. 1). The differences between PAH composition in non-molluscan tissue and source oil that were observed in eggs and adult herring tissues experimentally exposed to ANSCO (Carls et al. 1998; 1999; 2000) were anticipated by Bence and Burns (1995) who stated that their model was most applicable to samples of external surfaces and gastrointestinal tracts and that shifts in PAH composition in internal tissues lead to uncertainties in identifying EVO in tissue. Similarly, data from the same laboratory tests

could not be analyzed with the Short and Heintz model because some PAH essential for identification were absent. The Bence and Burns model correctly identified EVO in only 4% of these eggs and

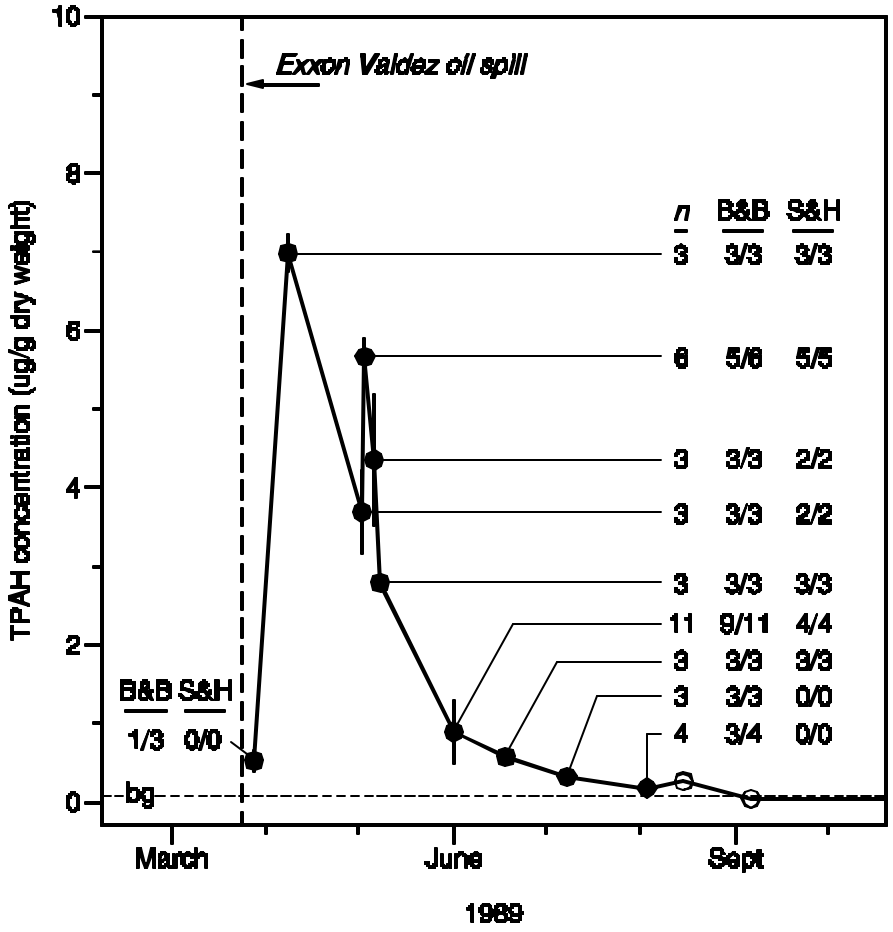


Fig. 1. Source of polynuclear aromatic hydrocarbons in mussels from Outside Bay on Naked Island in Prince William Sound: comparison of industry [Bence and Burns, 1995 (B&B)] and Natural Resource Damage Assessment [Short and Heintz, 1997 (S&H)] models. Plotted data are means ± SE, obtained from Short et al. (1996b). Solid symbols indicate *Exxon Valdez* oil (EVO) was identified in one or more samples; adjacent values indicate the total number of samples (*n*), and model results (number with EVO / number analyzable). Timing of the EVO spill on March 24, 1989 is indicated, and the line labeled "bg" is the estimated background concentration (0.089 $\mu g \bullet g^{-1}$ dry weight, Babcock et al. 1998).

did not identify EVO in herring muscle tissue or ova, thus identification of tissue contamination was usually erroneous because a source of oil is always assigned. Differences between PAH composition in water and source oil also cause difficulties in identification of the source oil because dissolution rates decline as the number of aromatic rings increases (Page et al. 1995; Short and Heintz 1997). Thus, composition of PAH in organisms contaminated by exposure to dissolved PAH may frequently not resemble composition in the source oil for multiple reasons. Because the Short and Heintz (1997) model is less likely to misidentify the source of contaminant oil, we prefer it and use it where applicable throughout the remainder of this paper.

Weathering alters composition of EVO

Oil weathering also alters PAH composition and recent herring experiments by Carls et al. (1998; 1999; 2000) closely modeled these changes in the laboratory. Although the rate at which oil weathers in PWS is extremely variable [the weathering index (w) of oil in beach sediment ranged from 0 to 10.7 in 1995 (Carls et al. 2001)], the close correspondence between PAH composition in the field and laboratory demonstrates that the weathering processes are fundamentally identical (Short and Heintz 1997). Carls et al. (2001) provide the following verbal descriptions for w: unweathered ($w = 0$), slightly weathered ($0 < w \leq 2$) moderately weathered ($2 < w \leq 8$), and highly weathered ($w > 8$). While the weathering model developed by Short and Heintz (1997) does not explicitly address the weathering of other petroleum hydrocarbons, e.g., alkanes and the unresolved complex mixture (UCM), there is no evidence at present to suggest that changes in concentrations and composition of these compounds in laboratory tests would be substantively different from changes in the field.

Short-term Consequences

Hydrocarbon data from industry and NRDA sources were reexamined to determine the temporal and spatial extent of EVO in areas where Pacific herring spawned in 1989. Data available and appropriate for assessment of site contamination include visual observations (Gundlach et al. 1990; Brady et al. 1991; Neff et al. 1995), and chemical analyses of seawater (Short and Harris 1996a), sediment (O'Clair et al. 1996; Short and Babcock 1996), molluscs (Andres 1995; Brown et al. 1996a; Short and Babcock 1996; Short and Harris 1996b), and herring eggs (Pearson et al. 1995a; Short et al. 1996b). Total PAH (TPAH) concentrations in seawater, sediment, and mussels from NRDA studies were obtained directly from the NRDA hydrocarbon database (Short et al. 1996b). Published data, including text and graphics from industry-sponsored research were included as appropriate, but detailed data from industry studies were generally not available. Sites where herring spawned that were nominally classified as oiled (the Naked Island area and northern Montague Island) were examined most closely. There were no arguments between industry and NRDA that sites in the northern and northeastern portions of PWS were not oiled (e.g., Fairmont Island and Tatitlek Narrows), and these sites were used in NRDA studies as EVO-free reference areas.

Spatial distributions of oil within spawning areas

Pacific herring spawn biomass and distances spawned in PWS were estimated by the Alaska Department of Fish and Game (Brady et al. 1991), and both industry and NRDA researchers accepted

these estimates (Bienert and Pearson 1995; Brown et al. 1996a,b). Of the total spawned biomass, 32% was in the northern Montague Island area, 19% was in the Naked Island area, and the remainder was in the North and Northeast areas (Brady et al. 1991). Distances of shoreline with spawn in oiled areas were relatively smaller, 29% and 14% of total spawn length, for the Montague Island and Naked Island areas, respectively (Brady et al. 1991). Industry estimates of potentially oiled herring spawn were based on spawn distance (Pearson et al. 1995a), while NRDA estimates were based on spawn biomass (Brown et al. 1996a,b).

Designation of potentially oiled spawn areas by NRDA and industry were identical (Brown et al. 1996a; Pearson et al. 1995a) (Table 1). The Naked Island area and northern Montague Island were both within the observed distribution of oil (Gundlach et al. 1990) (Figs. 2-3). Oiling was documented along the northern shoreline of Montague Island, including areas in Zaikof Bay, Rocky Bay, Montague Point, Graveyard Point, and Stockdale Harbor (Brady et al. 1991). Islands moderately or heavily impacted by oil included Naked, Storey, and Ingot Islands (Brady et al. 1991). In agreement with Brown et al. (1996a,b), Bienert and Pearson (1995) found 43% of the total miles of spawn were deposited within the slick trajectory, but based on a visual shoreline survey of oiling by Neff et al. (1995), Pearson et al. (1995a) concluded that only 4% of the total spawn distance was at risk of coming into direct physical contact with oil.

Biological availability of oil at spawning sites in 1989

Exxon Valdez oil was identified as the source of contamination in 1989 in herring spawning sites throughout the Naked Island and Montague Island areas, except for Zaikof Bay, where insufficient data were available (Figs. 2-3). In the Naked Island area, EVO was confirmed in mussel samples at the northwest and southwest corners of Cabin Bay, and in water from mid-bay (Short and Heintz 1997). *Exxon Valdez* oil was confirmed in mussels at 3 of 4 sites at the head of Outside Bay and in mussels tethered in the outer portion of the bay (Short and Harris 1996b; Short and Heintz 1997). [Total PAH concentrations in mussels tethered in Outside Bay demonstrate that EVO was biologically available to both intertidal and subtidal herring spawn. Mean TPAH concentrations in caged mussels in Outside Bay tended to be greatest at 25 m, but variance was high, thus concentrations did not differ significantly among depths ($P = 0.938$). In general, Short and Harris (1996b) found that hydrocarbons characteristic of EVO decreased with depth in tethered mussels, but EVO was detected at all depths (1-25 m) within the slick trajectory in PWS, May 1989.] In contrast, TPAH concentrations were not elevated in sediment collected at the head of Outside Bay. Mussel samples throughout Bass Harbor, on the north shore of Storey Island, and on a small islet to the south of Storey Island contained EVO (Short and Heintz 1997). On Montague Island, EVO was confirmed in mussel tissue at three locations along the southern shore of Rocky Bay, ranging from the inner to the outer bay, and bracketing most of the spawned area (Short and Heintz 1997) (Fig. 3). Total PAH concentrations in mussels were highest near the outer portion of Rocky Bay, as estimated on May 4, 1989, but concentrations in the inner bay exceeded background concentrations by 5-8 times (Fig. 4). Water samples provided additional evidence of EVO near the central southern shoreline of Rocky Bay. Total PAH concentrations in sediment from the inner bay (and extending subtidally to depths of 20 m) were slightly elevated (<0.5 $\mu g \bullet g^{-1}$), but EVO was not confirmed as the source of contamination. *Exxon Valdez* oil was confirmed in mussels collected from one of two sites in Stockdale Harbor (Short and Heintz 1997). In conclusion, EVO was identified in samples from most Pacific herring spawn sites (Short and Heintz 1997) within the

slick trajectory (Gundlach et al. 1990), providing justification for designating individual sites within the trajectory as oiled.

Table 1. Correspondence between sites sampled by industry researchers (Pearson et al. 1995) for total polynuclear aromatic hydrocarbons (TPAH) in herring eggs and those sampled by Natural Resource Damage Assessment (NRDA) researchers (Brown et al. 1996; Short et al. 1996b) for TPAH in mussel tissue. Classification of areas (oiled or reference) was identical for both industry and NRDA researchers.

Site	Sampled by	
	Industry Herring eggs	NRDA mussels
Oiled		
Naked Island area		
Bass Harbor	x	x
Cabin Bay	x	x
Storey Island	x	x
Outside Bay	x	x
Montague Island area		
Montague Point	x	
Stockdale Harbor	x	x[a]
Rocky Bay		x
Reference		
Fairmont Bay		x
Fairmont Island		x
Granite Bay	x	
Jack Bay	x	

[a]Samples collected by the US Fish and Wildlife Service. All other mussel samples were collected by Brown et al. (1996a).

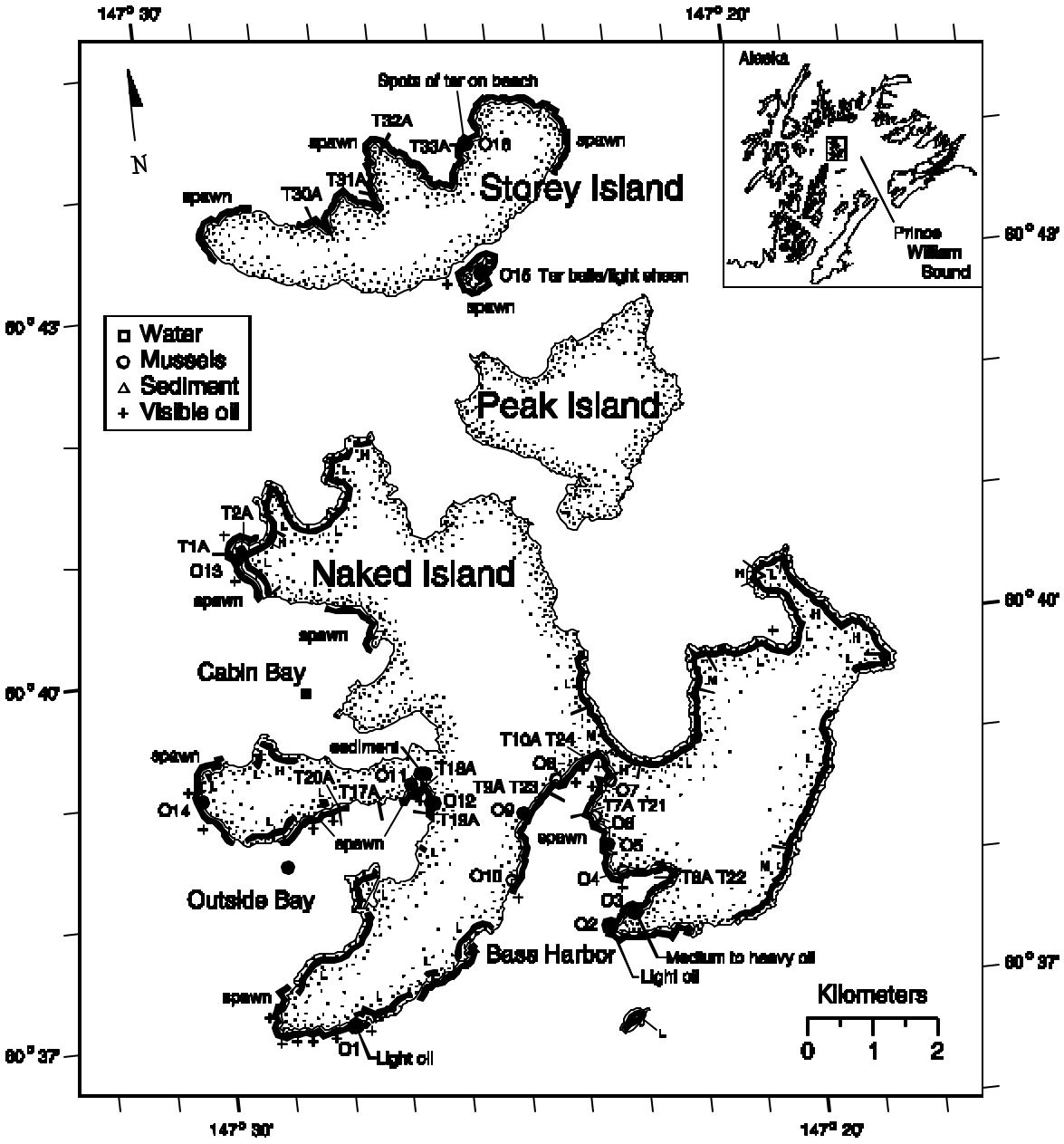


Fig. 2. Location of herring spawn, and water, mussel, and sediment hydrocarbon collection sites in 1989 in the Naked Island area. The mapped area was entirely within the *Exxon Valdez* oil (EVO) slick trajectory (Gundlach et al. 1990). Herring spawn data (bold lines drawn slightly offshore) are from Brady et al. (1991). Where symbols are solid, *Exxon Valdez* oil was confirmed as the source of contamination in one or more samples (Short and Heintz 1997). Sites labeled "O1-O16" were sampled by Brown et al. (1996a), "T1A-T33A" by Pearson et al. (1995a), and unlabeled sites represent a mixture of other studies (Short et al. 1996b). Visibly oiled samples are annotated (e.g., medium oil), and pluses (+) mark approximate positions of oil observed during herring spawn surveys (Brown et al. 1996b). Visible shoreline oiling (thick lines drawn

slightly onshore) was categorized as light (L), medium (M), or heavy (H) (Gundlach et al. 1990; Neff et al. 1995).

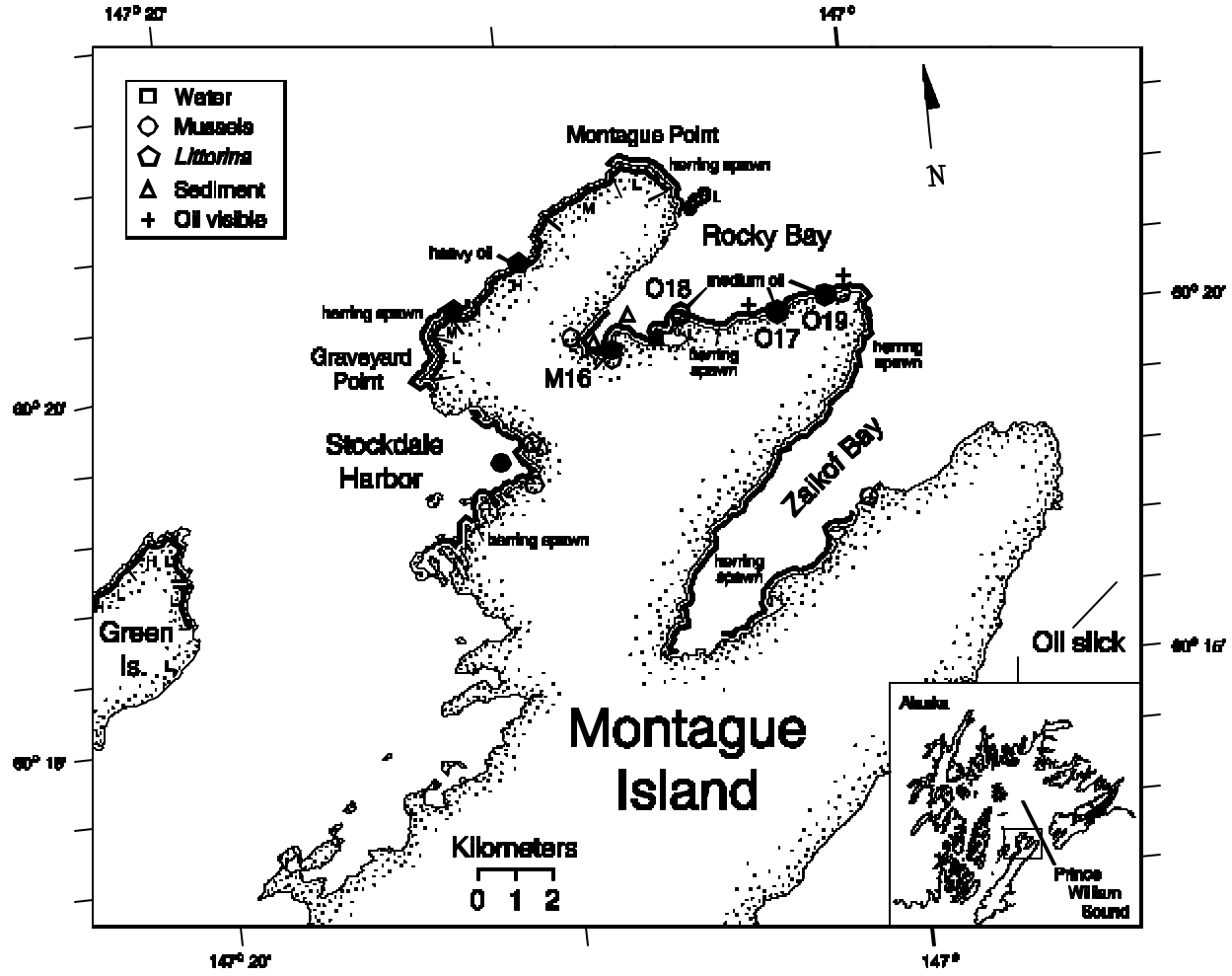


Fig. 3. Location of herring spawn, and water, mussel, *Littorina*, and sediment hydrocarbon collection sites in 1989 on northern Montague Island. Herring spawn data (bold lines drawn slightly offshore) are from Brady et al. (1991). Where symbols are solid, *Exxon Valdez* oil was confirmed as the source of contamination in one or more samples (Short and Heintz 1997). Sites sampled by Brown et al. (1996a) for mussels and herring are labeled as originally designated by the authors (O17-O19); unlabeled sites and site M16 represent a mixture of studies (Short et al. 1996b). Visibly oiled samples are annotated (e.g., medium oil), and pluses (+) mark approximate positions of oil observed during herring spawn surveys (Brown et al. 1996b). Visible shoreline oiling (lines drawn slightly onshore) was categorized as light (L), medium (M), or heavy (H) (Gundlach et al. 1990; Neff et al. 1995). Oil slick data (crosshatch) are from Gundlach et al. (1990).

Although there was little overlap between visibly oiled shoreline and herring spawn, there was strong evidence of biologically available oil in spawn areas in 1989 (Figs. 2-3). Industry estimates that 4% of the spawn length occurred along visibly oiled shorelines (Pearson et al. 1995a) are similar to our estimate (6%). [Herring spawn data (Brady et al. 1991) were combined with shoreline survey data (Gundlach et al. 1990; Neff et al. 1995) and geographic shoreline data in a computer-aided design program to estimate spawn distances and overlap between spawn and visibly oiled shorelines.] However, widespread contamination of mussels throughout much of the spawn length (as described in the preceding paragraph) provides evidence that EVO was biologically available in areas without visible shoreline oiling, as do elevated TPAH concentrations in herring eggs-on-kelp (Pearson et al. 1995a). For example, mean TPAH concentrations in mussels at the head of Outside Bay rose to a maximum of 7.0 $\mu g \bullet g^{-1}$ dry weight at a distance of 0.6-1.0 km from the nearest small (<0.1 km) patch of visible oil identified in shoreline surveys. Total PAH concentrations in intertidal sediment at the head of Outside Bay remained ≤0.2 $\mu g \bullet g^{-1}$, providing further evidence of minimal sediment contamination where oil was biologically available. Mean TPAH concentration in mussels on an islet south of Storey Island was 7.3 $\mu g \bullet g^{-1}$ dry weight in May 1989, yet these mussels were located 3.0 km from the nearest recorded visible shoreline oiling (site O15 in Fig. 2). Mussels in Rocky Bay with strongly elevated maximum mean TPAH concentrations (4.6-11.7 $\mu g \bullet g^{-1}$ dry weight) were located 2.2-3.5 km from the nearest reported oiled shoreline (sites O17 and O19 in Fig. 3). Mussels tethered 0.5 km offshore from the nearest oiled shoreline in Outside Bay at

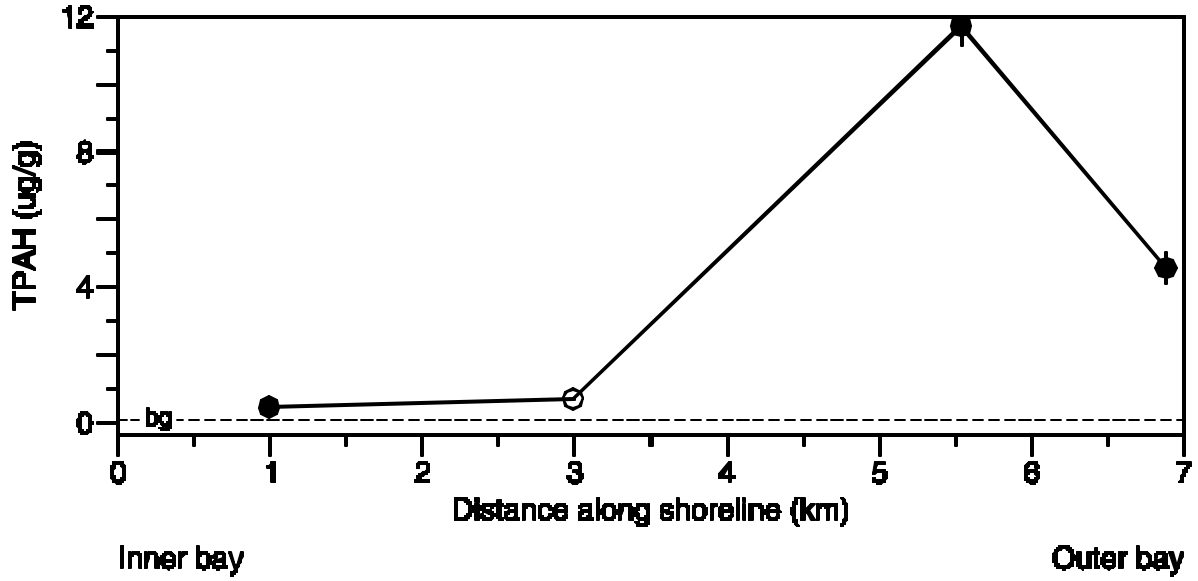


Fig. 4. Total polynuclear aromatic hydrocarbon (TPAH) concentrations in mussel tissue as a function of approximate shoreline distance in Rocky Bay on May 4, 1989. Solid symbols indicate EVO was

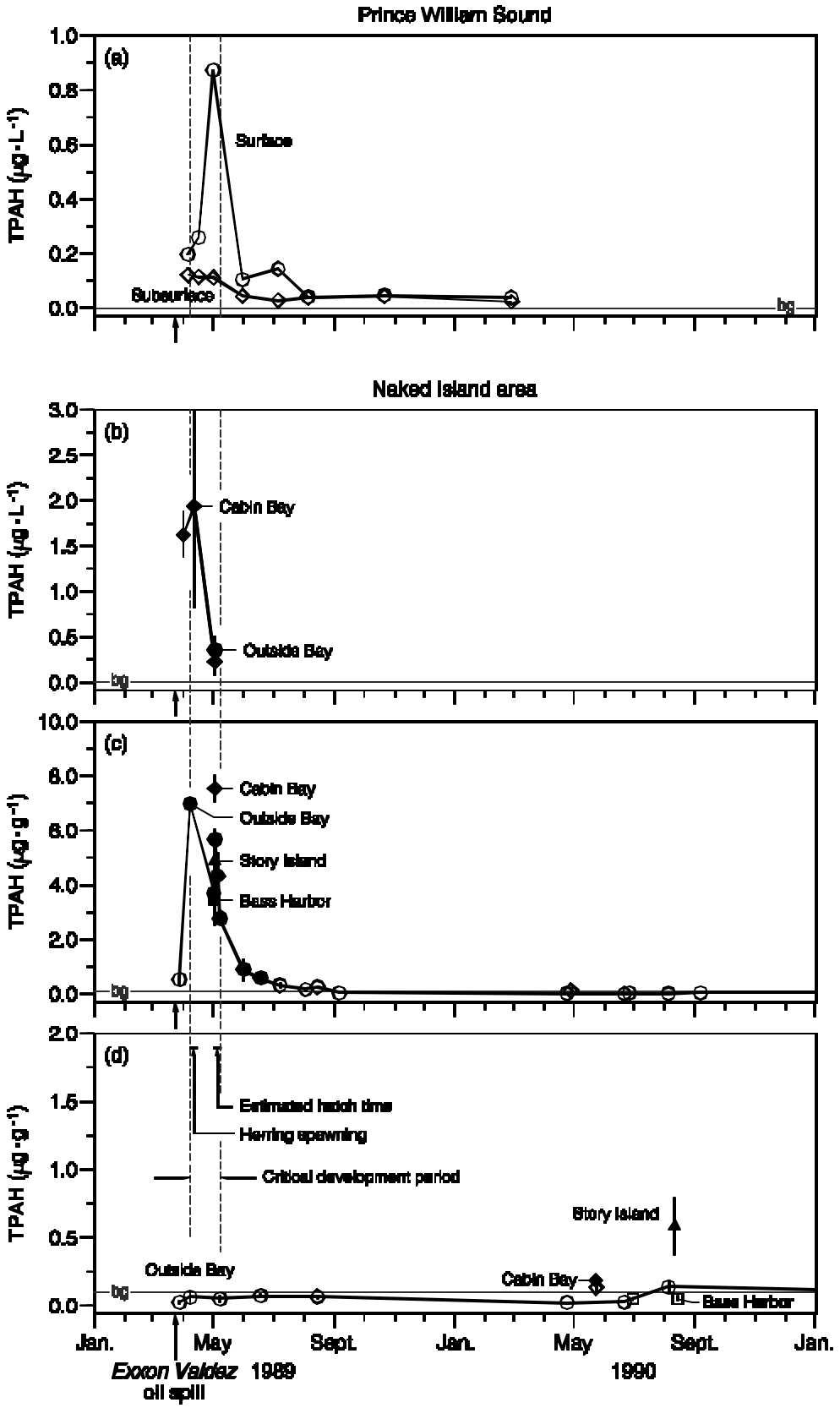
identified in one or more samples (Short and Heintz 1997). The line labeled "bg" is the estimated background concentration for mussel tissue (Babcock et al. 1998).

depths of 1-25 m clearly accumulated EVO (mean TPAH concentrations ranged from 0.6-1.5 $\mu g \bullet g^{-1}$ dry weight in May 1989) (Short and Harris 1996b), demonstrating that dispersed oil was biologically available in the water column. We conclude that oil was biologically available over a much broader range than suggested by visibly oiled shorelines or nearby sediment contamination.

Temporal changes in PAH

Seawater. Total PAH concentrations in seawater peaked in the Naked Island and Montague Island areas soon after the EVO spill, which was shortly before or at the time Pacific herring spawned in these areas (Figs. 5-6). Maximum TPAH concentrations in seawater were 1.9 $\mu g \bullet L^{-1}$ in Cabin Bay and 2.6 $\mu g \bullet L^{-1}$ in Rocky Bay (Short and Harris 1996a). Seawater samples at peak concentrations in Rocky Bay and all water samples in the Naked Island area contained naphthalenes, fluorenes, dibenzothiophenes, and phenanthrenes at concentrations above the method detection limit, strongly suggesting the source of contamination was EVO. Chrysenes were not always present, but absence of chrysenes was not unexpected because they are not always detected in water, even in carefully controlled experiments using progressively weathered ANSCO (Carls et al. 1997). Failure to detect chrysenes in water is explained by dissolution kinetics; e.g., when oil weathers from sediment into water, naphthalenes are lost from sediment most quickly, and higher molecular weight PAH, particularly chrysenes, are lost slowly (Page et al. 1995; Short and Heintz 1997). Expected movement of PAH from an oil slick into seawater follows a similar pattern, and is evident in the composition of mousse (Short et al. 1996b). Thus, PAH composition in seawater should be complimentary to the composition in weathering whole oil, and show enrichment of naphthalenes with relatively fewer higher molecular weight PAH, particularly chrysenes. The rapid increase in aqueous TPAH concentrations after the spill, followed by a decline, is unambiguous evidence for a single-event spill, and also suggests that EVO was the source of contamination. Peak TPAH concentrations in water occurred about the same time as peak concentrations in mussels in the Naked Island area (Fig. 5), but earlier than in mussels further south at Rocky Bay (Fig. 6).

Fig. 5 (*next page*). Mean total polynuclear aromatic hydrocarbon (TPAH) concentration in (a-b) seawater, (c) mussel tissue, and (d) sediment as functions of time (a) throughout Prince William Sound (Neff and Stubblefield 1995) and (b-d) at specific locations within the Naked Island area (Short et al. 1996b). Where mussel and sediment symbols are solid, *Exxon Valdez* oil was confirmed as the source of contamination in one or more samples (Short and Heintz 1997). Solid symbols for seawater data indicate one or more samples contained naphthalenes, fluorenes, dibenzothio-phenes, and phenanthrenes; composition data from Neff and Stubblefield (1995) were not available (a). Lines labeled "bg" are estimated background concentrations. Timing of herring spawn and estimated hatch times (assuming a 24 d incubation time) are indicated. The T/V *Exxon Valdez* spilled oil March 24, 1989.


Prince William Sound
(a)
TPAH (µg·L⁻¹)
1.0
0.8
0.6
0.4
0.2
0.0
Surface
Subsurface
bg
Naked Island area
(b)
TPAH (µg·L⁻¹)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Cabin Bay
Outside Bay
bg
(c)
TPAH (µg·g⁻¹)
10.0
8.0
6.0
4.0
2.0
0.0
Cabin Bay
Outside Bay
Story Island
Bass Harbor
bg
(d)
TPAH (µg·g⁻¹)
2.0
1.5
1.0
0.5
0.0
Estimated hatch time
Herring spawning
Critical development period
Story Island
Outside Bay
Cabin Bay
Bass Harbor
bg
Jan.
May
Sept.
Jan.
May
Sept.
Jan.
Exxon Valdez oil spill
1989
1990

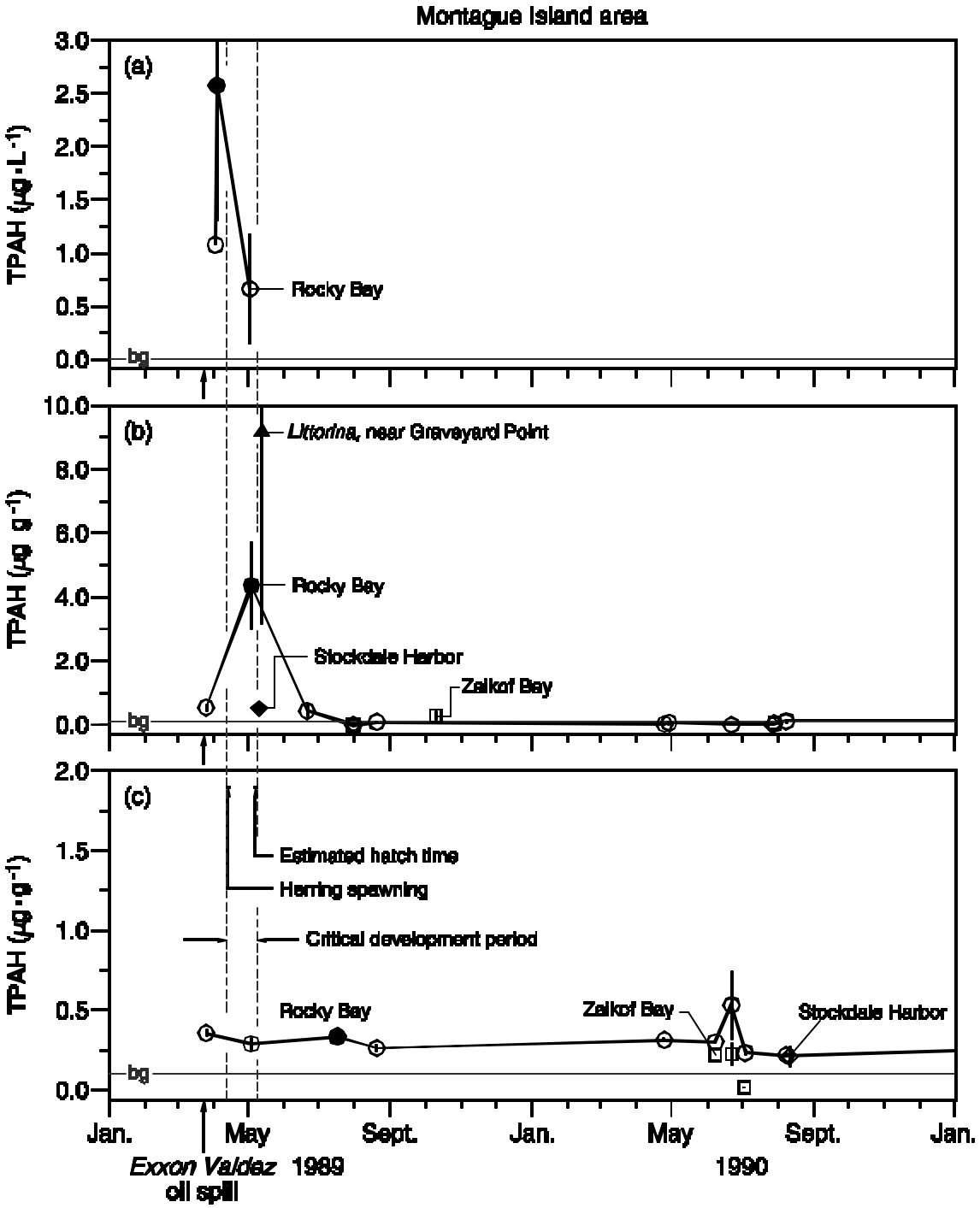


Fig. 6. Mean total polynuclear aromatic hydrocarbon (TPAH) concentration in (a) seawater, (b) mussel tissue, and (c) sediment as functions of time in Rocky Bay, Stockdale Harbor, and Zaikof Bay. Also included are TPAH concentrations in *Littorina* spp. near Graveyard Point. Where mussel and sediment symbols are solid, *Exxon Valdez* oil was confirmed as the source of contamination in one or more samples (Short and Heintz 1997). Solid symbols for seawater data indicate that one or more samples contained naphthalenes, fluorenes, dibenzothiophenes, and phenanthrenes. Lines labeled "bg" are estimated background concentrations. Timing of herring spawn and estimated hatch times (assuming a 24 d incubation time) are indicated. The T/V *Exxon Valdez* spilled oil March 24, 1989.

Sediment. EVO was infrequently detected in intertidal sediment from spawn areas within the oil trajectory, and TPAH concentrations were generally low throughout 1989 (Figs. 5-6). In 1989, TPAH concentrations were not elevated in sediment at the head of Outside Bay (≤0.2 $\mu g \bullet g^{-1}$), but concentrations at Rocky Bay were slightly greater than estimated background concentrations (<0.5 $\mu g \bullet g^{-1}$; Short and Babcock 1996), and remained elevated until mid 1991. Divers observed oil in sediment in 1989 at Outside Bay, Bass Harbor, Cabin Bay, and Montague Point to Graveyard Point (Pearson et al. 1995a), but intertidal sediments were not collected from Bass Harbor, Cabin Bay, Storey Island, Zaikof Bay, and Stockdale Harbor until 1990. *Exxon Valdez* oil was detected in sediment in 1990 at two of these sites (Cabin Bay and Storey Island) (Short and Heintz 1997), but not in the remaining sites. Although the extent of sediment samples in spawn areas was very limited, these data generally corroborate visible evidence of shoreline oiling in 1989 (Gundlach et al. 1990; Neff et al. 1995) or absence thereof.

Mussels. There was strong temporal evidence in mussel samples that oil was biologically available in herring spawn areas within or adjacent to the slick trajectory in 1989 despite little or no elevation of TPAH in sediment samples (Figs. 5-6). Total PAH concentrations in mussel tissue increased after the spill, peaked about the time Pacific herring spawned in the Naked Island area, and peaked about the time of embryo hatch in the Montague Island area (Figs. 5-6). The complete time series data for Outside Bay and Rocky Bay provide a frame of reference against which other mussel samples could be judged in the Naked Island and Montague Island areas. At Outside Bay, TPAH concentrations in mussels increased before herring spawned, were high during spawning (>6.1 $\mu g \bullet g^{-1}$ dry weight), and remained elevated through egg hatch (>2.5 $\mu g \bullet g^{-1}$) (Fig. 5). Total PAH concentrations in mussels at all other Naked Island sites were similarly high about the time of peak concentration in Outside Bay (3.4-7.5 $\mu g \bullet g^{-1}$) (Fig. 5). Total PAH concentration in mussels collected near the Ingot Island herring spawn, a site included in the Naked Island area, ranged from 9.9-15.4 $\mu g \bullet g^{-1}$ in September 1989, suggesting high exposures in the spring. [Both industry (Pearson et al. 1995a) and NRDA (Brown et al. 1996a) agree that the Ingot Island area was moderately to heavily oiled.] At Rocky Bay, TPAH concentrations in mussels increased during spawning and peaked at 4.4 $\mu g \bullet g^{-1}$ about the time of embryo hatch (Fig. 6). About the time of peak concentration in Rocky Bay, TPAH was high (9.2 $\mu g \bullet g^{-1}$) in *Littorina* spp. near Graveyard Point, but only slightly elevated (0.5 $\mu g \bullet g^{-1}$) in mussels at Stockdale Harbor. [Unlike mussels, where TPAH concentrations were measured in soft tissue only, *Littorina* were ground whole (including the shell), then analyzed for hydrocarbons, thus including both internal and external contamination.] Collection of mussels in Stockdale Harbor did not begin until about the time herring embryos hatched (May 11, 1989), and probably missed peak exposure. Total PAH concentration in *Littorina* also collected May 11 in Stockdale Harbor ranged from 0-1.2 $\mu g \bullet g^{-1}$ (mean = 0.3 $\mu g \bullet g^{-1}$). Mussel collection in Zaikof Bay began July 31, 1989, well after expected concentration peaks. The presence of EVO in mussel tissue was confirmed by Short and Heintz (1997) at every oiled site except Zaikof Bay. Mussels from both the Naked Island and Montague Island areas were contaminated with EVO even though sediment contamination was frequently undetectable.

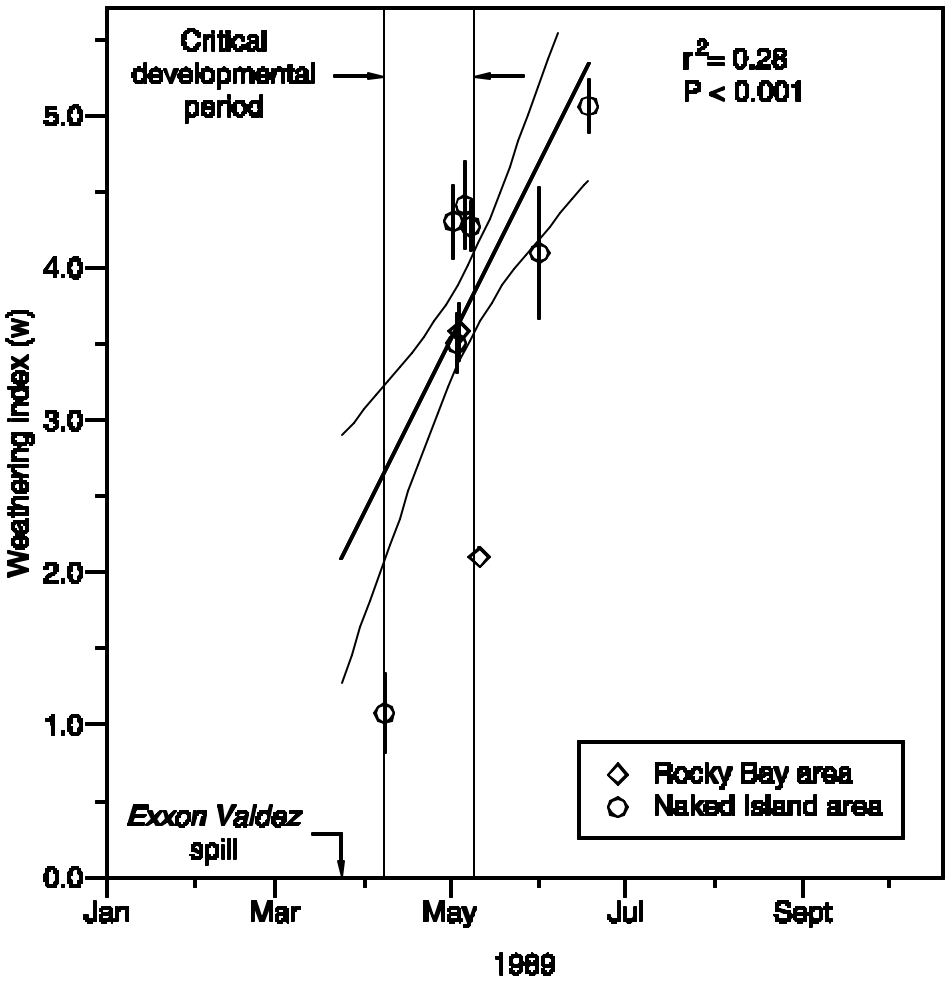


Fig. 7. Estimated state of oil weathering at herring spawn sites in Prince William Sound in 1989. Weathering index (*w*) was estimated from polynuclear aromatic hydrocarbon composition in mussel tissue collected from oiled areas by Brown et al. (1996a) with the methods of Short and Heintz (1997). Timing of herring spawn and estimated hatch times (assuming a 24 d incubation time) are indicated. The T/V *Exxon Valdez* spilled oil March 24, 1989.

Weathering. Increases in oil weathering (*w*), estimated from mussel tissue, were roughly linear in the first three months ($P < 0.001$), but correlation between *w* and time was poor ($r^2 = 0.28$; Fig. 7). Weathering (*w*) of EVO ranged from 0.7-5.7, in herring-spawn areas during the critical egg incubation period and averaged 3.7 (note: *w* is a unitless number).

Exposure of herring eggs to oil

Herring eggs were analyzed for hydrocarbon content by Pearson et al. (1995a) and Brown et al. (1996a) in 1989 and 1990. The latter group did not report their results, but the raw data are included in the NRDA database (Short et al. 1996b). Time series egg data were not collected, but several oiled and reference sites were sampled. Pearson et al. (1995a) subdivided hydrocarbon data into three tide zones (0.03 to 1.23 m, -2.43 to 0 m, and -6.15 to -2.46 m).

Pearson et al. (1995a) concluded that mean TPAH concentrations in eggs-on-kelp did not differ significantly between oiled and reference areas in 1989, although there were obvious concentration differences between many of the oiled sites and the reference sites (Fig. 8). Mean TPAH concentrations by tide elevation ranged from approximately 27-69 $ng \bullet g^{-1}$ at reference sites and 13-342 $ng \bullet g^{-1}$ wet weight at oiled sites; maximum concentrations ranged up to 1000 $ng \bullet g^{-1}$ at Cabin Bay where some eggs were visibly coated by oil (Pearson et al. 1995a). The highest mean TPAH concentrations were consistently in the upper tide zone Pearson et al. (1995a), but correlation of TPAH among tide zones was high ($r^2 = 0.93$), demonstrating that surface and subsurface oil concentrations were related (our analysis). (Total PAH concentrations for each tide zone at each site were normalized to the mid-tide zone concentration at that site. The mid zone was chosen as the denominator because there were no missing data points. Correlation was then tested between zones 1 and 3 across all sites.) Less than 4% of the eggs - all from Cabin Bay - were visibly coated with oil, and the proportion of developed eggs was inversely related to concentration at this site. Pearson et al. (1995a) concluded that only a minor portion of the 1989 spawn, the 2% of the spawn length at Cabin Bay, was affected by the spill.

Herring eggs collected by Brown et al. (1996a) were analyzed for hydrocarbons, but detection problems limited the usefulness of this collection and the results were not published. Total PAH concentrations in herring eggs collected by Brown's group were significantly elevated at Cabin Bay ($P_{ANOVA} < 0.001$), but not at other oiled sites (our analysis). Brown et al. (1996a) concluded that the mass of eggs sampled was insufficient for adequate detection of hydrocarbons, and thus relied on hydrocarbon concentrations in mussels collected within herring spawn areas as surrogate measures of exposure.

The source of PAH in herring eggs within the slick trajectory in PWS in 1989 was probably EVO because seawater, sediment, and mussels were all contaminated with EVO in these areas. We cannot directly test the hypothesis that the herring eggs-on-kelp samples collected by Pearson et al. (1995a) were contaminated with EVO because the data were unavailable. Pearson et al. (1995a) reported that EVO was identified in only three eggs-on-kelp samples, based on the Bence and Burns (1995) model - but this model failed to correctly identify ANSCO as the source of contamination in herring eggs exposed to this oil in laboratory tests by Carls et al. (1999; 2000). Moderately weathered EVO was identified as the source of contamination in a NRDA herring egg sample from Cabin Bay (*w* = 2.3; Short and Heintz 1997) and PAH composition was similar to mussels collected on the same day (May 3, 1989) from the same location (Fig. 9).

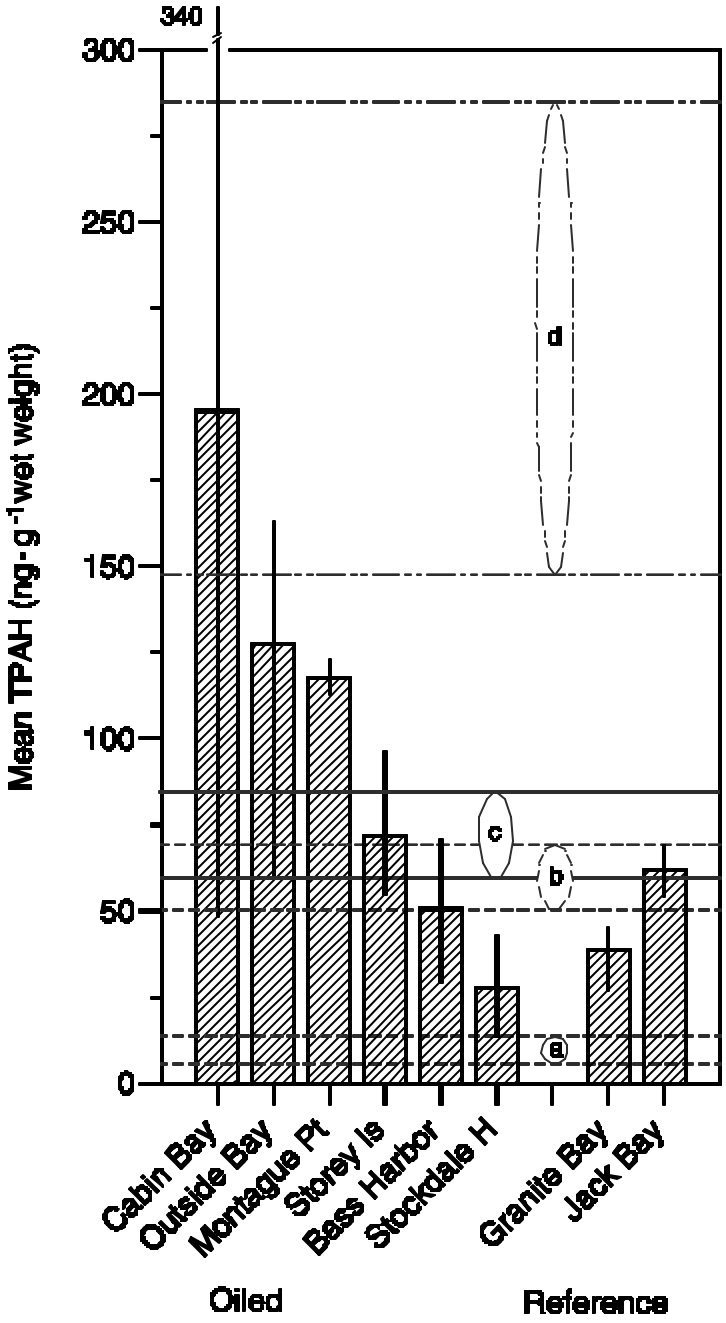


Fig. 8. Mean total polynuclear aromatic hydrocarbon (TPAH) concentration in Pacific herring eggs-on-kelp (Pearson et al. 1995a). Error bars indicate range of means by tide zone. Background TPAH concentrations in herring egg tissue estimated from (a) laboratory exposures (Carls et al. 1999), and (b) PWS (Pearson et al. 1995a) and are indicated. To compensate for differences in background concentrations, estimates of damaging concentrations were scaled to field background estimates. The minimum estimated zone of concentrations sufficient to cause abnormalities (c) is based on several estimates of the lowest observed effective concentrations in the laboratory (Carls et al. 1999) applied to two estimates of field background concentrations (see text). Estimation of minimum concentrations sufficient to be directly lethal (d) were calculated similarly.

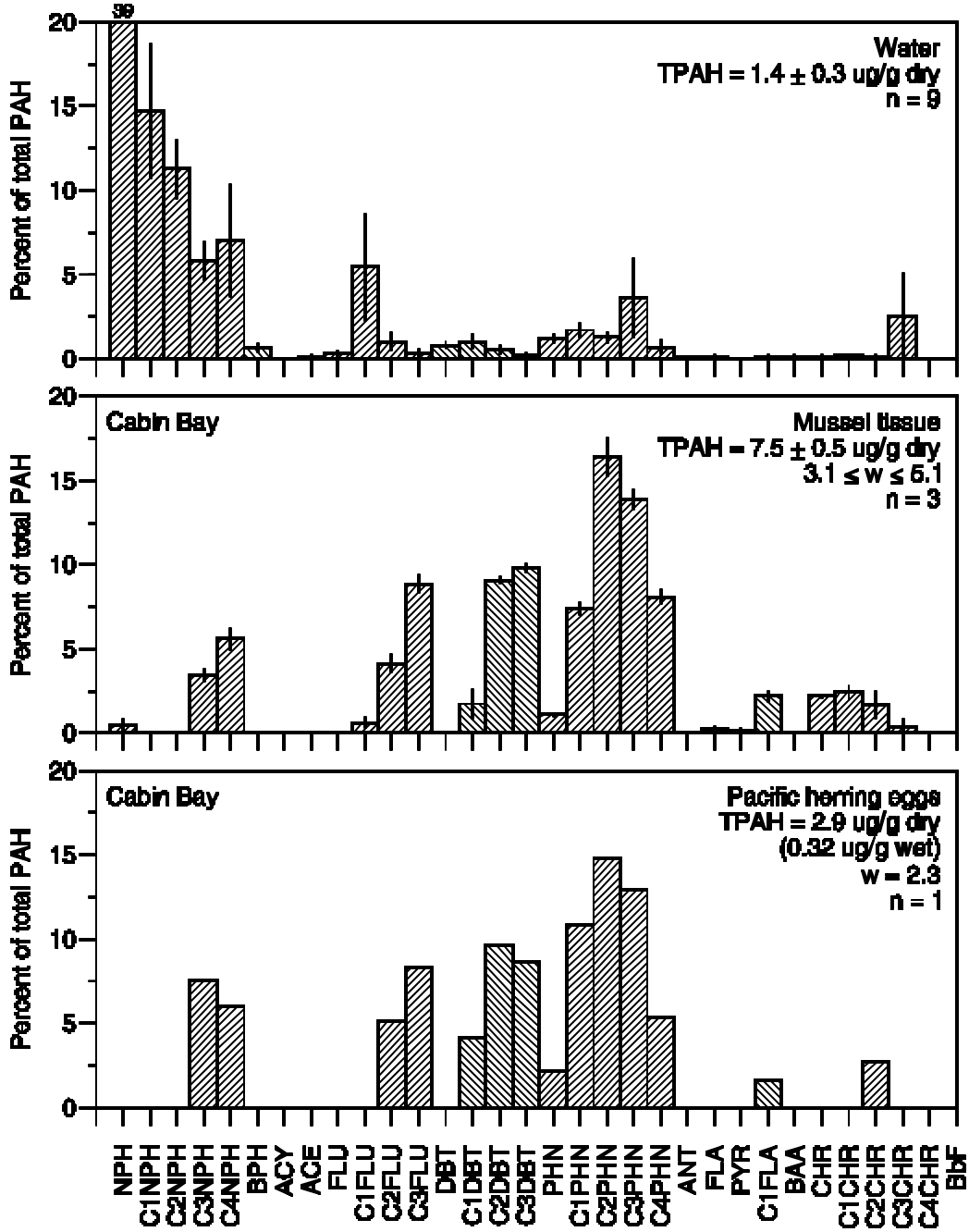


Fig. 9. Composition of polynuclear aromatic hydrocarbons (PAH) in water, mussel, and Pacific herring egg samples collected by Natural Resource Damage Assessment (NRDA) researchers in Cabin Bay. Tissues were collected on May 3, 1989; water data summarize contamination leading to this date (April 1, 1989 - May 3, 1989). Listed for each sample are total PAH (TPAH) concentrations, sample size (*n*), and weathering index (*w*), (except *w* was not calculable for water samples).

Exposure of mussels and eggs to oil in spawn areas was water-mediated

We infer that EVO was biologically available primarily from the water column in herring spawn areas, because hydrocarbon concentrations were elevated in water but not in intertidal sediment. MacKay et al. (1980) suggested the major toxic effects of an oil spill may be from hydrocarbons dissolved from dispersed oil. Oil can be dispersed in the water column by wind or wave activity (Mackay et al. 1980; Payne et al. 1991; Wolfe et al. 1994), and 3 days after the *Exxon Valdez* spill a 3-day storm dispersed substantial quantities of oil into the water (Wolfe et al. 1994). This dispersed oil was clearly accumulated by mussels tethered at depths to 25 m (Short and Harris 1996b). Phytane was present in 77% of Naked Island area mussels and 58% of Montague Island area mussels in 1989, confirming the presence of particulate oil in the majority of samples (Short et al. 1996b). Dispersion of oil into water greatly increases the surface area of the oil, thus accelerating the weathering processes described by Short and Heintz (1997) and increasing the concentrations of dissolved hydrocarbons, particularly of the higher molecular weight, less soluble compounds.

All available evidence indicates that the primary source of herring egg contamination by oil in PWS was water-mediated. The PAHs in nearly all eggs-on-kelp samples from PWS (Pearson et al. 1995a) must have been accumulated from surrounding water because 1) TPAH were present in the water column (Neff and Stubblefield 1995; Short and Harris 1996a), 2) TPAH were accumulated from water by both caged (Short and Harris 1996b) and native mussels (Brown et al. 1996a) in spawn areas, 3) composition of TPAH in the only herring egg sample with detectable hydrocarbons available to us (Short et al. 1996b) is consistent with contamination by dissolved PAH, 4) < 4% of herring eggs had visible tarry oil deposits (Pearson et al. 1995a), and 5) oil in beach sediment was infrequently encountered and present only at low concentrations where detectable (Pearson et al. 1995a; Short and Babcock 1996; Short et al. 1996b). The argument put forward by Pearson et al. (1999) that the ascites (an accumulation of fluid in the body cavity) observed in herring larvae in PWS by Marty et al. (1997) must have resulted from direct coating of eggs by oil is unlikely. A coating incidence of < 4% cannot explain the > 16% elevation in the incidence of ascites in oiled areas. Furthermore, Hay et al. (1995) reported that herring eggs in direct contact with oil invariably died, an observation corroborated by Pearson et al. (1995a) who found higher mortality in eggs coated with oil at Cabin Bay. Thus, the damaged larvae observed by Marty et al. (1997) likely resulted from exposure of eggs to aqueous PAH, not oil coating.

EVO concentrations in mussels and herring eggs were correlated in 1989

Total PAH concentrations in mussels collected by Brown et al. (1996a) from spawn sites were correlated with TPAH concentrations in herring eggs (Pearson et al. 1995a) at the same sites, justifying the use of mussel data as surrogates for herring egg exposure to EVO in PWS. Brown et al. (1996a) and Hose et al. (1996) did not report TPAH concentrations in herring eggs, but rather used TPAH concentrations in mussels adjacent to herring egg collections as surrogate measures of hydrocarbon exposure of herring eggs because mussels and other suspension feeding bivalves are frequently used for monitoring sporadically distributed hydrocarbons in seawater (NRC 1980; Phelps and Galloway 1980; Wolfe et al. 1981; Armstrong et al. 1995). To determine if using TPAH in these mussels as surrogates for herring egg exposure was justifiable, mussel and herring egg data from sites sampled in common by

both research teams were regressed (Fig. 10). These sites included Bass Harbor, Outside Bay, Cabin Bay, Storey Island,

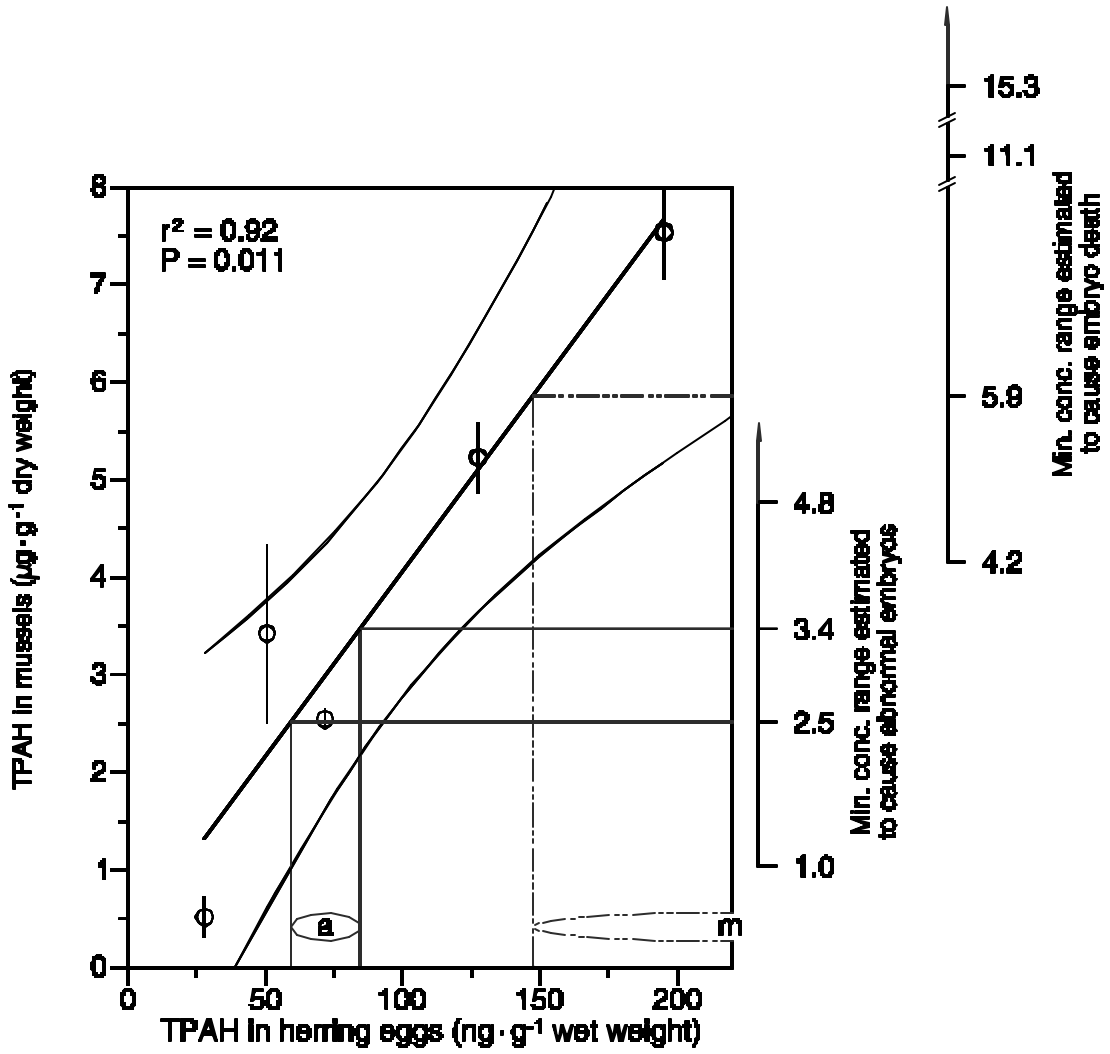


Fig. 10. Mean total polynuclear aromatic hydrocarbon (TPAH) concentrations in herring eggs-on-kelp (ng•g^{-1} wet weight; Pearson et al. 1995a) and in adjacent mussels (µg•g^{-1} dry weight; Brown et al. 1996a) were correlated (r^2 = 0.92; bounding curves are the 95% confidence bands). Data are means ± standard error. Minimum concentrations in mussels sufficient to predict damage in herring embryos were estimated from minimum concentrations sufficient to cause (a) abnormalities or (m) mortality in Prince William Sound herring embryos (see Fig. 6 and text).

and Stockdale Harbor. [Total PAH data for mussels in Stockdale Harbor were collected by the US Fish and Wildlife Service and reported by Short et al. (1996b).] Transects O1, O14, and O15 (McGurk et al. 1990; Brown et al. 1996a) were considered outside areas of overlap and were not included (Fig. 2). Mean TPAH concentrations in mussels (Brown et al. 1996a) were correlated with those in herring eggs (Pearson et al. 1995a) ($r^2 = 0.92$, $P = 0.011$) (Fig. 10). Bienert and Pearson (1995) previously argued that mussel data may be of limited value in estimating exposure of herring eggs deposited subtidally, but demonstration 1) that hydrocarbons were available to subsurface marine fauna after the *Exxon Valdez* spill (Short and Harris 1996b), 2) that TPAH concentrations in herring eggs were correlated across tide zones, and 3) that TPAH concentrations in mussels were correlated with those in herring eggs suggests otherwise. Thus, we conclude that the use of TPAH concentrations in mussels as surrogates for herring egg exposure to EVO in PWS by Brown et al. (1996a) and Hose et al. (1996) was valid.

Oil effects in PWS herring eggs

Adverse reactions of Pacific herring eggs to EVO in PWS were documented at hatching and in newly hatched larvae by NRDA studies (Brown et al. 1996a), but the industry study concluded oil effects were generally negligible (Pearson et al.1995a). Response data in all studies focused primarily on the condition of larvae hatched from exposed eggs. (Eggs naturally spawned in PWS were collected late in development by both NRDA and industry researchers and incubated in laboratories until hatch.) Premature hatch (Brown et al. 1996a) and abnormalities in Pacific herring larvae (Hose et al. 1996), effects consistent with exposure to crude oil (Smith and Cameron 1979; Pearson et al. 1985; Weis and Weis 1989; Kocan et al. 1996a; Marty et al. 1997; Carls et al. 1999), were significantly more frequent in oiled areas than in reference areas of PWS. Compared to reference embryos, oiled embryos hatched earlier, producing less mature larvae (Brown et al. 1996a). The proportion of larvae with jaw deformities varied significantly with oil concentrations in resident mussels (Hose et al. 1996; Brown et al. 1996a). Oiled larvae were longer at hatch, but weighed less than reference larvae (Brown et al. 1996a). Hose et al. (1996) found that the severity of skeletal, craniofacial, and finfold abnormalities was significantly higher in oiled areas compared to a reference site, Fairmont Bay. The severity of skeletal abnormalities, certain types of craniofacial defects (jaw abnormalities, microphthalmia, and absence of otic capsules) and the total severity index were significantly correlated to log-transformed TPAH concentrations in adjacent mussels (Hose et al. 1996). Not clear is why Pearson et al. (1995a) generally did not observe similar effects in embryolarval herring. Possibly the statistical power of tests by Pearson et al. (1995a) was too low (designed to detect a 30% change in the frequency of abnormal larvae at a power of 0.8 and a significance level of 0.05, although the power achieved was not reported). With regression analysis, Pearson et al. (1995a) detected a reduction in the proportion of developed eggs at Cabin Bay, but no other biological variables correlated with TPAH concentrations in eggs-on-kelp. Comparison of biological responses among sites by Pearson et al. (1995a) was limited to a single statistical contrast between oiled and reference areas, but because mean TPAH concentrations in eggs-on-kelp (and in mussels) varied considerably among oiled sites, this comparison may have missed significant inter-site variation. Because Pearson et al. (1995a) only presented the proportions of biological response variables without reporting the total number of individuals examined, and generally did not provide site-specific and tide zone detail, direct comparison to NRDA data is not possible.

Oil effects in laboratory-exposed eggs

Extensive research has consistently demonstrated that aqueous exposure of fish eggs to oil is damaging [e.g., see reviews by Moore and Dwyer (1974); Neff and Anderson (1981); Weis and Weis (1989)]. However, much of this literature focused on acute toxicity and one- to two-ring aromatics. Because one-ring compounds were quickly lost from the water of PWS (Wolfe et al. 1994; Neff and Stubblefield 1995), subsequent toxicity research was specifically tailored to study constituents remaining after the initial weathering [e.g., Marty et al. (1997); Carls et al. (1999); Heintz et al. (1999)].

The quantitative laboratory studies of Carls et al. (1999) provide a link between field and laboratory toxicity for embryolarval herring and can be used to hindcast biological responses in PWS to EVO in 1989. Herring eggs were exposed to concentration series of less weathered and more weathered oil in two consecutive experiments where seawater (32 ppt) was contaminated by passage through oiled gravel. Originally reported weathering estimates ranged from 0.04-1.28; amended ranges are $0.1 \leq w \leq 1.0$ (less weathered test) and $0.5 \leq w \leq 1.5$ (more weathered test). The resultant oil concentrations and composition closely modeled those observed in PWS after the spill (Short and Heintz 1997), and TPAH concentrations declined exponentially during tests. Total PAHs accounted for 36-79% of total hydrocarbons dissolved in seawater in the less weathered test, and 3-13% in the more weathered test. Concentrations of some higher molecular weight and more substituted PAH were at least as high in the upper two treatments of the more weathered test as in the less weathered test, e.g., C3- and C4-phenanthrene and C2-chrysene (Carls et al. 1997). Exposure of eggs to 0.7 $\mu g \bullet L^{-1}$ TPAH of the more weathered oil caused malformations, genetic damage, mortality, decreased size, and inhibited swimming in herring larvae. Total aqueous PAH concentrations as low as 0.4 $\mu g \bullet L^{-1}$ caused sublethal responses such as edema (ascites) and immaturity consistent with premature hatching. Responses to less weathered oil, which had relatively lower proportions of high molecular weight PAH, generally paralleled those of the more weathered oil, but lowest observed adverse effect concentrations (LOAECs) were higher (9.1 $\mu g \bullet L^{-1}$), demonstrating the importance of composition. Based on total measured aqueous hydrocarbons (TPAH + total alkanes + UCM), LOAECs in the less and more weathered herring egg tests were 25 and 14 $\mu g \bullet L^{-1}$ (previously unreported by Carls et al. 1999). Correlations (r^2) between biological responses and TPAH were consistently greater than correlations to either total alkanes or the UCM in these experiments, and were significantly greater in 3 of 4 comparisons ($0.001 \leq P \leq 0.013$); $P = 0.154$ for the remaining comparison. The UCM in the LOEC concentration did not exceed 10.4 $\mu g \bullet L^{-1}$ in the less weathered experiment, and fell from 9.8 $\mu g \bullet L^{-1}$ to 0 within 4 d in the more weathered experiment.

These results provide a framework to interpret field data. To properly match laboratory and field results, the state of oil weathering, summarized by w, was considered. Mean w, as estimated from mussel tissue, was 3.7 (range 0.7-5.7, $n = 51$) during the critical egg development period in PWS (1989) and 2.3 in herring eggs ($n = 1$), thus generally exceeding weathering in both experiments reported by Carls et al. (1999). However, Heintz et al. (1999) provide evidence that ANSCO remains toxic with further weathering ($w = 4.9$), and continues to be more toxic per unit mass than unweathered oil. Thus, use of the more weathered test by Carls et al. (1999) for interpretation of field results is more justifiable than use of the less weathered test, but may underestimate the true toxicity due to continued weathering. True toxicity may also be underestimated because exposure to ultraviolet light was

negligible during incubation but developing embryos in PWS were likely exposed to ultraviolet light from sunlight (Barron and Ka'aihue 2001) and absorption of ultraviolet light can increase PAH toxicity 2-1000 times (Pelletier et al. 1997). Tidally influenced fluctuations in salinity were not modeled by the laboratory experiment, but could also have influenced toxicity in PWS. For example, Vines et al. (2000) found that the increased incidence of abnormalities of herring embryos exposed to creosote was proportionately less at 28 ppt than at lower salinities (16 and 8 ppt).

Laboratory toxicity estimates suggest toxicity occurred in PWS

Three independent estimates of herring egg exposure to hydrocarbons in PWS in 1989, TPAH concentrations in 1) water, 2) herring eggs, and 3) mussels, interpreted by laboratory exposures, suggest exposure to EVO was damaging. These predictions of damage were confirmed by observation of larvae collected from oiled areas in PWS with abnormalities consistent with exposure to oil (Marty et al. 1997).

Water. Aqueous TPAH concentrations in PWS in 1989 exceeded concentrations detrimental to herring embryos and contaminated water contained high molecular weight compounds (e.g. phenanthrenes and chrysenes). Directly measured peak aqueous TPAH concentrations in Cabin Bay and Rocky Bay (1.9 to 2.6 $\mu g \bullet L^{-1}$; Short and Harris 1996a) exceeded the lowest observed effective concentrations (LOECs) causing abnormalities in herring embryolarvae (0.4 $\mu g \bullet L^{-1}$) in controlled laboratory experiments (Carls et al. 1999). Arguments that imply that EVO did not harm marine life because TPAH concentrations never exceeded water quality standards (e.g., Pearson et al. 1995a; Neff and Stubblefield 1995; Neff and Burns 1996; Pearson et al. 1999) are invalidated by post-spill field and laboratory observations to the contrary (e.g., Hose et al. 1996; McGurk and Brown 1996; Marty et al. 1997; Carls et al. 1999; Heintz et al. 1999). Water quality standards obviously need revision as a result of recent research.

Herring eggs. Interpretation of 1989 field results requires comparison of observed TPAH concentrations in eggs-on-kelp from PWS with biologically significant concentrations observed in laboratory tests. Recently published estimates by Carls et al. (1999) indicate that the mean LOEC causing abnormalities was 22 ± 4 ng$\bullet g^{-1}$ wet weight TPAH (mean of 4-16 d exposure data). Average area under the curve extending over all exposure days (0-16 d) might have been a more appropriate estimate of LOEC causing abnormalities, 19.7 ng$\bullet g^{-1}$, and provides a lower estimate for minimum damaging exposure concentrations. The peak LOEC causing abnormalities was 26.0 ng$\bullet g^{-1}$, providing an upper estimate of minimum damaging exposure concentrations. The published LOEC causing embryolarval mortality was 108 ± 35 ng$\bullet g^{-1}$ in herring egg tissue (mean of 1-16 d data; Carls et al. 1999). Again, a more appropriate estimate would be the average area under the curve (0-16 d), 123 ng$\bullet g^{-1}$. The peak LOEC causing mortality was 226 ng$\bullet g^{-1}$.

Complicating application of biologically significant laboratory concentrations to field results is a difference in field and laboratory baseline concentrations. The inferred background TPAH concentration for PWS eggs-on-kelp was the mean reference concentration (50.4 ng$\bullet g^{-1}$; our calculation). Reasons why this estimate of background concentration in eggs-on-kelp was higher in PWS (Pearson et al. 1995a) than in the laboratory (10.7 ± 1.9 ng$\bullet g^{-1}$; Carls et al. 1999) are not clear, but the possibility of contamination at reference sites in PWS cannot be discounted. Other possibilities include differences in analytical and sampling procedures [e.g., kelp was included in samples analyzed

by Pearson et al. (1995a), but not in laboratory samples (Carls et al. 1999)]. To compensate for these background differences, we calculated the difference between each laboratory estimate and the mean laboratory background concentration, and added these differences to the field background concentration. To estimate an upper limit for minimum concentrations causing abnormalities, we increased the estimated background concentration for PWS to the maximum mean concentration reported for any reference tide zone in 1989 (69 $ng \bullet g^{-1}$; Pearson et al. 1995a).

Direct measurement of TPAH in Pacific herring eggs in 1989 (Pearson et al. 1995a), interpreted by recent experiments (Carls et al. 1999), suggests that exposure of eggs to EVO in five of six oiled sites in PWS was detrimental. In contrast, Pearson et al. (1995a) concluded that mean TPAH concentrations in eggs-on-kelp from PWS in 1989 did not differ significantly between oiled and reference areas and that only three samples, representing two sites, were contaminated with EVO. Estimated minimum concentrations that caused abnormalities in PWS herring eggs, 59.4-84.6 $ng \bullet g^{-1}$ wet weight, included four or five of six oiled sites; observed concentrations in Bass Harbor fell within the zone of uncertainty (Fig. 8). However, larvae at Bass Harbor were shorter and grew slower than larvae from reference sites and a number of histopathological and cytogenetic scores were elevated (finfold, cytologic, and craniofacial lesions, yolk, ascites, and anaphase aberration; Marty et al. 1997). McGurk and Brown (1996) found mean egg-larval mortality at Bass Harbor was greater than at reference sites, also demonstrating that these biological effects were consequential. All of these differences are consistent with exposure to oil, and confirm that the model accurately predicts occurrence of negative effects, including those in Bass Harbor, a site within the zone of uncertainty. One or two sites may have experienced minimum concentrations sufficient to be directly lethal (147-285 $ng \bullet g^{-1}$), Cabin Bay and portions of Outside Bay (Fig. 8). Observation of eggs in contact with oil at these two sites (Pearson et al. 1995a) together with the observation by Hay et al. (1995) that eggs in direct contact with oil invariably die early in development suggests our interpretation that oil concentrations at these two sites could be directly lethal is accurate. We conclude that oil-induced abnormalities, which likely presaged delayed mortality, were plausible at 83% of the oiled sites sampled by Pearson et al. (1995a) and were confirmed in the oiled site least likely to produce significant differences (Marty et al. 1997).

Mussels. Interpretation of TPAH concentrations in mussels, which were correlated with TPAH concentrations in herring eggs, provides a third estimate of toxicity, and demonstrates that damage to herring embryos was plausible in Rocky Bay, a site not sampled by Pearson et al. (1995a; 1999). Minimum concentrations in mussel tissue sufficient to predict adverse reaction in adjacent herring embryos were estimated from the regression between TPAH concentration in herring eggs-on-kelp and mussels (Fig. 10). Ranges of critical exposure concentrations estimated from TPAH in mussel tissue are greater than corresponding ranges estimated directly from herring eggs due to uncertainties in the linear fit. Minimum concentrations sufficient to predict abnormalities ranged from 1.0-4.8 $\mu g \bullet g^{-1}$ dry weight and those sufficient to be directly lethal ranged from 4.2-15.3 $\mu g \bullet g^{-1}$ (Fig. 10). The mean TPAH concentration in Rocky Bay mussels was >3.4 $\mu g \bullet g^{-1}$ but <4.8 $\mu g \bullet g^{-1}$, suggesting negative effects were plausible. Indeed, Rocky Bay larvae were shorter and grew slower than larvae from reference sites and histopathological and exhibited histopathological and cytogenetic damage consistent with oil exposure (Marty et al. 1997). McGurk and Brown (1996) found mean egg-larval mortality at Rocky Bay was greater than at reference sites, demonstrating that these biological effects were consequential. Elevated TPAH concentration in Naked Island mussels was also consistent with observed larval damage.

Laboratory exposure experiments provide useful modeling of real-world effects

That the laboratory results of Carls et al. (1999) predicted response of PWS herring larvae to EVO with considerable precision also demonstrates that these tests accurately model real-world spill conditions and are not misleading as implied by Neff et al. (2000). Hydrocarbons other than PAH may contribute to petroleum toxicity, but the toxicity of PAH is well recognized. At a minimum, TPAH serves as an index of the presence of oil, and was the only measure of oil concentration common to the studies presented in this synthesis. In most cases acute toxicity of a petroleum product is directly correlated to its content of soluble aromatic derivatives (Anderson et al. 1974; Moore and Dwyer 1974; Neff 1979). Neff et al. (2000) reported that PAHs accounted for 3-94% of estimated hazard indices of four oils, and that the contribution increased with weathering: TPAH contribution to toxicity in the most weathered fractions ranged from 57 - 94% (omitting a 99% observation for one oil because of analytical errors). Phenols contributed < 15% to the toxicity in any test by Neff et al. (2000), and did not vary consistently as a function of weathering. Similarly, Barron et al. (1999) also found that the least toxic of three oils had by far the highest phenol concentration. Retention of the higher molecular weight, more refractory and more toxic PAHs in aqueous solution as the oil weathers appears to best explain the increased toxicity of weathered oil (Black et al. 1983; Carls et al. 1999; Heintz et al. 1999; 2000). Although m estimated microbial contribution to toxicity in tests by Carls et al. (1999) is minimal. Concentration of WSF varied among microbial cultures and increased with time (Middaugh et al. 1996; 1998; Shelton et al. 1999); these concentration changes explained > 70% of the variation in toxicity (our analysis). Furthermore, microbial degradation of oil is typically nutrient limited, [e.g., Gibbs and Davis (1976); Atlas (1995)]; as was the case in water-column tests (Middaugh et al. 1996; 1998; Shelton et al. 1999) and in experimentally oiled sandy-gravel columns (Gibbs and Davis 1976). Biodegradation rates may be increased by 5-10 times by nutrient additions (Bragg et al. 1994; Atlas 1995; Shelton et al. 1999). The time required for hydrocarbon-utilizing microbes to begin to degrade substantive quantities of hydrocarbons in beach substrate may require roughly 1-3 weeks (Gibbs and Davis 1976). Degradation rates for a given microbial population can be far less at low temperatures than at high temperatures [e.g., ZoBell (1969); Gibbs and Davis (1976); Atlas (1981)]. Experimental conditions used by Carls et al. (1999) were less favorable for microbial growth than those by Middaugh et al. (1996; 1998) and Shelton et al. (1999): temperatures were colder, (4-7°C versus 20°C), water flow was rapid (5-6 L/minute versus static), and nutrients were not added. Dividing the potential contribution of metabolic byproducts (<30%; estimated from Shelton et al. 1999) by 3 to 5 to account for slower degradation rates without nutrient supplement (Atlas 1995) and by 3.7 to account for colder temperatures (estimated from Gibbs and Davis 1976), we estimate that metabolic byproducts accounted for <2-3% of the toxicity reported by Carls et al. (1999).

Prediction of oil effects in PWS herring eggs-on-kelp using laboratory results was restricted to 1989 because the primary oil reservoir, PWS water, was essentially uncontaminated in spawn areas after 1989 (Fig. 5) except at two islands where intertidal sediment had been heavily oiled. Total PAH concentrations in mussels at Cabin Bay and Rocky Bay, fell in 1989 and remained at baseline in the following years (Figs. 5-6). EVO was not verifiable in 47 of 48 mussel samples collected from oiled spawn sites in 1990 (Short et al. 1996b). Biologically available EVO was plausible in some (7 of 13) mussel samples at Green Island (0.02-4.8 $\mu g \bullet g^{-1}$) and Smith Island (0.07-5.3 $\mu g \bullet g^{-1}$), sites that received spawn in 1990 but not in 1989; however, samples were too weathered and the source was verifiable only once (w = 7.70 at Green Island, Short et al. 1996b). These areas received a relatively

small proportion of the total spawn [roughly 6% at Green Island, and 3% at Smith Island; our estimates from Brady et al. (1991)]. Because mussels are effective water samplers, there is little reason to believe aqueous PAH concentrations were elevated a year or more after the spill at most herring spawn sites, and Neff and Stubblefield (1995) reported little or no TPAH in PWS water in 1990 (Fig. 5). The 1990 herring eggs-on-kelp data are perplexing because there is continued evidence of elevated TPAH concentrations at both oiled and reference sites in PWS (Pearson et al. 1995a), yet concentrations in eggs did not correlate with those in mussels at sites in common geographically and temporally (r^2 = 0.19, P = 0.562, n = 4). The composition of this contamination in PWS and at Sitka Sound reference sites is unknown to us because the data were not published (Pearson et al. 1995a). Available evidence suggests < 10% of the herring eggs were exposed to EVO in 1990. Thus, application of laboratory results (Carls et al. 1999) to PWS herring egg data is not advisable beyond 1989 and the lack of embryo response in 1990 (Hose et al. 1996) suggests contaminant hydrocarbon composition was not the same as in 1989.

Differences in industry and NRDA perspectives explain conflicting conclusions

Some of the differences in industry and NRDA embryolarval studies may be explained by intensity of effort. The parallel studies in 1989 lead by Pearson et al. (1995a) (industry) and McGurk (1992) (NRDA) both initially reported minor impacts. Industry studies stopped at this point, but NRDA studies were extended. Hose et al. (1996) examined larvae hatched in the McGurk study for genetic and morphological damage. Field efforts to directly examine PWS herring adults and larvae were also added (McGurk and Brown 1996; Marty et al. 1997; 1999). These extended studies demonstrated significant damage occurred in embryolarvae from oiled areas compared to those from reference areas. In their update of McGurk (1992), McGurk and Brown (1996) reported that differences in growth and mortality rates between oiled and reference areas supported the hypothesis of injury to herring embryos and larvae. The philosophy behind the science was apparently different - industry initially found minor effects, stopped testing, and concluded the spill caused little damage, but the NRDA group was not convinced that initial equivocal results could be interpreted as no harm, continued testing, and ultimately concluded that there were major spill effects.

The effects of the *Exxon Valdez* oil spill on herring eggs extended beyond the restricted areas where oil was in contact with eggs. Pearson's group concludes that oil must adhere to (or coat) herring eggs to cause effects (Pearson et al. 1985; 1995a; 1996; 1999), but this perspective conflicts with the large body of literature indicating significant oil toxicity in the absence of coating [e.g., Moore and Dwyer (1974), Neff (1979), and Neff and Anderson (1981)]. Hay et al. (1995) provide a link between coating and chemical toxicity effects. They report that direct contact with oiled substrate invariably killed eggs, but because oiled substrate negatively impacted many eggs even though most were not in direct contact with oil, the primary exposure mechanism must have been via contaminated incubation water (Hay et al. 1995). Dissolved hydrocarbons are clearly toxic to fish embryos [e.g. Mironov (1967); Kuhnhold (1974); Kocan et al. 1996a; Carls et al. (1999); Heintz et al. 1999; 2000]. Our conclusion is that herring eggs in PWS were exposed primarily to dissolved oil in water, and that this was the principal route of toxicity after the *Exxon Valdez* spill.

While both industry and NRDA researchers relate egg exposure to TPAH concentrations, the two groups draw upon different literature to interpret EVO toxicity and reach opposite conclusions

concerning its toxic potential in PWS. Industry researchers estimated the toxic potential of dissolved EVO using water-soluble and water-accommodated fraction research (Pearson et al. 1999; Neff et al. 2000). Aromatic compounds present in short-term, unweathered water-soluble or water-accommodated fraction tests are typically dominated by single-ring compounds [(94% in WSF) Rice et al. 1979; (27-99% in WSF) Neff and Anderson 1981; (95% in WSF) Rice et al. 1987; (38-97% in WAF of unweathered products) Neff et al. 2000]. Neff et al. (2000) conclude that "under mild conditions with little physical dispersion of petroleum into the water column, volatile monocyclic aromatic hydrocarbons dissolving from the surface slick would be the main contributors to any toxicity observed in water column organisms." In their review, Pearson et al. (1999) argue that "the main hydrocarbons present in the water-soluble fraction include the low relative molecular mass PAHs that are responsible for most of the toxicity of crude oil (Rice et al. 1977; Neff and Stubblefield 1995)" and conclude that the water-soluble fraction used in toxicity tests can be compared with the composition of the hydrocarbon fraction present in the PWS water column following the spill. Problems with these assertions are that 1) oil and water in PWS did not mix under calm conditions, 2) monoaromatic hydrocarbons all evaporated rapidly from PWS, and 3) toxic high molecular weight PAHs were present in PWS in addition to low molecular weight PAHs. Three days after the *Exxon Valdez* spill a 3-day storm dispersed substantial quantities of oil into the water and accelerated the evaporation process (Wolfe et al. 1994). By the time herring spawned, composition of oil in PWS did not resemble WSFs, because monoaromatics had evaporated (Wolfe et al. 1994; Neff and Stubblefield 1995) and high molecular weight PAHs were present (Neff and Stubblefield 1995; Short and Harris 1996a; Short and Heintz 1997). The technique used by Carls et al. (1999), passage of water through oiled gravel, produced water with a PAH composition similar to that observed in PWS (naphthalenes through chrysenes), including the weathering patterns characterized by Short and Heintz (1997). Because high molecular weight PAHs are more toxic than low molecular weight PAHs (Rice et al. 1977; Neff 1979; Black et al. 1983), Carls et al. (1999) and Heintz et al. (1999) observed that PAH from EVO was about 1000 times more toxic than previously studied WSFs. Reliance of industry on traditional acute toxicity tests, which focus on narcosis induced by the most water soluble components of oil, does not explain the suite of toxic effects in embryonic life stages caused by exposure to PAH, where the toxicity mechanism is oxidative cellular damage that can be passed on to daughter cells during development (Livingstone et al. 1990; Akcha et al. 2000). Thus, while industry concludes that the roughly 1 $\mu g \bullet L^{-1}$ aqueous concentrations of EVO in PWS were not toxic, NRDA researchers conclude that 1 $\mu g \bullet L^{-1}$ aqueous concentrations were sufficient to have caused significant damage.

Reassessment of the fraction of herring eggs exposed to EVO in PWS

We reassessed the fraction of PWS herring eggs exposed to EVO in 1989 by determining toxic body burdens of EVO at various oiled sites, and related them to those of industry (4-10% oiled, Pearson et al. 1995a; 1999) and NRDA (40-52%, Brown et al. 1996a,b) (Table 2). [Zaikof Bay was included in the high estimate by Brown et al. (1996a,b), but excluded from the low estimate because of insufficient chemical analysis.] Earlier we noted that the industry assessment

Table 2. Reassessment of percentages of Pacific herring eggs exposed to *Exxon Valdez* crude oil in Prince William Sound after the spill, and comparison to previous estimates. Published Natural Resource Damage Assessment (NRDA) estimations were based both on spawn distance and egg mass; the industry estimate was based only on spawn distance (NE = not estimated). The reassessment of egg exposure to oil by the current authors is more conservative than the previous NRDA estimate, but much larger than the industry estimate.

	Spawn		Percent	
Oiling	distance (km)	mass (metric tons)	based on distance	based on mass
Industry estimate[a]				
moderate-heavy	0.9	NE	1	NE
very light-light	5.2	NE	3	NE
none	152.2	NE	96	NE
total	158.3			
NRDA estimate[b,c]				
oiled[d]	54.7	20,886	35	40
Zaikof Bay	13.8	5,998	9	12
Not oiled	89.8	25,351	57	48
total	158.3	52,235		
Re-assessment				
significant[e]	30.6-38.1	13,230-15,911	19-24	25-30
likely significant[f]	2.7	984	2	2
unknown[g]	19.0	6,823	12	13
likely not significant[h]	8.8-16.2	3,167-5,847	6-10	6-11
None	89.8	25,351	57	48
total	158.3	52,235		

[a]Pearson et al. 1995a; [b]Brown et al. 1996a; [c]Brady et al. 1991.

[d]Brown et al. (1996b) stated range of eggs exposed to oil was 41-52%, based on spawn mass

[e]Includes the Naked Island area plus Rocky Bay and Graveyard Point.

[f]Oiling at Montague Point was likely biologically significant, and most of the spawn overlapped visibly oiled shoreline (Neff et al. 1995), but TPAH concentrations were not documented by chemical analysis.

[g]Insufficient data were collected in Zaikof Bay to either support or refute exposure of Pacific herring eggs in 1989.

[h]There were indications of slight oiling in Stockdale Harbor, but the weight of evidence suggests concentrations were probably too low to damage herring eggs.

was based on spawn distance, while the NRDA estimate was based on spawn biomass; the difference in estimation methods is <10%. We concur that biomass is the more appropriate measure of egg exposure and report only this measurement in the following text. Evidence from all portions of the Naked Island area suggested biologically significant oiling, i.e., TPAH concentrations in mussels and herring eggs were high enough to cause developmental abnormalities in Pacific herring embryos. Concentrations were similarly significant in the outer portion of Rocky Bay, although concentrations in the inner portion of the bay may have been below biological significance. [Total PAH concentrations in mussels in the inner portion of the bay (generally west of, and including site O18 in Fig. 3) were less than the 2.5 $\mu g \bullet g^{-1}$ level estimated to signal biological significance, but TPAH concentrations in water samples collected in the eastern portion of the inner bay were consistently above 0.4 $\mu g \bullet L^{-1}$, a level that caused adverse biological response in herring embryolarvae in laboratory tests (Carls et al. 1999).] We conclude that 25-30% of the spawn biomass was oiled at biologically significant levels and that oiling was likely significant in an additional 2% of the spawn biomass at Montague Point. Oil was visually identified at Montague Point (Gundlach et al. 1990; Brady et al. 1991; Neff et al. 1995), but not documented by chemical analysis. Graveyard Point may have acted as a natural barrier to shield Stockdale Harbor from most of the oil slick, where evidence suggests that oil concentrations were too low to be detrimental to herring egg development (6% of spawn mass). There were insufficient data to determine if herring egg exposure was significant in Zaikof Bay (13% of spawn mass). Our estimate of the percentage of Pacific herring eggs exposed to biologically meaningful amounts of EVO in 1989 (25-32% of spawn mass) falls between the original estimates.

Identical oil effects were observed in older herring larvae from oiled PWS sites

Pacific herring larvae from oiled areas of PWS were also adversely affected by EVO in 1989, and the causal relationship between oil exposure and detrimental effects was confirmed through laboratory study. Major oil-associated effects in larvae captured from oiled sites in spring 1989 included small size, ascites, pericardial edema, delayed development, and genetic damage (Marty et al. 1997). Microscopic lesions were consistent with decreased growth and increased mortality of herring larvae collected near oiled beaches (McGurk and Brown 1996), and were similar to those observed in laboratory studies of herring larvae exposed to ANSCO as eggs [e.g., Kocan et al. (1996a), Marty et al. (1997), Carls et al. (1999)]. Because of the low aqueous concentrations of hydrocarbons in PWS and variable environmental conditions, the link between ascites and oil exposure in field-sampled larvae was controversial (Pearson et al. 1999). Recent laboratory studies, however, provide evidence that aqueous PAH concentrations of 0.4 - 1 $\mu g \bullet L^{-1}$ cause ascites and edema in herring exposed as developing eggs (Carls et al. 1999) -concentrations well within documented TPAH levels in PWS in April and May 1989 (Neff and Stubblefield 1995; Short and Harris 1996a). Growth of older larvae from offshore areas also decreased throughout PWS in 1989, but a direct link to oil exposure was not possible because these larvae likely originated from a mixture of oiled and reference areas (Norcross et al. 1996). In contrast, the frequency of genetic defects was low and jaw size was within normal limits in PWS larvae six years after the spill (Norcross et al. 1996). Pacific herring larvae that hatched in PWS were not studied by industry. We conclude that NRDA information on embryolarval effects, coupled

with identical responses observed in the laboratory and in free-swimming herring larvae, provides consistent documentation of oil toxicity in early life stages of PWS herring in 1989.

Possible oil effects in juvenile Pacific herring not were evaluated

No studies were performed on the effects of the oil spill on juvenile Pacific herring. This decision was based in part on the lack of baseline data on juvenile abundance, distribution, and movements. The only observations concerning exposure of juvenile herring to EVO are anecdotal. Schools of juvenile herring were observed inhabiting the same contaminated intertidal habitat as juvenile pink salmon (Carls, unpublished data). EVO accumulated in the tissues of juvenile pink salmon in these intertidal areas (Carls et al. 1996), suggesting the possibility that juvenile herring may have been similarly exposed. Studies since 1994 revealed that juveniles are distributed in small schools within bays throughout PWS year-round (Evelyn Brown, University of Alaska, Fairbanks; personal communication). Therefore, the possibility that some juveniles were affected by oil spill cannot be excluded, but we have no information on which to evaluate EVO exposure or effects. Pearson et al. (1999) concluded effects on juveniles were minor, based on extrapolation from adult data.

Adult PWS herring accumulated hydrocarbons and exhibited histopathological changes

In 1989, adult Pacific herring in oiled areas of PWS exhibited significant oil-associated lesions and evidence of hydrocarbon exposure, but fish from reference sites did not (Moles et al. 1993; Marty et al. 1999). When the oil spill occurred, herring were beginning to congregate in shallow bays for their annual mass spawning in April. Fish from oiled sites had hepatic necrosis and elevated PAH concentrations (primarily naphthalenes) in their tissues (Marty et al. 1999). Naphthalenes were also preferentially accumulated in muscle tissue in laboratory exposures of adult herring to PAH in water (Carls et al. 2000) - evidence of metabolism (Thomas et al. 1997) and differential uptake. Herring from oil-exposed areas had fewer nematode parasites in their body cavity than did fish from reference sites (apparently because stressed nematodes migrated into muscle tissue) and the potential link to acute oil exposure was confirmed through laboratory study (Moles et al. 1993). In a recent study, wild-caught Pacific herring exposed to crude oil had higher prevelences of hepatic necrosis, increased mortality, and viral hemorrhagic septicemia virus (VHSV) was isolated from exposed fish but not control fish (Carls et al. 1998). Also, aqueous PAH concentrations in the range of those measured in PWS in spring 1989, ≥ 0.6 $\mu g \bullet L^{-1}$, cause immunotoxicity in rainbow trout (Karrow et al. 1999). Although significant mortality of adult herring in 1989 as a result of the spill was never documented in PWS, lesions in fish sampled from oiled sites in 1989 were consistent with lesions in fish from which VHSV was isolated in more recent studies (Marty et al. 1998; Marty et al. 1999), and a small fraction of the population probably died as a result of this viral outbreak. In 1990 and 1991, fish sampled from oiled sites had neither oil-related lesions nor significant PAH concentrations in their tissue (Marty et al. 1999).

Long-term Consequences were Indirect or Not Associated with the Spill

Adult biomass in PWS was at historically high levels in 1989 and continued at high levels through 1992 (Fig. 11a). During this period, the spawning population was composed of fish that were adults during the 1989 oil spill because Pacific herring in PWS first enter adult schools in the fall of their second year of life and first spawn when 3 years old. A preliminary study in 1992 provided evidence that among fish that were yearlings at the time of the spill (1988 year class), reproductive success was less in fish from previously oiled sites than in fish from reference sites (Kocan et al. 1996b). However, potential spill effect studies were not funded for 1993 because a record biomass was predicted for that year and recovery of PWS herring was presumed.

Collapse of the PWS herring population in 1993

Contrary to predictions made before the spring of 1993, the adult Pacific herring population of PWS apparently declined more than 75% from an estimated 11×10^7 kg in 1992 to 1.7×10^7 kg in 1994, and most of the decline occurred during the winter of 1992-1993 (Marty et al. 1998; Fig. 11a). The valuable commercial roe fishery was closed in 1993 and did not reopen until 1997; it was closed again in 1999 and has remained closed through 2001. Large numbers of dead fish were not observed in 1993, thus determination of the cause of mortality is difficult. Although natural variation in clupeid population size can be large and unpredictable (Blaxter and Hunter 1982; Cole and McGlade 1998), recruitment failure does not explain the collapse (fish from all year classes were involved), and the magnitude of the 1992-1993 collapse suggests external factors played a critical role.

Several hypotheses have been advanced to explain the population collapse, including both spill-related and natural reasons and combinations of the two. Fishery management decisions may have also played a role. Spill-related hypotheses include direct and residual oil toxicity and delayed response to previous oil exposure: 1) exposure of larvae and juveniles to oil in 1989 caused permanent immunosuppression, and these year classes increased the susceptibility of the entire adult population to disease when they recruited and spawned for the first time; 2) exposure of larvae or juveniles caused short-term immunosuppression sufficient to initiate an increase in disease transmission, followed by a gradual spread of the pathogen and ultimately an epizootic, 3) residual oil caused immunosuppression, expression of VHSV, and mortality of adult herring; 4) exposure to oil in 1989 reduced reproductive success in subsequent years; and 5) oil affected food supplies. Hypothetical natural causes include disease, poor nutrition, large-scale environmental change, and increased predation. High population biomass may have been a contributing variable for disease and poor nutrition.

Immunosuppression as a result of exposure to oil does not appear to explain the 1993 population collapse, a conclusion also reached by Pearson et al. (1999). The hypothesis is that exposure of larvae and juveniles to oil in 1989 (i.e., the 1989 and 1988 year classes) caused permanent immunosuppression; when faced with the added stress of their first spawn in 1993, they were unable to avoid or recover from disease, and the epizootic occurred (Brown et al. 1994). Had oil caused low-grade but irreversible immunosuppression, survival of affected individuals for 2-3 years to recruitment would be unlikely. Instead, exposure to disease during the intervening years should have resulted in mortality before recruitment. Furthermore, permanent immunosuppression is unlikely; for example, immune function in mussels exposed to

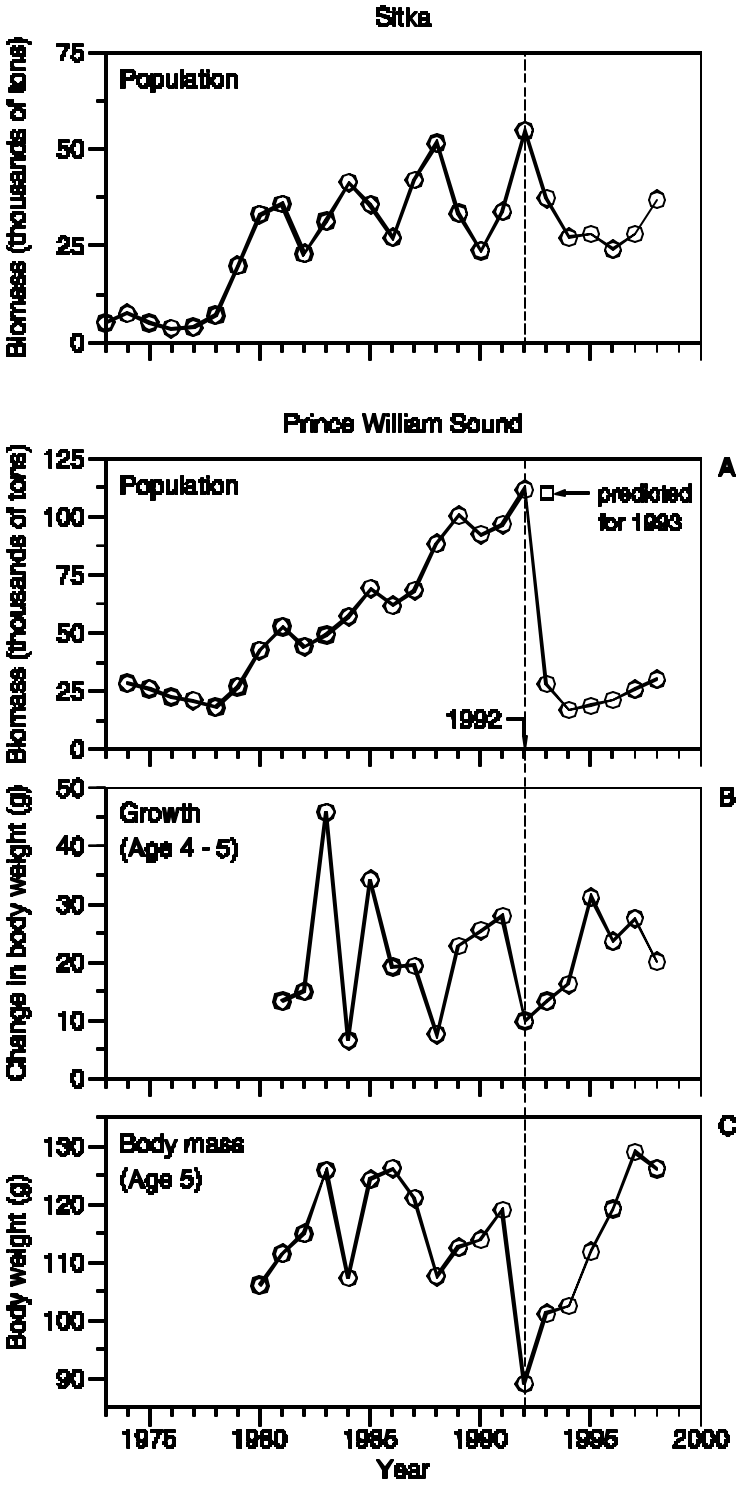


Fig. 11. Biomass estimates of mature Pacific herring in Prince William Sound (PWS), Alaska (Marty et al. 1998) (A) compared to estimates for Sitka, Alaska (unpublished data, Dave Gordon, Alaska Department of Fish and Game, Sitka, AK) (D). Unexploited spawning biomass was estimated using an age-structured assessment model. Included are the annual growth of 5-year-old PWS herring (i.e. change in body weight from age 4 to age 5) (B) and the weight of 5-year-old fish each year, as measured before spawning in the spring (C). The vertical reference line emphasizes conditions in 1992, the year prior to the population collapse.

crude oil recovered as PAH levels declined (Dyrynda et al. 2000). The possibility that short-term oil-induced immunosuppression of larvae or juveniles ultimately caused the 1993 collapse is also unlikely. A comprehensive study (1994-1999) indicates that Pacific herring infection with VHSV is an annual event in PWS and that the severity of the infection depends on the general condition of the fish (i.e., new recruits in poor condition are more likely to become infected with VHSV; G.D. Marty, unpublished observations). For example, 14% of the herring sampled in spring 1997 and in 1998 (total n = 510) were positive for VHSV, but none sampled in fall 1997 and 1998 (total n = 180) were positive for VHSV (GD Marty, unpublished observations). Juvenile Pacific herring that survived VHSV infection were strongly protected against reinfection (Kocan et al. 1997). After 1990, continued exposure of herring to residual oil concentrations sufficient to cause toxicity or immunosuppression is implausible. The proportionately large recruitement of the 1988 year class (John Wilcock, Alaska Department of Fish and Game, Cordova, Alaska; personal communication) also argues against a significant immunosuppression effect. Additionally, evaluation of disease by age class in 1994 (Marty et al. 1998; Elston et al. 1997) suggests permanent immunosuppression did not occur because parasite and disease prevalence was lower among the 1988 year class than among any other age groups. However, only survivors of the collapse were observed by these studies, and most affected fish could have died before evaluation.

Reproductive success (fertilization success, hatching success, larval viability, swimming ability, and lack of spinal abnormalities) of the 1988 and 1989 year classes in 1995 was no different than in other year classes (Johnson et al. 1997), arguing against a long-term, delayed oil effect. If oil had affected reproduction, then the effect should have been greatest in the 1988 and 1989 year classes, and less in year classes born before or after significant concentrations of EVO were present in the environment. As with the previously cited disease studies, however, only the survivors of the collapse could be studied. Senescence of the herring population as a result of reproductive failure or destruction of specific year classes, does not explain the sudden drop in the population because fish from all year classes were involved in the collapse (i.e., there were no unusual shifts in age structure between 1992 and 1994). Recruitment failure would have caused the population to decline more slowly than was observed.

Reduction of food supply in 1992-1993 as a result of oil toxicity is highly unlikely. Spring plankton abundance reached a historical high in 1989 (Pearson et al. 1999), the time of maximum potential oil exposure. No difference in plankton abundance between oiled and reference areas was detected in 1989 (Wertheimer and Celewycz 1996). Meiofauna populations were apparently affected by oil for only short periods of time (Fleeger et al. 1996), and there were indications that epibenthic harpacticoid copepod populations maintained or increased in abundance in oiled areas during the year after the spill (Wertheimer et al. 1996). Given the negligible PAH concentrations in the water column after 1989 (Neff and Stubblefield 1995), oil-related changes in food supply at the time of the herring population collapse are unlikely. No differences in plankton abundance between oiled and reference areas were observed 1 year after the spill (Wertheimer and Celewycz 1996), and a 4-year delayed reduction in plankton abundance as a result of earlier exposure is implausible.

Prey abundance has fluctuated as a result of natural factors, and Pearson et al. (1999) could not reject the hypothesis that poor nutrition was limiting. Observed spring zooplankton abundance in PWS was lowest in 1991 (observations began in 1981) and somewhat higher in 1992-1993 (Pearson et al. 1999). Mean zooplankton abundance in the Gulf of Alaska was also lower in 1991 than in the

preceding decade; 1992 was a relatively strong year, and abundance in 1993 was about the same as in 1991 (Bailey et al. 1995). Although Bailey et al. (1995) found major changes in the Gulf of Alaska ecosystem that may be due to the El Niño-Southern Oscillation, these effects on specific species are unpredictable.

Causes that do not involve oil may best explain the 1993 PWS herring population collapse. Overfishing does not appear to explain the collapse. Estimated fishing rates were 20-25% of the spawning biomass when the fishery was open (Fritz Funk, Alaska Department of Fish and Game, Juneau, Alaska; personal communication), conforming with best estimates that exploitation at this rate will not cause stock collapse when the environmental changes or poor recruitment occurs (Doubleday 1985). Increased predation on herring by other species is possible, but has not been adequately documented (Pearson et al. 1999). Interspecific competition for prey may also have had negative effects but data are insufficient for adequate interpretation. For example, the large numbers of pink salmon fry released into PWS by hatcheries may result in lower zooplankton availability for herring. Large scale environmental changes could have played a role in the population decline. For example, declines in average seawater salinity in PWS from 1989 through approximately 1993 have been observed (Evelyn Brown, University of Alaska, Fairbanks, personal communication). El Niño-Southern oscillation events may adversely affect the abundance of small pelagic fish such as herring, but herring landings were not anomalous in 1992-1993 except in PWS (Bailey et al. 1995). Thus, the most likely causes for the population collapse may involve disease, high population density, and poor nutrition.

Disease was associated with the population decline

Pathogenic VHSV was isolated from sick adult herring in 1993, and this was the first published report of the virus in this species (Meyers et al. 1994). Subsequent efforts revealed that VHSV infection is common in Pacific herring populations throughout northwest coast of North America (Meyers and Winton 1995), and although it can be lethal for herring (Kocan et al. 1997), VHSV infection was associated with a dramatic population decline only in PWS.

Disease epizootics in wild fish populations involve complex interactions of the pathogen, host (Pacific herring), and environmental stressors (Hedrick 1998), and most likely a combination of all three tipped the balance toward a population collapse in 1993. In addition to the presence of the virus, the Pacific herring biomass in 1992 was the highest since reliable estimates were first made in the early 1970s (Fig. 11a), primarily due to recruitment of a very large 1988 year class augmenting a large 1984 year class. The high biomass was associated with low annual growth (Fig. 11b) and low weight-at-age (Fig. 11c), providing evidence that food availability limited fish growth in 1991 and 1992, and that the population was either near or had exceeded carrying capacity. Winter starvation, a known annual stress factor (Hurst and Conover 1998; Schultz et al. 1998), may have contributed substantially to the population decline. Prey availability is generally low in the winter and herring survive on stored energy reserves (Blaxter and Holliday 1963; Paul et al. 1998). The potential zooplankton food supply for herring in PWS was below average from 1990-1993, possibly as a result of large-scale oceanographic changes (Pearson et al. 1996). Although young herring are more vulnerable to overwinter stress than older fish (Paul et al. 1998), energy reserves may have been sufficiently limiting over the winter of 1992-1993 that the entire population was stressed, thereby increasing vulnerability to disease. Based on results of the fall food/bait fishery in 1992, the population had not collapsed prior to winter (John

Wilcock, Alaska Department of Fish and Game, personal communication), thus providing constraints on the timing of the collapse.

Population collapse was most likely caused by high population density and disease

The most likely primary cause of the 1992-1993 population collapse was that the Pacific herring population exceeded carrying capacity in PWS, leading to a disease event during the winter of 1992. The oil spill forced the closure of the herring fishery in 1989, possibly contributing to the increased population size. Fishery management typically tries to maintain a population near, but below carrying capacity, thus allowing for maximal harvest. However, exactly what is carrying capacity is ill-defined, and depends on environmental fluctuations, including exposure to pathogens. Some of the adults that were not fished in 1989 still contributed to the record biomass in 1991 and 1992. Although the incremental contribution of these survivors to the adult biomass in 1992 was about 6%, this might have been significant in initiating an epizootic in a population already at carrying capacity. Pearson et al. (1999) concluded that closure of the commercial fishery was not a major cause of the population collapse but could not completely dismiss it as a contributing factor. Regardless of whether the high herring population in the early 1990s resulted from fishery closures after the spill, or whether it was simply the result of decadal trends, biomass estimates suggest the herring population was near carrying capacity. Basic epidemiologic principles describe the risk of a disease epizootic increasing as population density increases (Wobeser 1994). In other herring populations, severe population decline has been associated with disease in Atlantic herring *Clupea harengus* (Fish 1934; Sindermann 1958; Rahimian and Thulin 1996; Mellergaard and Spanggaard 1997) and at least once previously in Pacific herring (Tester 1942). In all cases, the population biomass was estimated to be at or near historical highs when the epizootic occurred. Therefore, the 1993 population collapse of Pacific herring in PWS is most consistent with historical epizootics associated with population biomass exceeding carrying capacity, and delayed reaction to the oil toxicity is unlikely. In comparison, Pearson et al. (1999) suggest that increased biomass and decreased food supply caused the population decline, but could not eliminate other contributing natural factors including disease.

Evidence that the population collapse was related to oil cannot be entirely discounted

The strongest evidence that the oil spill caused the PWS herring population to collapse is location and timing. The population collapse in the winter of 1992-1993 was restricted only to PWS, and generally did not occur in other parts of Alaska (Funk 1995b). The collapse coincided with a collapse in the 1992 and 1993 pink salmon fishery in PWS (Brannon and Maki 1996), which also did not occur elsewhere in Alaska (Geiger and Savikko 1993; Geiger and Simpson 1994). This is weak evidence at best. While the collapse in Pacific herring and pink salmon populations suggests that PWS ecosystem was perturbed between 1992 and 1993, direct linkage of the herring population collapse to the oil spill is unlikely. [The case for linkage is strong for wild pink salmon; retention of oil in intertidal habitat caused long-term damage (Bue et al. 1998; Heintz et al. 2000).] However, indirect effects of oil on the ecosystem cannot entirely be ruled out. The simultaneous collapses in Pacific herring and pink salmon populations may have been due to 1) delayed or indirect oil effects, 2) ecological or oceanographic changes unrelated to oil that affected both populations in a similar manner, or 3) different causes unrelated to oil for each population. Declines in both populations suggests, but does not prove,

that the underlying cause was the same (although mechanisms may have been different). Hypotheses 1 and 2 both suggest a common cause, but available data are insufficient to adequately support or refute either one. Although direct linkage of the herring population collapse to the oil spill seems unlikely, indirect effects of oil on the ecosystem cannot entirely be ruled out.

Both broad- and regional-scale events influence herring populations, complicating interpretation of size fluctuations. For example, there is circumstantial evidence that broad-scale environmental change similarly affected PWS and Sitka Sound (SE Alaska) herring populations from the mid 1970s through the 1990s. Both areas adjoin the Gulf of Alaska, but are located about 750 km apart, yet herring population mass and year-class fluctuations were similar. Although the herring population mass increased considerably more in PWS than in Sitka Sound through the 1980s and early 1990s, total mass in both areas tended to exhibit a sawtooth pattern, and there was a minor synchronous low in PWS for each low in Sitka Sound (1982, 1986, and 1990) (Fig. 11). Populations peaked in 1992 in both areas and declined rapidly in the following two years. The collapse in PWS was major, and mass fell abruptly to pre-1980 levels, but the loss of biomass in Sitka was unremarkable. The synchrony of major year-class strength (1976, 1980, 1981, 1984, 1988, 1992, and 1994) between the two areas, is strong evidence that broad scale environmental factors affect herring recruitment. However, the swift collapse of the PWS population in 1992-1993 demonstrates that regional influences also have dramatic effects on local populations. The PWS herring population collapse was apparently caused by a major regional event, but was also influenced by broad-scale controlling factors. However it is not possible to determine if the regional event in PWS was related to oil, or some other unknown factor.

Because the PWS ecosystem is highly dynamic, identification of oil-related problems has become increasingly difficult with time. Declines in salmon and herring populations, especially rapid events such as the 1993 collapse of the herring population and a similar drop in salmon abundance in 1992 and 1993, raised the specter of lingering spill effects in the minds of many. The collapse stimulated further study, but by 1994 and 1995 finding links to the spill would have been difficult even if oil were the primary cause. The impacts of the oil spill may have had a dominant influence on the 1989 herring year class, but by the time survivors recruited in 1992-1993, high population size and disease appear to have been more important factors.

Summary and Conclusions

Reassessment of response of Pacific herring to oil in PWS suggests that observed impacts could not have been predicted from routine toxicological monitoring methods in use at the time of the spill. This review emphasizes the need to thoroughly study pollutant exposure, adequately characterize pollutant composition, estimate toxic effects using appropriate laboratory models, critically examine sensitive life stages over appropriate time intervals, and place the findings within broad ecological perspectives. That the PWS oil spill occurred in a nearly pristine environment (Karinen et al. 1993) yields much information that can be advantageously used in situations complicated by previous habitat degradation. Adverse biological responses were observed orders of magnitude below predicted response in part because the spill chemistry had not been accurately predicted, in part because sensitive early life stages were exposed during critical development periods, and because sufficient funding and

controversy ensured intensive study. Because oil typically persists for at least several days after a spill, the research paradigm should shift away from an emphasis on mechanisms of acute toxicity (such as narcosis) to long-term toxicity (e.g., oxidative cellular damage). As a result of this reassessment, we recommend that safety standards for dissolved polynuclear aromatic hydrocarbons should be revised to reflect a new toxicity threshold of < 1 $\mu g \bullet L^{-1}$ (part per billion) TPAH to adequately protect aquatic organisms and habitat. Assessment of risk should also consider population dynamics (e.g., a population at carrying capacity may be at greater risk than one below capacity), season (e.g., spill effects are more deleterious when they impact critical early life stages), location of the spill (such as spawning grounds), local ecology, hydrographic conditions, and large-scale ecological processes. Results of the many *Exxon Valdez* studies have broad applicability to other situations, such as the combined industrial and non-point source runoff that results in elevated aquatic PAH loads near urban areas (Rice et al. 2001).

We do not recommend that routine monitoring hydrocarbons in other species (such as mussels) be substituted for direct measurements in the species of interest (e.g. herring eggs). For this particular spill we made use of the data available, and the close correspondence between TPAH concentrations in herring eggs and mussels allowed us to verify the assertions of NRDA researchers that mussels were adequate proxies for direct measurement in herring eggs. However, we have used the mussel data with caution and based our conclusions regarding adverse reaction of PWS herring embryos to oil principally on observed TPAH concentrations in those eggs, responses to similarly low TPAH concentrations in experimentally exposed herring eggs, and directly observed effects in PWS larvae. That TPAH concentrations in herring eggs and mussels were correlated suggests that correlations may be found in other spills, but the nature of such correlations may depend on many factors, such as mixing energy and duration, temperature, salinity, oil viscosity, and organic content of receiving water (Rice et al. 1977; Gustafson & Dickhut 1997), and thus may not be consistent among oil spills. Mussels, which are filter feeders, preferentially accumulate particulate or colloidal oil (Short & Harris 1996b; Axelman et al. 1999), but the majority of exposed herring eggs in PWS likely responded to dissolved oil. Particulate and dissolved hydrocarbons concentrations can be related, i.e., the large surface area presented by small particles will accelerate dissolution (Short and Heintz 1997). However, because petroleum hydrocarbons are sparingly soluble, contact time is also very important, and when contact time is short the correlation between particulate and dissolved compounds may be substantially different than when longer contact times are involved (Axelman et al. 1999). In general, routinely monitored 'indicator' species (e.g. mussels) can identify likely environmental problem areas, but should not simply be substituted for direct measurements in the species of interest.

Differing perspectives between industry and NRDA researchers led to disparate conclusions concerning the consequences of the *Exxon Valdez* spill to PWS herring in 1989. Because initial results were equivocal and environmental oil concentrations (roughly 1 $\mu g \bullet L^{-1}$ aqueous TPAH) were below previously published toxicity thresholds, industry researchers stopped testing and concluded that the spill caused little damage. In contrast, NRDA researchers were unconvinced that initial results could be interpreted as harmless and continued their study, experimentally determining that 1 $\mu g \bullet L^{-1}$ aqueous TPAH concentrations can cause embryo abnormalities and mortality and ultimately concluded that short-term spill effects were major.

This reassessment shows that the short-term consequences of the spill were detrimental to herring in PWS, but that possible long-term consequences of the spill are difficult to discern. Results from these analyses have refined the nominal classification of visually-oiled spawn areas using site-specific chemical evidence. Because TPAH concentrations in mussels were correlated with TPAH in herring eggs (justifying their use as a surrogate index of exposure by NRDA researchers), we inferred that herring eggs from almost all spawning sites within the oil trajectory in 1989 were exposed to EVO in the water column. The incubation of herring eggs roughly coincided with peak aqueous hydrocarbon concentrations in PWS, and the bioaccumulated oil was detrimental to 25-32% of the spawned biomass. Herring larvae collected from oiled PWS areas throughout spring 1989 were malformed, small in size, and had higher mortality rates than in reference areas, consistent with field measurements of EVO above laboratory toxicity thresholds. Embryolarval toxicity appeared to be limited to 1989, and the adult herring biomass continued at high levels through 1992. The 1993 population collapse was likely caused by a combination of factors including high population size, disease, and suboptimal nutrition, but indirect links to the spill cannot be ruled out. The population collapse underscores the importance of formulating resource management decisions that integrate knowledge of the historical fishery. When exposed to additional stress, such as oil, populations already stressed by other factors may be at greater risk than unstressed populations.

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Appendix 1
Estimated toxic contribution of microbial metabolites in oiled-rock tests

Although microorganisms throughout the marine environment have evolved the ability to metabolize hydrocarbons (Atlas 1981; Floodgate 1984), and the resultant metabolic byproducts can be toxic (Middaugh et al. 1998; Shelton et al. 1999), the estimated microbial contribution to toxicity in tests where seawater was contaminated by passage through oiled rock is minimal. The purpose of this appendix is to demonstrate how this conclusion was reached. In particular, we estimate the contribution of microbial metabolites to toxicity in tests by Shelton et al. (1999), and contrast conditions that favor microbial growth (warm temperatures, static water conditions, and nutrient supplements) with standard conditions in oiled-rock toxicity tests conducted by Natural Resource Damage Assessment researchers.

Contamination of seawater by passage through oiled rock, a novel toxicity testing method developed after the *Exxon Valdez* oil spill, successfully models the composition of polynuclear aromatic hydrocarbons (PAH) observed in the Prince William Sound (PWS) after the spill (Short and Heintz 1997). Standard procedure for many toxicity studies completed by researchers at the Auke Bay Laboratory has been to pass seawater through oiled rock columns [e.g., Marty et al. (1997), Short and Heintz (1997), Carls et al. (1998; 1999; 2000), and Heintz et al. (1999; 2000)]. This procedure was designed to emulate PAH composition and concentration observed in PWS after the *Exxon Valdez* oil spill [e.g., Neff and Stubblefield (1995), Short and Babcock (1996), and Short and Harris (1996ab)].

A criticism of oiled-rock toxicity tests is that the effluent may contain toxic metabolites produced by microbial growth, and that these polar metabolites either contribute to toxicity or are the major cause of toxicity (Neff et al. 2000).

Microorganisms capable of metabolizing hydrocarbons as sources of energy and carbon are widely distributed in nature, including seawater and beach sediments [e.g., Gibbs and Davis (1976), Atlas (1981), and Floodgate (1984)]. A portion of these ubiquitous microbes are capable of degrading numerous petroleum hydrocarbons [e.g., Floodgate (1984) and Atlas (1995)]. Thus, in addition to abiotic factors, the fate of spilled oil is influenced by microbial action of which bacteria are by far the most important part (Floodgate 1984). In some cases, bacteria in the water column degrade oil hydrocarbons more effectively than bacteria in sediment (Walker et al. 1975).

Visual evidence suggests microbial growth was slight in oiled-rock columns. Macroscopic bacterial accumulations were not encountered when passing water through oiled rock, in sharp contrast to earlier work with water-soluble fractions (WSF) [e.g. Rice et al. (1987)], which required routine removal of *Pseudomonas* accumulations from apparatus. Our experience with *Pseudomonas* in WSF tests suggested that the presence of the bacteria reduced toxicity, if for no other reason than it occluded the delivery system. There was no evidence that *Pseudomonas* growth enhanced toxicity. Microbial growth on oiled rock was not evident in one month tests (e.g., Carls et al. 1999), but there was some evidence of microbial or algal growth after several months (unpublished data, Ron Heintz, Auke Bay Laboratory).

Demonstrable microbial growth does occur in laboratory tests when conditions for such growth are favorable, and the resultant metabolic byproducts can be toxic (Middaugh et al. 1996; 1998; Shelton et al. 1999). Alkanes were clearly metabolized in tests by Middaugh et al. (1996) and Shelton

et al. (1999), and aromatics were also metabolized in one culture (Shelton et al. 1999). As a result of microbial activity, the concentration of the water-soluble fraction increased, and smaller compounds were apparently present in the UCM. (Microbial action may have increased WSF concentrations as a result of surfactant secretion, thus increasing the surface area of the weathered oil and allowing more compounds to dissolve into the water.) "Toxicity occurred only on exposure to neutral material accumulated by active, oil-degrading cultures and not with material washed from weathered crude oil" (Shelton et al. 1999). The authors of these papers largely ignore the fact that changes in WSF composition as a result of microbial activity were consistently confounded with changes in WSF concentration. For example, in their abstract Shelton et al. (1999) state that "these results imply that unique compounds were accumulated during degradation that may have been responsible for increased toxicity."

Changes in WSF concentration alone explain the majority of the toxicity reported by Shelton et al. (1999) (our analysis, Fig. A1.1). Sufficient data were presented by Shelton et al. (1999) to examine the effect of variable WSF concentration on toxicity. Survival of grass shrimp was estimated from their figure 4, and WSF concentration was estimated from their table 1 (mg recovered water-solubles divided by 16.5 L to yield reconstituted concentration; authors state that WSF was reconstituted for toxicity tests in seawater to the same concentrations found in the original incubations). Not all groups tested by Shelton et al. (1999) could be tested because the data were not presented (SBMIX and GOMEX NP control). Survival was correlated with WSF concentration ($r^2 = 0.72$, $P = 0.007$). Survival adjusted for control response was similarly correlated with WSF concentration ($r^2 = 0.74$, $P = 0.006$). Thus, concentration changes explain most of the variation in survival, and the toxicity of metabolic byproducts must account for <30% of the variation in response.

The type of hydrocarbons degraded, alkane or aromatic, did not appear to influence the resultant toxicity in tests by Shelton et al. (1999). Toxicity of WSF from the aromatic-degrading culture was reportedly the lowest, but appeared to be intermediate and predictable when plotted as a function of concentration (our analysis, Fig. A1.1). If metabolites were truly the primary cause of toxicity, we suspect there would be differences between aromatic- and alkane-degrading cultures, yet no differences in toxicity were evident.

Microbial degradation of oil is typically nutrient limited, particularly by nitrogen, and addition of fertilizer is required to significantly increase natural degradation rates (Gibbs and Davis 1976; Oudot and Dutrieux 1989; Bragg et al. 1994; Atlas 1981; Atlas 1995). Such was also the case in experiments by Shelton et al. (1999); nutrient-limited bacterial cultures were essentially inactive, and the toxicity and concentration of the resultant WSF was indistinguishable from control response (>90% embryo and larval survival). Microbial action was only significant where cultures were provided with additional nitrogen and phosphate (Shelton et al. 1999), and nutrients were routinely added by Middaugh et al. (1996; 1998). Similarly, nutrient addition (nitrate and phosphate) was required to initiate biodegradation in experimentally oiled sandy-gravel columns (coarse beach material, 0.5-8 mm diameter; Gibbs and Davis 1976). Biodegradation rates may be increased by 5-10 times by nutrient additions (Bragg et al. 1994; Atlas 1995; Shelton et al. 1999). Microbial degradation rates in our oiled-rock columns were probably nutrient-limited because we used natural water sources and did not add nutrients. The coarse, washed gravel in our apparatus was of terrestrial origin (glacial till) and did not include fine-grained material, and thus was unlikely to furnish the nitrogen necessary for bacterial growth.

The time required for hydrocarbon-utilizing microbes to begin to degrade substantive quantities of hydrocarbons in beach substrate may require roughly 1-3 weeks (Gibbs and Davis 1976). Bio-oxidation in their oiled sandy-gravel columns did not begin for more than 2.5 weeks after light oiling and 1.5 weeks after the addition of more oil (Gibbs and Davis 1976). Furthermore, nutrient addition (nitrate and phosphate) was apparently required to initiate substantive biodegradation (Gibbs and Davis 1976). [Flow rates in these columns (60 ml/hr) were much slower than in our apparatus (5-7 L/hr) but were operated under aerobic conditions as were ours. An earlier experiment by Johnston (1970) explored bacterial action in sand columns, with very slow water exchange rates (7 L/day), and heavy oiling caused anoxic conditions. In fine-grained material, oxygen is the principal factor limiting microbial degradation; in coarse material where oxygen is not depleted, microbial growth is generally nutrient limited (Gibbs and Davis 1976)]. Shelton et al. (1999) reported that metabolites accumulated faster in the second week of microbial incubation at 20EC under static conditions (including cultures from PWS), suggesting that changes in bacterial abundance occurred within this time scale. The rate of biological degradation in oiled substrate also declines with increasing hydrocarbon concentration, thus physical removal of oil may initially be dominant (Fusey and Oudot, 1984).

There was no evidence of time-dependent toxicity increases due to microbial byproducts from on week-length time scales in oiled-rock column tests by Carls et al. (1999). If microbial growth had contributed significantly to toxicity, then the toxicity-time relationship would likely have been nonlinear over weekly time scales, but responses were highly linear (Carls et al. 1999). Another clue that microbial growth likely played a minor role in oiled-rock column experiments is that toxicity was highly similar in herring egg and pink salmon egg tests, yet far more time was available for colonization and degradation in the salmon tests (roughly 6 months) versus 2-16 d in herring tests (Heintz et al. 1999; Carls et al. 1999). However, both species developed past the blastula stage within the first week, a time when developing embryos are very sensitive, so these differences in time scales must be interpreted cautiously. For example, *Menidia beryllina* and Pacific herring embryos exposed shortly after fertilization (8-16 cell stage) were more vulnerable to damage than those exposed 48-96 h post fertilization (Middaugh et al. 1998).

Low temperature may limit the growth of oil-degrading microbes in the marine environment, and although indigenous populations may be cold adapted, degradation rates for a given microbial population can be far less at low temperatures than at high temperatures (ZoBell 1969; Mulkins-Phillips and Stewart 1974; Gibbs and Davis 1976; Atlas 1981). For example, ZoBell (1969) reported that degradation was more than an order of magnitude faster at 25EC than at 5EC. In experimentally oiled sand-gravel columns, net oxidation rates were 3.7 times faster at 21EC than at 6EC (estimated from Gibbs and Davis 1976).

Experimental conditions in oiled-rock tests were less favorable for microbial growth than those by Middaugh et al. (1996; 1998) and Shelton et al. (1999). For example, temperatures were colder, (4-7°C versus 20°C), water flow was rapid (5-6 L/minute versus static), and nutrients were not added in tests by Carls et al. (1999). Dividing the potential contribution of metabolic byproducts (<30%; estimated from Shelton et al. 1999) by 3 to 5 to account for slower degradation rates without nutrient supplement (Atlas 1995) and by 3.7 to account for colder temperatures (estimated from Gibbs and Davis 1976), we estimate that metabolic byproducts accounted for <2-3% of the toxicity reported by Carls et al. (1999). Predictions for other oiled-rock toxicity tests should be similar (e.g., Heintz et al.

1999). Thus, we conclude that microbial contribution to toxicity in tests where seawater was contaminated by passage through oiled rock is minimal.

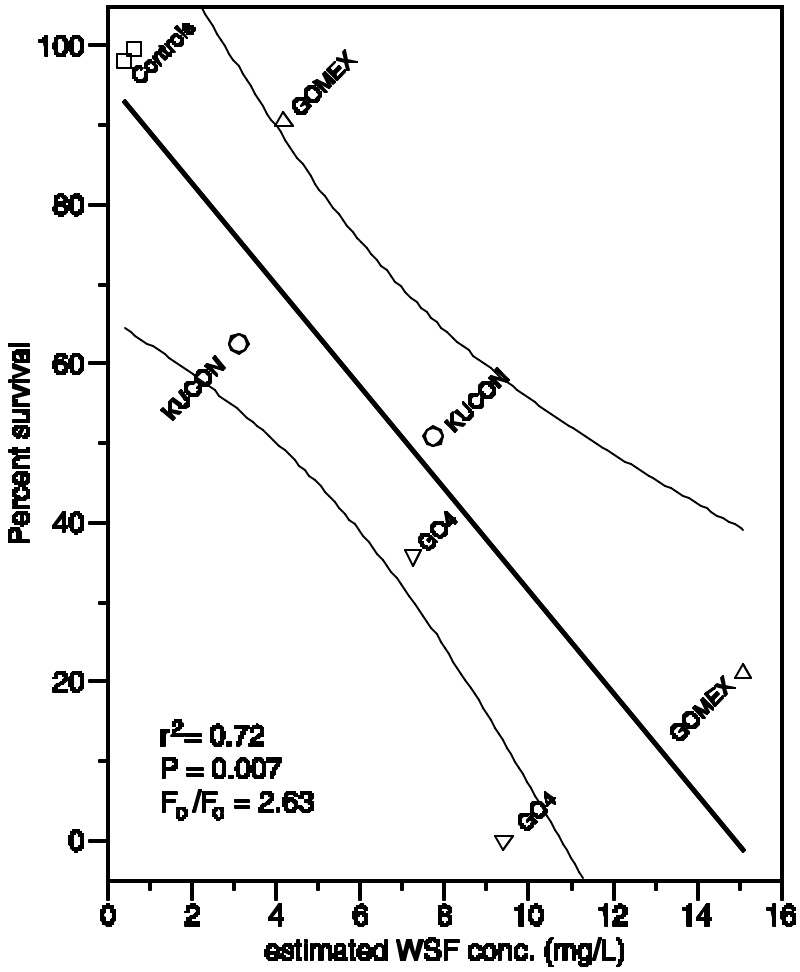

Fig. A1.1. Survival of grass shrimp as a function of concentrations of water-soluble fractions (WSF) of oil (derived from the bacterial study of Shelton et al. 1999). Microorganism cultures were from the Gulf

of Mexico (GOMEX), the Arabian Gulf (KUCON), and Prince William Sound, Alaska (GO4). The KUCON culture degraded primarily aromatics, the other cultures primarily degraded alkanes.

Appendix 2

Are estimations of high Alaska North Slope crude oil toxicity reasonable?

Several studies have indicated Alaska North Slope crude oil (ANSCO) dissolved in water is highly toxic; lowest observed effective concentrations (LOEC) of total polynuclear aromatic hydrocarbons (TPAH) in water were $\geq$ 4.4 $\mu g \bullet L^{-1}$ (Marty et al. 1997), $\geq$0.4-9.1 $\mu g \bullet L^{-1}$ (Carls et al. 1999), $\geq$1.0 $\mu g \bullet L^{-1}$ (Heintz et al. 1999), and $\geq$ 5.4-18 $\mu g \bullet L^{-1}$ (Heintz et al. 2000). Toxicity per unit mass of PAH increased as weathering increased in these studies. Other researchers, both published (Neff et al. 2000) and anonymous, have suggested these results are anomalous and do not describe the toxicity of oil spilled into the natural environment. These researchers have suggested that TPAH measurements are not indicative of oil toxicity because other compounds present in oil may be toxic including alkanes, phenol, or the unresolved complex mixture (e.g., Neff et al. 2000). In this appendix we compare the oiled-rock column studies of Marty et al. (1997), Carls et al. (1998; 1999), and Heintz et al. (1999; 2000), which hereafter are identified as NRDA studies in this appendix, with other toxicity studies, and in particular with the recently published study by Neff et al. (2000) which explores the influence of weathering on oil toxicity.

Numerous studies support the validity of using TPAH to monitor potentially toxic oil concentrations [e.g., Moore and Dwyer (1974); Anderson et al. (1974); Neff (1979); Neff and Anderson (1981)]. Because the acute toxicity of a petroleum product is directly correlated to its content of soluble aromatic derivatives (Anderson et al. 1974; Moore and Dwyer 1974; Neff 1979), aromatic concentrations are the best, most widely recognized indicator of toxic oil components. Neff et al. (2000) reported that PAHs accounted for 3-94% of estimated hazard indices of four oils, and that the contribution increased with weathering: TPAH contribution to toxicity in the most weathered fractions ranged from 57 - 94% (omitting a 99% observation for one oil because of analytical errors). Phenols contributed < 15% to the toxicity in any test by Neff et al. (2000), and did not vary consistently as a function of weathering. Similarly, Barron et al. (1999) also found that the least toxic of three oils had by far the highest phenol concentration. Retention of the higher molecular weight, more refractory and more toxic PAHs in aqueous solution as the oil weathers appears to best explain the increased toxicity of weathered oil (Rice et al. 1977; Neff 1979; Black et al. 1983; Carls et al. 1999; Heintz et al. 1999; 2000).

A critical difference between traditional acute bioassays and NRDA assays is the composition of hydrocarbons to which animals are exposed. Aromatic compounds present in short-term, unweathered water-soluble or water-accommodated fraction tests are typically dominated by single-ring compounds [e.g., Rice et al. (1979); Neff and Anderson (1981); Rice et al. (1987)]. However, monoaromatic hydrocarbons all evaporated rapidly from PWS but high molecular weight PAHs remained in the water column until late summer (Short and Harris 1996b). The bioassay technique used by NRDA investigators, passage of water through oiled gravel, closely mimicked the PAH composition observed in PWS (naphthalenes through chrysenes), including the weathering patterns characterized by Short and Heintz (1997). Consequently, NRDA studies focused on exposure of embryonic life stages to PAH, where the toxicity mechanism is oxidative cellular damage that can be passed on to daughter cells during development (Livingstone et al. 1990; Akcha et al. 2000), rather than acute toxicity tests which focus on narcosis induced by the most water soluble components of oil.

When the data reported by Neff et al. (2000) were converted to the same measure of oil (TPAH) reported in NRDA studies, similarities between toxicity were immediately evident. [Neff et al. (2000) completed 4-day acute, static bioassays of four oils on fish (*Amphiprion clarkii*, and *Menidia beryllina*), crustaceans [*Penaeus vannamei* and *Americamysis bahia*), sea urchin larvae (*Arbacia punctulata* and *Strongylocentrotus purpuratus*) and sand dollar larvae (*Dendraster excentricus*). Each oil, Wonnich crude, Campbell condensate, Agincourt crude, and Australian diesel, was artificially weathered and unweathered plus two or three progressively weathered fractions of each oil were assayed.] The data reported by Neff et al. (2000) were converted from percent water-accommodated fraction (WAF) values to median lethal concentrations of TPAH using the values provided in their paper. The average LC50 derived from all Neff et al. (2000) tests was 255.7 $\mu g \bullet L^{-1}$ TPAH (range 2.6-1112 $\mu g \bullet L^{-1}$). Because percentages of monoaromatic hydrocarbons decreased as weathering increased (Neff et al. 2000), composition in the most weathered oils were more similar to composition in NRDA tests: the average LC50 for the most weathered oil treatments was 384.8 $\mu g \bullet L^{-1}$ total PAH (range 5.7-1112 $\mu g \bullet L^{-1}$). In both cases, the lower end of the LC50 range overlaps the LOEC range (0.4-18 $\mu g \bullet L^{-1}$ reported in NRDA studies. Further factors must be considered, however, in order to compare the results of Neff et al. (2000) with NRDA studies.

Four key differences between recent bioassays of Neff et al. (2000) and NRDA tests may explain the observed toxicity differences: test duration, type of test (static versus flow-through), measures of biological response, and life stage examined (Moles 1998; Heintz et al. 2000). Conversion factors for each of these differences can be estimated from published literature (Table A-3.1). Conversion of the mean 4-day, static, acute LC50, estimated for adult or larval animals (Neff et al. 2000) to long-term, flow-through, sublethal tests with eggs results in an estimated mean EC50 of 28 $\mu g \bullet L^{-1}$ (= 384.8 $\mu g \bullet L^{-1} \times 0.498 \times 0.499 \times 0.424 \times 0.703$). This estimate falls within the EC50 range (19-35 $\mu g \bullet L^{-1}$) observed in the less weathered test of Carls et al. (1999). Larva in tests of Neff et al. (2000) were frequently not more responsive to oil than adult forms and unknown inter-species differences may be an explanation for this. However, the estimated mean EC50 (40 $\mu g \bullet L^{-1}$) for tests by Neff et al. (2000) remain similar to NRDA estimates when the life-stage conversion term is dropped. Thus, the results of Neff et al. (2000) appear to be in general agreement with NRDA results.

Other investigators have reported toxic responses to low concentrations of crude oil or specific PAH found in crude oil. Pearson et al. (1985) observed that the frequency of abnormal herring larvae was elevated at 4.4 $\mu g \bullet L^{-1}$, and although he did not discuss this result in early *Exxon Valdez* oil spill papers, included it in his 1999 review. Solberg et al. (1982a,b) found the growth and feeding of larval cod (*Gadus morhua*) was reduced when exposed to the WSF of Ekofisk crude oil at concentrations as low as 50 $\mu g \bullet L^{-1}$ (dichloromethane-extractable hydrocarbons), the lowest concentration tested, and suggest that effects might occur at lower concentrations. Water-soluble fractions from both unweathered and weathered oil were tested by Solberg et al. (1982a,b). Benzene, toluene, and xylene were the main constituents in the unweathered WSF (60-70%), but <5% of the weathered WSF was composed of monoaromatics. Solberg et al. (1982b) conclude that the weathered WSF had a more potent growth-reducing effect than the unweathered WSF. Carls (1978) reached a similar conclusion in tests with weathered diesel oil. Middaugh et al. (1996) reported that cardiovascular malformation (edema or tube hearts) in *Menidia beryllina* was significantly elevated at the lowest tested dose of ANSCO, 75 $\mu g \bullet L^{-1}$. These conditions resulted in reduced cardiac output and cessation of circulation. TPAH concentrations of approximately 130 $\mu g \bullet g^{-1}$ wet weight (range: 34 - 844 $\mu g \bullet g^{-1}$) were

associated with increased genetic defects and mortality of early winter flounder embryos (Longwell et al. 1996). In that study, the only PAH that yielded strong statistical correlations with mitotic effects and mortality was 1-methylphenanthrene with a mean concentration of 1 $\mu g \bullet g^{-1}$ wet weight; this PAH is highly toxic in its unmetabolized form (NRC 1985). Maximum 1-methylphenanthrene concentrations in herring egg tissue exceeded this value in several doses tested by Carls et al. (1999). White et al. (1999) reported survivorship of F2 *Pimephales promelas* larvae was significantly reduced by exposure of grandparent fish to 1 $\mu g \bullet L^{-1}$ benzo[a]pyrene; a clear dose-response relationship was evident at the lowest concentration tested, 0.1 $\mu g \bullet L^{-1}$ demonstrating that very low concentrations may have multigenerational effects. We expect that further investigations will continue to find that PAHs dissolved in water are highly toxic.

Table A-2.1. Comparisons between different types of toxicity tests.

Conversion	multiplier	range	Response ratios	Comment	Source
Length of exposure	0.498	(0.444 - 0.552)[a]	long-term (16-28 d) / short term (4 d)	LC50, sublethal tests	1,2
Static vs flow-through	0.499	(0.353 - 0.706)[b]	flow-through / static	4 day tests	1
Lethal vs sublethal	0.424[c]	(0.196 - 0.648)[a]	sublethal / lethal		2,3,4
Life Stage	0.703[d]	(0.652 - 0.754)[a]	egg or larval / adult		4,5

[a]range of means, [b]range of individual observations, [c]estimate by Moore and Dwyer (1974), 0.006, was not included. This would reduce the mean to 0.319. [d]Estimate by Moore and Dwyer (1974), 0.02, was not included. This would reduce the estimate to 0.475.

Sources of data are: 1) Moles 1998, 2) Carls et al. 1999, 3) Di Toro et al. 2000, 4) Carls 1987, 5) Rice et al. 1987.

Appendix 3
Estimating aqueous hydrocarbon concentrations from mussel tissue

As an argument that Pacific herring eggs in Prince William Sound (PWS) were never exposed to biologically meaningful concentrations of oil following the *Exxon Valdez* spill, Pearson et al. (1999) present estimates of aqueous total polynuclear aromatic hydrocarbon (TPAH) concentrations based on back-extrapolations of hydrocarbons sequestered in mussel tissue. (Direct aqueous TPAH concentration measurements were apparently not available to Pearson et al. 1999.) In this appendix we examine the validity of this argument, and conclude (along with the authors who originally developed the back-extrapolation technique) that the technique underestimates initial TPAH concentrations.

To determine the relationship between directly measured aqueous TPAH concentrations in PWS after the spill (Short and Harris 1996a) and those estimated from TPAH accumulated by mussels, we applied the method of Neff and Burns (1996) to mussel tissue collected from two oiled spawn areas, Naked Island and Rocky Bay (Brown et al. 1995a,b). Concentrations of all 39 PAHs routinely reported in the Natural Resource Damage Assessment database were included (Short et al. 1996b). The estimation model proposed by Neff and Burns (1996) relies on steady state conditions, and they recognized that estimated concentrations would not match true aqueous concentrations after a spill (Fig. A-4.1).

Back-extrapolation estimates of aqueous TPAH concentrations based on Naked Island and Rocky Bay mussel data underestimated true peak aqueous concentrations in these herring spawn areas. The relationship between true and estimated concentrations was similar to that predicted by Neff and Burns (1996) (Fig. A-4.1). Mean back-extrapolated concentrations reached 0.5 $\mu g \bullet L^{-1}$ and underestimated true aqueous concentrations by as much as 5.3 times. Total PAH concentrations in mussels did not change as rapidly as those in water, thus estimated aqueous concentrations essentially represent a smoothed curve, extending over a broader time interval than true aqueous TPAH concentrations, but underestimating peak concentrations. We concur with Neff and Burns (1996) that direct measurements of water samples provide the most accurate values for total dissolved and particulate TPAH concentrations in the water column and conclude that aqueous concentrations in herring spawn areas in 1989 peaked at 1.9-2.6 $\mu g \bullet L^{-1}$ (Short and Harris 1996a).

The difference between back-extrapolated estimates of aqueous TPAH and direct measures is important because the lowest observed adverse effective concentration for experimentally exposed herring eggs was 0.4 $\mu g \bullet L^{-1}$ (Carls et al. 1999). Thus, while back-extrapolations based on the Neff and Burns (1996) technique would suggest limited damage, direct measures of aqueous TPAH (Short and Harris 1996a) indicated considerable damage was possible. Adverse reactions of Pacific herring eggs to EVO in PWS were documented at hatching and in newly hatched larvae by NRDA studies, e.g., Brown et al. (1996a) and Marty et al. (1997), and demonstration that the short-term (1-year) consequences of the spill were detrimental to herring in PWS, is contained in the main portion of this report.

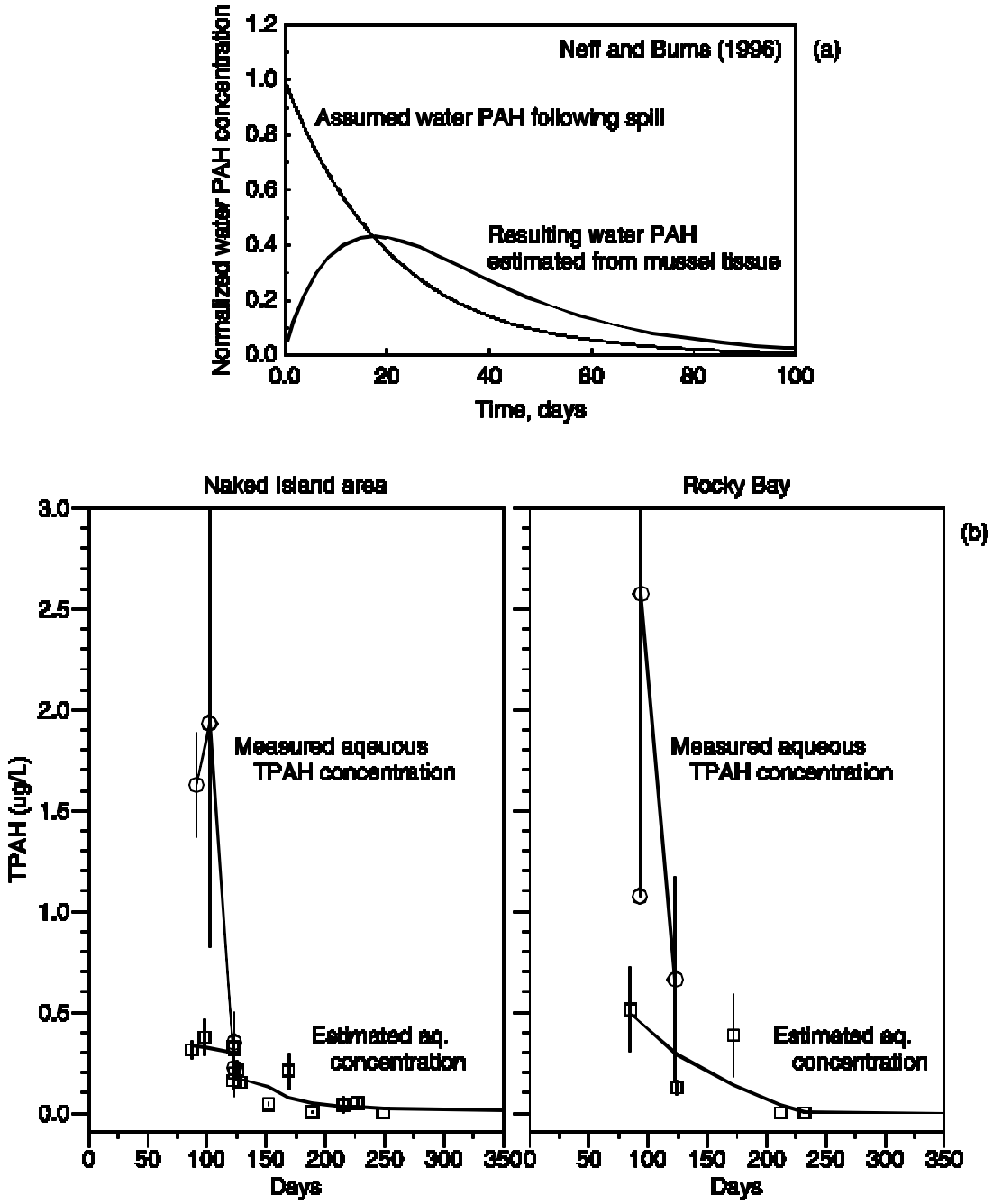

Fig. A-3.1. Comparison of aqueous theoretical total polynuclear aromatic hydrocarbon (TPAH) concentrations in water and estimates back-extrapolated from hydrocarbons sequestered in mussel tissue (Neff and Burns 1996) (a) with directly measured and back-extrapolated aqueous TPAH concentrations from two 1989 herring spawn sites in Prince William Sound (Short et al. 1996b) (b).

Curves drawn for estimated aqueous concentrations which smoothed (4253H filter; Velleman and Hoaglin 1981)

Appendix 4
Publications specific to Prince William Sound, Pacific herring, and the *Exxon Valdez* oil Spill

Published studies of Pacific herring in Prince William Sound, Alaska, after the March 1989, *Exxon Valdez* oil spill. Studies listed in bold type were industry sponsored, all others were sponsored by Natural Resource Damage Assessment funds. Studies listed in italics are reports or other non-peer reviewed literature. Reported study years separated by a "-" include intervening years; those separated by "&" do not include intervening years. The type of study, field or laboratory is indicated for primary papers, but not in summary or review papers. Only studies where live eggs, larvae, or fish were maintained were considered to have a laboratory component. Laboratory studies were categorized as observational (O) or experimental (E). Studies were considered observational if there were no experimental treatments in the laboratory. Types of experimental treatment, exposure to oil or to viral hemorrhagic septicemia virus (VHSV), are indicated in parentheses.

Study	Years Observed	Biological Observation	
		Field	Lab
Bienert and Pearson 1995	**1989 - 1990**	**Summary/review**	
Brown et al. 1996a	1989 - 1990	summary/review	
Brown et al. 1996b	1989 - 1991	summary/review	
Carls et al. 1998	1994		E(oil)
Carls et al. 1999	1995		E(oil)
Carls et al. 2000	1994		E(oil)
Elston et al. 1997	**1994**	**x**	
Havnes et al. 1993	*1989 - 1990*	*x*	
Hose et al. 1996	1989 - 1991		O
Hose and Brown 1998	1989 - 1995	summary/review	
Johnson et al. 1997	1995	x	O
Kocan et al. 1996a	1991 - 1992		E(oil)
Kocan et al. 1996b	1992		O
Kocan et al. 1997	-		E(VHSV)
Kocan and Hose 1997	1989 - 1991	summary/review	
Marty et al. 1997	1989	x	E(oil), O
Marty et al. 1998	1994	x	
Marty et al. 1999	1989 - 1991	x	
McGurk et al. 1990	*1989*		*O*
McGurk et al. 1991	*1990*		*O*
McGurk 1992	*1989*		*O*

McGurk et al. 1993	1989	summarv/review	
McGurk & Brown 1996	1989	x	
Mevers et al. 1994	1993	x	E(VHSV)
Moles et al. 1993	1989	x	E(oil)
Norcross et al. 1996	1989 & 1995	x	
Pearson et al. 1995a	1989 - 1990	x	O
Pearson et al. 1995b	1989 - 1990	summarv/review	
Pearson et al. 1996	1989 & 1993	summarv/review	
Pearson et al. 1999	1989 - 1994	summary/review	
Thomas et al. 1997	-		E(oil)