Exxon Valdez Oil Spill
Restoration Project Final Report

Prince William Sound Herring: An Updated Synthesis of Population Declines and Lack of Recovery

Restoration Project 050794
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Study history: The Prince William Sound (PWS) Pacific herring population remains depressed, and may be unique in remaining vulnerable to disease and demonstrating a general lack of recovery for the past 14 years. Prior to the spill, the population was behaving in concert with the Sitka Sound stock; periodically, both stocks had good recruitment years. This suggests that regional oceanographic factors were dominant factors in limiting the populations. Since the spill, the two populations have not been fluctuating in concert, suggesting that there are now other dominant factors limiting this struggling population. A previous synthesis suggested oil related effects contributed to the poor recruitment of the 1989 year class, and that disease was a major contributing factor to the 1993 stock collapse (Carls et al. 2002). The PWS stock continues to show poor recovery. In this synthesis, holistic population dynamics modeling was applied to determine if the population trend is unique. The contribution of oil exposure in limiting present day recovery was evaluated and other potentially limiting factors were considered such as disease, recruitment failure, and genetic restriction. A panel of experts was convened to examine these issues and their views are included in this synthesis.

Abstract: The PWS herring population collapsed 4 years after the *Exxon Valdez* oil spill, igniting debate about the cause. Fishermen who once depended on this stock for income and some investigators are convinced that the spill was causal, others are not. Our re-examination of the data demonstrates that polynuclear aromatic hydrocarbons (PAH) are highly toxic and that the oil spill significantly damaged herring embryos in 1989. These effects were no longer detectable after 1990 and strong recruitment of the 1988 year-class (in 1991) marked population recovery from the direct toxic effects of the spill. No plausible oil-related mechanisms have been developed to explain a delayed response after intervening years of no response. By 1993, recruitment to an expanding population (plus an additional 1 to 2% because the stock was not fished in 1989) helped precipitate a catastrophic disease outbreak. Epidemiological analysis identifies three significant risk factors for the 1993 population crash: 1) relatively large biomass from 1988 to 1992 (i.e., a susceptible host); 2) relatively low zooplankton production in 1991

and 1992 (i.e., environmental conditions contributing to poor overwinter condition); and 3) the presence of disease (VHSV and filamentous bacteria). Timing of the population collapse was questioned by some, who extrapolated hydroacoustic data (a time series that began in the mid-1990s) to dates earlier than sampled and suggested the collapse began in 1989, adding fuel to the controversy that the oil spill was linked to the collapse. However, this particular hindcast fails to explain the observation of lethargic survivors with external hemorrhages in 1993 and is inconsistent with a competing age-structured model. Although linkage of the 1993 collapse with the oil spill cannot be proved or disproved with certainty, reasons for poor recovery since the collapse remain perplexing. Natural factors, including climate, inter-species competition, suboptimal recruitment, condition prior to entering the winter starvation period, disease, and predation may be important. Disease measurements through 2002 continued to indicate the population was restricted by chronic disease; recruitment was negatively affected by VHSV and life spans were shortened by *Ichthyophonus,* although reasons why disease continues to cycle in the population are unresolved. Aside from limited measurement in a reference population (southeast Alaska), comparable disease measurements in other fish populations do not exist. The PWS herring population is not genetically discrete within the Gulf of Alaska. Thus, genetic diversity within the population and exchange with surrounding populations is adequate and does not explain the lack of population recovery, despite the sudden population collapse in 1993. Continued disease cycles may be the most parsimonious reason why the PWS herring population has failed to recover in the past 13 years but the root causes for this are unknown. **The resulting lack of recovery is more than likely the contribution of several factors, not just one factor.**

Key words: Pacific herring, *Clupea pallasii*, Prince William Sound, *Exxon Valdez* oil spill, population collapse, recovery failure, disease, *Ichthyophonus hoferi,* VHSV, population dynamics, population trends, age-structured assessment model, genetics, diversity, bottleneck, heterozygosity, discreteness, effective population size, gene flow.

Project data: *Description of data* – This document synthesizes other published work and does not contain new data. Corresponding author: S.D. Rice, NOAA/NMFS, Auke Bay Laboratory, 11305 Glacier Highway, Juneau, AK 99801 (work phone: (907) 789-6020, FAX: (907) 789-6094, or email jeep.rice@noaa.gov. *Availability* - Copies of the report are available on CDROM for the cost of duplication.

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Executive Summary

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Problem

The Pacific herring population in Prince William Sound (PWS) remains depressed, 14 years past the collapse in 1993. Herring are a valuable fishery resource and have great ecological importance; they are valuable prey for many species, ranging from other fish to birds and marine mammals. The health of the PWS ecosystem is in question when the population of herring, typically the most abundant forage fish present, remains depressed.

Herring biology is complicated. All life stages are preyed upon by many different species and all life stages are influenced by a suite of environmental factors. The basic survival strategy for herring is to swamp predators at all life stages. Consequently, recruitment is highly variable, ranging from bust to boom. Populations are the net result of many factors, each operating with different intensities on different life stages. These factors vary in importance (impact) on different life stages and vary from one year to the next, including their relative importance. Thus, identification of the specific factors contributing to recruitment, population growth, and poor stock recovery is complex.

This project has two primary goals: (1) determine if the continued depressed state of the PWS herring population is related to the oil spill, and (2) determine if the current genetic diversity is critically low and limits recovery. A wide variety of hypotheses have been advanced to explain poor population recovery. Some of these are explored in depth, others are relatively implausible and receive little attention. **1)** Broad scale natural factors certainly play a role in recruitment and population fluctuations; the synchronization with Sitka stock prior to the spill is strong evidence of the importance of large-scale factors. However, because the Sitka stock is currently experiencing growth but the PWS stock is not, other factors must also be influential in restraining PWS population recovery. **2)** The herring population is reacting to the continued presence of low oil concentrations in PWS with unknown impacts on spawning success, population productivity, and sustainability. Although there is evidence of biologically available *Exxon Valdez* oil in PWS (Short et al. 2004; Springman et al. in preparation), these intertidal areas do not include historical herring spawning habitat (Carls et al. 2002) and their zone of influence is relatively small. In general, water in PWS is characteristic of the least-contaminated

portions of the world's oceans (Carls et al. 2006), suggesting that continued oil exposure is inconsequential for Pacific herring. **3)** Oil metabolites, produced by microbial action, chronically affect the herring population. There is no evidence and no mechanism to suggest that more chemically reactive, thus more readily degraded, metabolites would be present after the hydrocarbon source (oil) is gone, thus we find no support for this hypothesis. **4)** Inter-species competition may be limiting PWS herring recovery; other species may have expanded to fill the ecological niche lost by herring when the population collapsed. Inter-species competition may be more significant at some life stages than others, but needs to be significant in only one life stage to be a major limiting factor. **5)** Similarly, significant predation on the small herring population may hamper recruitment and population growth. Study is underway to determine if humpback whales are a major contributing factor; other candidates include piscivorous fish, pinnipeds, and birds. Hypotheses 4 and 5 are viable, but quantitative data to evaluate are generally not available pre or post spill, and are beyond the scope of the current synthesis. **6)** Failure of the Pacific herring population in PWS to recover may be a result of age-dependent mortality from three pathogens: the mesomycetozoan *Ichthyophonus hoferi*, viral hemorrhagic septicemia virus (VHSV), and filamentous bacteria (associated with cutaneous ulcers). However, reasons why disease would continue to play a dominant role are unresolved and there are no comparative long-term disease data for other herring populations. **7)** Suboptimal recruitment. Restricted spawning areas may reduce the probability of larval survival because they drift into fewer potential rearing habitats than would occur under more extensive spawning, thus reducing subsequent recruitment. This possibility is discussed in Chapter 7. **8)** Cascade effects. Possible cascade effects are highly speculative. We are unaware of any reports of oil-related cascade effects in pelagic fish species or their prey. The primary support for a cascade effect is the persistent population depression, coupled with the persistent association with disease for unknown reasons. Also supporting a cascade effect is the simultaneous collapse in the pink salmon population in PWS in 1992 - 1993. Populations of these two species with very different life histories and survival strategies collapsed in the same localized region (PWS) but did not collapse elsewhere in Alaska suggesting unusual circumstances in PWS. This fuels speculation of a cascade effect linked to the oil spill with no known mechanism. **9)** Insufficient genetic diversity in the PWS herring population. This hypothesis is refuted in Chapter 6; genetic diversity in PWS herring is high and does not contribute to poor recovery.

Previous Herring syntheses

Possible responses of Pacific herring (*Clupea pallasii*) to *Exxon Valdez* oil in Prince William Sound (PWS), including a possible delayed population collapse, generated numerous primary papers. These were previously summarized and synthesized twice, once by industry-sponsored researchers (Pearson et al. 1999) and once by Natural Resource Damage Assessment (NRDA) researchers (Carls et al. 2002). Industry researchers concluded that oil concentrations were too low to be toxic to most herring embryos; NRDA researchers concluded that oil concentrations in PWS were sufficient to damage or kill a substantial number of the embryos. Conclusions regarding the collapse of the PWS herring population were more similar. Industry researchers concluded increased biomass and decreased food supply were the most likely factors. To these, government researchers added disease as an important factor, but did not rule out indirect links to the oil spill.

In contrast to some large Alaskan herring stocks, the PWS herring population remains depressed (Appendix 10). While increases and decreases in herring populations are expected, with or without fishing pressure, the continued depression is unexpected. The low herring population in PWS continues to produce hypotheses that it is related to the oil spill. Fueling some of this conjecture is a recent retrospective study by Thomas and Thorne (2003) that hypothesizes that the herring population collapse started in 1989 but was not detected until 1993. They suggest that the oil spill was responsible for uncoupling the various biological parameters used to model the stock, and as a result, the models used to determine the fishery harvest in the years immediately prior to the population collapse (1990 to 1992) were in error.

The current synthesis reviews recent literature to re-examine the possible connection of the herring population collapse to the oil spill, and in light of the Thomas-Thorne paper, takes more of a stock modeling approach.

1. Review of toxicity literature relevant to PWS herring.

For oil to be a cause of the current population depression, (a) lingering oil must have continued to exert new effects, or (b) the oil exposures of 1989 must have caused a persistent biological effects.

(a) Lingering oil effects are not suspected. There is no evidence of significant herring exposure to oil in PWS after 1990. Unlike the habitat of certain other species (pink salmon, sea

otters, and harlequin ducks), oil was not stranded in herring habitat, thus there is no chronic source of lingering oil for herring and their habitat.

(b) Initial effects are reinforced, persistent effects are speculative. The initial effects of the spill have been reinforced by recent published literature. Recent published papers strengthen the arguments for a toxicological impact in 1989 on herring eggs and larvae. Damage to fish embryos is caused by low aqueous total polynuclear aromatic hydrocarbon concentrations (0.4 to 23 µg/L (Marty et al. 1997; Carls et al. 1999; Heintz et al. 1999, 2000; Colavecchia et al. 2004; Rhodes et al. 2005). A previous synthesis estimated that approximately a third of the herring eggs were exposed to detrimental concentrations of dissolved or particulate oil (Carls et al. 2002), and published literature on controlled exposures indicates these are realistic exposure routes for both eggs and larvae (e.g., Carls et al. 1999; Barron et al. 2003). The field studies of 1989 are limited but the observed adverse responses in PWS herring embryos and larvae are consistent with those observed in the laboratory (e.g., Brown et al. 1996; Carls et al. 2002; Barron et al. 2003).

Persistent effects from the initial oil spill in 1989 are speculative. For oil exposures in 1989 to have had a continuing effect in PWS herring, either of two criteria would have to be met: (a) long term immune suppression leading to disease, or (b) a possible cascade effect. While disease issues continue for the PWS herring and are probably limiting herring recovery, there are no studies (in PWS or elsewhere) linking a long-term immune suppression in fish to contaminant exposure. The plausibility of immune-compromised individuals surviving for long periods is small. Disease challenge would likely remove impaired individuals from the population, particularly after annual winter starvation events when fish are least resistant. Each fall VHSV drops to undetectably low levels only to rebuild in the spring. This natural cycling does not require individuals damaged as a result of oil exposure to introduce disease into the population.

Possible cascade effects are highly speculative. We are unaware of any reports of oil-related cascade effects in pelagic fish species or in their prey. The primary support for a cascade effect is the persistent population depression, coupled with the persistent association with disease. The causes for the persistent disease are not understood, suggesting an unknown cascade effect. Also supporting a cascade effect is the simultaneous collapse in the pink salmon population in PWS in 1992 - 1993. Populations of two species with very different life histories and survival strategies collapsed in the same localized region (PWS) but did not collapse

elsewhere in Alaska. Thus, these collapses appear to be a PWS phenomenon. This fuels speculation of a cascade effect linked to the oil spill with no known mechanism.

2. Evidence that the *Exxon Valdez* oil spill did not cause the 1993 disease epidemic in the PWS Pacific herring population.

The conclusion best supported by the evidence is that strong recruitment of the 1988 year-class in 1991 marked population recovery from the toxic effects of the spill. By 1993 that recruitment—plus an additional 1 to 2% not harvested by the closed fishery in 1989 —helped precipitate a catastrophic disease outbreak. Epidemiological analysis identifies three significant risk factors for the 1993 population crash: 1) a susceptible host (the relatively large biomass from 1988 to 1992); 2) relatively low zooplankton production in 1991 and 1992 (i.e., environmental conditions contributing to poor overwinter condition); and 3) the presence of VHSV and filamentous bacteria. For unknown reasons, the effects of that outbreak continue to cycle through the population, preventing recovery 13 years later.

3. Comparison of Pacific herring recruitment in PWS with other western North America stocks.

To determine if the low recruitment patterns after the 1993 population collapse are unique to PWS or whether they are within the expected range of natural variability, historical and contemporary information about herring recruitment in PWS were examined and compared to recruitment patterns elsewhere on the west coast of North America.

The current history of low herring recruitment in PWS is not without precedent in other west coast herring populations, though these consecutive low recruitment events are rare (on the order of once every 50 years). The continued existence of herring populations is threatened when the number of consecutive low recruitments approaches the reproductive lifespan. Herring in PWS came dangerously close to the reproductive lifespan threshold with 4 successive years of near-zero recruitment in the late 1990s, following previous low recruitment in the early and mid-1990s. Moderate recruitment in 1999 may sustain the population provided adult mortality is not excessive, at least for the short term. Recovery of PWS herring will require further above-average or strong recruitment events, combined with increased adult survival from disease and

other sources. Because we do not know the cause of the current series of low recruitment events, it is not possible to predict if recruitment will get better or worse.

4. Conflict in Population Dynamic modeling.

Estimation of Pacific herring standing stock in PWS with population dynamic modeling originally relied on age-composition, miles of spawn, and egg deposition data. Acoustic estimation of populations was not done prior to 1994. Miles of spawn and egg deposition data are related, but it was thought the egg deposition data probably provided better estimates of spawn intensity and thus numbers of spawning herring. Disease data were added to the ages-structured model (ADFG) after the collapse of the herring population in 1993. The disease parameter is necessary to explain the variability in this disease-limited herring population, and the data exist through 2002. Also, about 1995, Thomas and Thorne (2001, 2003) began acoustic estimates of population size in winter (when herring collect into tighter schools), and noted a close correlation with mile-days of spawn after1995. Using the correlation of miles-days of spawn data and acoustic estimates after 1995, they hindcast the numbers of herring present between 1989 and1995, and deduced that the population collapse started in 1989 and was exacerbated in 1990-1992 by overfishing the standing stock, an error caused by failure of the ADFG age-structure models to identify the timing of the collapse.

The ADFG age-structure and Thomas-Thorne models disagreed between 1989 and 1993, because the parameters measured during this time period became uncoupled, possibly because of the oil spill, but that is speculative. The modeling highlights their divergence, but does not resolve which model is correct or which parameters are more dependable in estimating biomass. Prior to 1995 and without acoustic estimates of biomass, the age-structure model was believed to be a robust measure of population biomass, therefore commercial fishing was permitted on the stocks in 1990, 1991, and 1992. (Note: none of these estimates are an exact measure of population biomass and each can be biased by circumstance. Acoustic estimates are dependent on calibrations for tightly schooled fish, and estimates may be biased if herring are not tightly schooled, calibrations are off, or not all schools are located. Mile days of spawn and egg deposition are each correlated with fish biomass but estimates may be biased if environmental factors change reproductive output or prevent significant numbers of reproductive fish from spawning. Age structure can be biased if the various schools are not proportionately sampled.)

The Thomas and Thorne hypothesis is very important. If the herring population collapse started in 1989, then the oil spill can be implicated as a likely cause, even if not detected until 1993. If so, then present day depressed population levels are related to the oil spill and unexpected damages, a key criterion of the re-opener clause, would be satisfied. However, hindcasting does not solve conflicting population model estimates.

Histopathology of adult Pacific herring in PWS provides evidence that the population was relatively healthy in 1991 and 1992 but experienced a massive disease-related population decline in the spring of 1993 that continued into 1994. Histopathology of PWS Pacific herring in the spring of 1991 (n = 60) and spring of 1992 (n = 100) revealed no fish with lesions related to VHSV (viral hemorrhagic septicemia virus), and prevalence of *Ichthyophonus hoferi* was low (< 10%) (Kocan et al. 1996; Marty et al. 1999). By comparison, lesions associated with viral hemorrhagic septicemia virus were common in 1993 (Meyers et al. 1994) and 1994 (Marty et al. 1998). Protracted population collapse as hindcast by the hydroacoustic model does not explain this abrupt change in disease status and associated mortality.

From 1995 on, incorporation of hydroacoustic data in the age-structured model as a relative index of total herring biomass improved stability and confidence estimates by adding winter population data, collected 6 to 8 months after the last spawning event. The hydroacoustic information had no substantial effect on the original biomass trends and estimates by the agestructured model. Although the age-structured model remains the best population estimator prior to the collapse, substantial uncertainty about population parameters during 1989 to 1992 remains due to irresolvable conflicts between spawning data (egg deposition and mile-days of milt). Consequently, the magnitude of population declines for that period depends on the weights chosen for the various datasets, particularly mile-days of milt.

5. Disease was a primary contributing factor to population collapse and is the most likely reason the Pacific herring population in PWS has failed to recover.

Disease incidence in PWS herring was prominent at the time of the 1993 population collapse and stimulated 10 years of monitoring. Disease was and is the most likely reason the herring population has failed to recover; although field measurements were discontinued after 2002, disease probably remains a limiting factor (Marty et al. 2003). The reason why disease continues to exert this level of impact on the PWS population is unknown.

The failure of the Pacific herring population to recover is a result of age-dependent mortality from three pathogens: the mesomycetozoan *Ichthyophonus hoferi*, viral hemorrhagic septicemia virus (VHSV), and filamentous bacteria (associated with cutaneous ulcers). Beginning in 1993 with a severe outbreak of VHSV and ulcers, epidemics have cycled through the Pacific herring population in PWS about every 4 years. Unfortunately there are no long-term disease data sets for other herring populations, or other species with which to make comparisons.

In general, newly recruiting 3 year olds have the highest VHSV infection rates in spring, which may attenuate recruitment of that year class, but VHSV infection rates decrease in older fish and do not appear to contribute significantly to mortality in the older age classes. (Infection rates in 1- and 2-year old PWS herring are unknown, but are likely to be significant when rates affecting the 3-year old recruits are significant). Young fish, with smaller biomass and less reserves, are plausibly the most vulnerable to VHSV disease impacts after the overwinter starvation period. In contrast, *Ichthyophonus hoferi* infection rates increase as herring age and probably increase the mortality rates of the older fish, which are the most effective spawners (larger mass means more reproductive products).

The causes for sustained disease problems from 1993 through 2002 are not apparent. Immune suppression can be caused after acute exposure to toxicants, but no herring living today in PWS were alive and exposed to oil in 1989, and no exposure to lingering oil is suspected. Evidence for oil-related immune suppression over long time periods, particularly from one generation to the next, does not exist. At present, the relationship of disease and other factors, such as the lack of food, is not apparent (but data to evaluate these relationships, particularly with larval and juvenile stages, are not generally available for most years). Food may have become limiting between 1986 and 1993 a period of declining zooplankton biomass and exceptionally high herring biomass, but this argument does not explain continued disease problems. Traditional fish population estimates have shown prolonged depression of population numbers in other areas, and this depression might be related to contaminant exposure or disease (e.g., Hershberger et al. 2005). However, disease has never been followed systematically in other fish populations for more than a few years, thus limiting our ability to compare results to other studies. The PWS Pacific herring population remains too low to allow commercial fishing and there is no hypothesis to explain the continuing disease or adequate information to predict when disease problems will abate.

6. Low genetic diversity does not appear to be a factor limiting population recovery.

Genetic diversity appears adequate to support recovery. A hypothesis was presented at a previous EVOS Lingering Oil workshop that questioned the available genetic diversity for PWS herring stocks. The concept is that if there were a genetic bottleneck, caused by a combination of overfishing in recent historic times coupled with the population collapse in 1993, the genetic diversity would be inadequate to cope with changing environmental and disease stressors. Genetic information was gathered in 1995 and 1996 in PWS, in an attempt to clarify whether there was a "PWS stock" or if the individual spawning areas represented different stocks. Several techniques were used. Using these data, the issue of genetic diversity (in contrast to genetic bottleneck) was re-examined to look for evidence to support limited genetic diversity. Limited genetic diversity would indicate the availability of too few genes to cope with the ever changing environmental parameters, including disease challenges. Where available, genetic diversity in impaired herring populations (such as Cherry Point in Puget Sound, Washington, and San Francisco Bay, California) were also examined to determine if there was a pattern among stressed stocks.

By all available measures, genetic diversity in PWS herring collected in 1995 and 1996 (shortly after the 1993 population collapse) was comparable to that of other healthy Northeast Pacific herring populations in Alaska, British Columbia and Puget Sound. Both gene diversity (heterozygosity) and allelic diversity (the number of alleles per locus) are high in PWS herring. The genetic diversity of PWS herring is similar to that of herring from Cherry Point but significantly higher than that of herring from San Francisco Bay. Both of the latter stocks are stressed. All measurements examined fail to demonstrate evidence of a genetic bottleneck among PWS herring capable of reducing recruitment success. According to observed genetic diversity, the 22,000 metric ton minimum spawning biomass threshold needed to conduct a commercial fishery is expected to protect the long-term genetic diversity of PWS herring. Even currently low population levels appear to be at least one thousand times higher than the upper bound on the evolutionarily effective population size of PWS herring.

Although the data are limited, the genetic diversity is apparently more than adequate in PWS herring. The years sampled were shortly after the 1993 population collapse, and if diversity were low, one would expect it to be detected in those years. However, lower diversity was not discovered. Migration rates from other populations were calculated (Seeb et al. 1999)

and the estimated genetic flow from other Gulf of Alaska herring populations would appear to be sufficient to maintain the relatively high diversity among PWS herring.

One caveat may exist: the many genes responsible for immune response to various diseases were not directly examined (technology does not exist for that information); hence we were unable to assess whether PWS herring possess the genetic diversity needed to combat common diseases. Reduced disease resistance could explain the continued disease problems for the PWS herring populations; these diseases are frequent throughout the west coast and reduced disease resistance for PWS herring would explain their long term susceptibility to the diseases. However, this does not seem likely.

7. Survival of Pacific herring eggs and larvae in PWS, particularly the latter, are key factors controlling recruitment success.

An early life history model which integrated published egg, larval, and juvenile data predicted survival after the first year to be 118 herring out of one million eggs, with a 95% confidence interval of 5 to 2,822. Hjort's concept of mortality in the larval stages as the most critical period in determining year-class strength of herring fisheries was supported by the model. Estimates of survival of the egg stages, fall juvenile stage and winter juvenile stage were two orders of magnitude greater than the survival of the larvae stage. A single-stage sensitivity analysis demonstrated that the largest influence on total survival was daily mortality in the larval stage. An interaction sensitivity analysis of all possible paired life stages showed that the combination of the egg stage with the larval life stage contributed the most to total survival. Environmental processes, including food availability, water temperature, and transport processes, are key factors in the larval stage.

Although speculative, a potentially important hypothesis concerning larval dispersal and reproductive success emerges from this model and suggests a second major reason (other than disease) why current PWS herring stock has failed to recover. A contraction in spawning range may have three negative effects, 1) increased mortality due to higher egg deposition density, 2) poorer hedge-betting as larval are dependent of oceanographic processes to distribute them to adequate nursery areas, and 3) increased density of adult spawners. Contraction in spawning area is clearly evident; the spawning area in 1988 was more than 10 times larger than in 1994, the smallest observed extent. Egg deposition density may have increased between 1988 and

1992, a time when the spawning biomass apparently remained high but spawning area contracted. By 1993 disease clearly challenged the adult population; we can only speculate whether contraction in spawning range increased crowding, thus promoting disease transmission. Finally, there are no direct data in support of reduced larval dispersal as a contributing factor, yet because modeling suggests larval survival is the most critical pre-recruitment factor and because recruitment in PWS has been consistently low since 1993, larval mortality has likely contributed to chronic recruitment failure. How this may relate to dispersal by oceanographic processes, and how it may have differed had spawning occurred over a larger area is unknown and leaves an interesting question open for study.

Summary:

The current PWS herring population is depressed, and recovery is limited by diseases that affect survival. The argument that oil negatively affected PWS herring in 1989 is reasonable but the argument for a direct continuing oil effect is not supported. No fish alive today in PWS were alive and exposed in 1989; chronic exposure from lingering oil is not suspected because the lingering oil in intertidal PWS (Northern Knight Island area) is too distant from the herring spawning and over-wintering habitat to cause effects.

Likewise, long term (multi-year) damage caused by oil exposure in 1989 is not supported by the data. Delayed response hypotheses are not supported by data, rather declines in food availability, changes in ecosystem structure (such as increased inter-species competition and predation pressure, or increased disease provide non-oil explanations for the population collapse and recovery failure. While a nearly simultaneous 1992-1993 collapse of both salmon and herring populations in PWS suggests a possible cascade effect resulting from the oil spill, a simpler explanation may be simultaneous declines in food supply (supported by data) or other localized changes that negatively affected both species. By 1994, the pink salmon population rebounded although the Pacific herring population did not. Some suggest that continued vulnerability to diseases is evidence of an oil-related cascade effect yet no plausible or substantiated hypotheses have been advanced in support of this speculation.

The causes for sustained disease problems from 1993 through 2002 are not apparent and there is no hypothesis to explain the continuing disease or predict when disease problems will

abate. Young recruiting age classes have elevated VHSV, while older age classes have elevated rates of *Ichthyophonus* infections. Although these diseases are common on the west coast, infections limiting populations over a 10 year period were not expected. Causes for the continued vulnerability to the diseases are unknown. Links between disease and protracted immune suppression has never been demonstrated. Any fish with immune systems permanently impaired by oil exposure likely died years before major disease event in 1993. Genetic diversity in PWS herring is apparently adequate and does not explain population recovery problems.

The argument that the population collapse began in 1989 is interesting, and if that were the case, would provide the basis for a link to the oil spill. However, two different population modeling efforts diverge in the important time period of 1989 to 1993 and discerning which model and which data are correct is unlikely. These models are based on different parameters (age structure, mile-days of spawn, egg deposition, and starting in 1995, acoustic estimates). The parameters measured during the spill year and just prior to the collapse diverged, suggesting a possible disturbance by the spill. Our modeling efforts recognize conflicts between population assessment data but cannot resolve them.

The lack of recovery of the PWS herring population is perplexing. Insufficient genetic variability is unlikely. While the cause may be linked to continuing disease problems, the reasons why disease may have a long continuing influence are not clear. Range contraction and crowding due to the behavior of remaining fish may contribute to current disease transmission. Disease problems in both new recruits and older fish may constrain the reproductive biomass; contracted spawning ranges may attenuate larval survival (the most important pre-recruit factor) by limiting access to appropriate rearing habitat. Delayed, post-spill disease has not been demonstrably linked to oil. While we have limited ability to monitor and model herring populations, we clearly do not understand all the factors that control their populations and recovery. Because we do not know the quantitative significance of all of the environmental variables (disease, prey, predation, intra- and inter-species competition, pre- and post winter condition of the early year classes, etc.) on each life stage for each brood year, prediction of if or when recovery of the PWS population will occur is not possible with the present data sets.

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Chapter 1

Oil toxicity: review of contemporary literature

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Abstract

This review addresses a long-standing controversy between government and industry researchers concerning the toxicity of *Exxon Valdez* crude oil. Contemporary research continues to find polynuclear aromatic hydrocarbons (PAH) are highly toxic (0.4 to 25 μg/L aqueous total PAH concentration); support comes from multiple independent groups, including an industry study that replicated government studies. For example, pink salmon embryos not in contact with oiled substrate were damaged by part-per-billion concentrations of dissolved PAH (<8 μg/L) in the industry study. Contemporary petroleum toxicity research is underpinned by the observation that dissolved PAH are toxic, a concept supported by toxicity models developed by researchers affiliated with industry. Alternative explanations to PAH toxicity posited by industry researchers were eliminated by a combination of industry and government studies. Notably, the same industry researchers who claim non-hydrocarbon sources caused toxicity in government studies did not consider these sources to be contributory in their own study, designed to replicate government studies. Induction of cytochrome P4501A clearly demonstrates that these planar hydrocarbons enter embryonic tissue. The high toxicity of PAH to fish embryos continues to support the original conclusions of government researchers that the *Exxon Valdez* oil spill damaged exposed Pacific herring embryos in 1989, the year of the spill. However, herring habitat did not retain oil and effects were no longer detectable after 1990; strong recruitment of the 1988 year-class (in 1991) marked population recovery from the toxic effects of the spill.

Keywords – Pacific herring, *Clupea pallasi*, Prince William Sound, *Exxon Valdez* oil spill, PAH toxicity, cytochrome P450, immune suppression.

Introduction

Global reliance on fossil fuels for energy and other purposes has profound environmental consequences. The effects of combustion, including soot, smog, and attendant human health risks, have long been obvious. Although the effects of increasing atmospheric CO_2 levels have taken longer to recognize, global warming is becoming one of the major challenges facing humans in the 21st century. The environmental consequences of spilled oil are an increasing challenge as the global rate of consumption continues to rise. Petroleum released into the environment by consumers is the largest source of oil entering the sea (53 to 63%) followed by transportation of petroleum (24 to 39%; NRC 2003). In the latter category, the *Exxon Valdez* oil spill was the largest ever in U. S. waters and has been the subject of intense scientific study. Damage to birds and mammals that rely on feathers or pelage for insulation was immediate and obvious. Damage to species that live below the surface, such as fish, was neither as dramatic nor as easy to document.

In this paper we focus on the environmental consequences of oil in water, and specifically on a single species of fish, Pacific herring (*Clupea pallasi*). The *Exxon Valdez* oil spill bracketed herring spawning in spring 1989, placing them at risk. Three or 4 years later the PWS herring population collapsed (Pearson et al. 1999; Carls et al. 2002).

Were Pacific herring in Prince William Sound (PWS) damaged by exposure to *Exxon Valdez* oil? The previous answer depended on the funding source. Industry researchers concluded that oil concentrations were biologically significant for a small percentage of herring embryos (<2%, Pearson et al. 1995; revised to <10% by Pearson et al. 1999). Government researchers concluded that oil concentrations in PWS were sufficient to damage or kill a substantial number of the embryos (52%, Brown et al. 1996; revised to 25 to 33% by Carls et al. 2002). Researchers on both sides agreed that effects to the sensitive early life stages were limited to 1989 (Pearson et al. 1995; Carls et al. 2002). The synthesis of Pacific herring data by Carls et al. (2002) was based on evidence that polynuclear aromatic hydrocarbons (PAH) in crude oil are highly toxic. The experimental results of Marty et al. (1997a), Carls et al. (1999) and Heintz et al. (1999, 2000) were key to data interpretation. Opposing the conclusions of Carls et al. (2002) are several industry-based researchers who discount these studies (Pearson 2002; Neff 2002; Page et al. 2002a).

Was the collapse of the PWS herring population also related to *Exxon Valdez* oil? Here industry and government answers were more similar. Industry researchers concluded increased biomass and decreased food supply were the most likely factors (Pearson et al. 1999). To these, government researchers added disease as an important factor, but could not rule out indirect links to the oil spill (Carls et al. 2002).

The purpose of this paper is to re-examine the controversy, review hydrocarbon toxicity research with particular emphasis on publications subsequent to the 2002 synthesis, and again examine the possibility that the 1993 population collapse was linked to the 1989 oil spill using new information on the long term consequences of PAH exposure.

Aromatic hydrocarbons and toxicity

Although well established is that the hydrocarbon composition of petroleum-based oils is related to toxic potential in aquatic ecosystems, the design of traditional aquatic toxicity tests has hampered recognition of the true toxicity of the most toxic components in oil, the aromatic hydrocarbons (Appendix 1). The earliest toxicity tests utilized short exposure regimens (typically 2 to 4 d) suitable only to detect effects caused by the highly volatile and rapidly toxic mononuclear aromatic hydrocarbons (MAH) such as benzene and its derivatives. The MAH act as narcotics in aquatic organisms, a reversible effect caused by the partitioning of hydrophobic chemicals into cell membranes and nervous tissue, disrupting the central nervous system (Barron et al. 2004). Relatively high aqueous concentrations (mg/L) are required to elicit these effects. Because acute toxicity tests are standardized and yield data quickly, predictive models based on narcosis are available and remain in use by regulatory agencies (e.g., Di Toro et al. 2000; Long et al. 2000; Fairey et al. 2001; Hansen et al. 2003) and by some petroleum industry researchers (e.g., McGrath et al. 2004; Page et al. 2002a; Neff 2002). Although the narcosis approach has validity in some instances, non-narcotic toxins with specific modes of action may be present, playing important or even dominant roles in organism responses. The PAH pose such a risk, particularly because they remain in aquatic environments longer than the more volatile MAH and are toxic at far lower concentrations. These toxic effects, however, frequently do not occur within the 4 d observation period of traditional acute toxicity tests.

Progressively higher molecular weight PAH are increasingly toxic (e.g., Anderson et al. 1974; Moore and Dwyer 1974; Rice et al. 1977; Hutchinson et al. 1980; Black et al. 1983; Neff 1985; 2002) but marine organisms are progressively less likely to respond rapidly (NRC 1985). Rather, effects of higher molecular weight PAH (such as cell damage, mutagenesis, teratogenesis, and cancer; Neff 1985), require prolonged observation to discern. Tricyclic PAHs (e.g., phenanthrenes) are highly toxic (Barron et al. 2004; Sundberg et al. 2005; Incardona et al. 2005) and have entered the environment through oil spills and as urban pollution for decades (Lima et al. 2003). By 1985, Birtwell and McAllister recognized that part-per-billion concentrations of crude oil cause a variety of lethal and sublethal effects in fish eggs and larvae, yet this report was not formally published until 2002. Key papers by Marty et al. (1997a), Carls et al. (1999), and Heintz et al. (1999; 2000) demonstrated that 0.4 to 18 µg/L total PAH dissolved from crude or weathered crude oil are toxic to Pacific herring and pink salmon (*Oncorhynchus gorbuscha*) embryos. These toxic concentrations were about three orders of magnitude smaller than anticipated by earlier, short-term tests using water-soluble fractions of oil (e.g., Rice et al. 1977) and are within the range of environmental exposures generated during oil spills (Birtwell & McAllister 2002; Fatima et al. 2002) including the *Exxon Valdez* oil spill (Neff & Stubblefield 1995; Short & Harris 1996). The source of dissolved oil used in the studies of Marty et al. (1997a), Carls et al. (1999), and Heintz et al. (1999) was water passed through oiled rock columns. Negative effects occurred in the absence of physical contact with oil (Carls et al. 1999, 2005; Heintz et al. 1999), demonstrating that toxicity may occur in the absence of oil coating and in areas removed from visible oil slicks and sheens.

Controversy

The finding that toxicity in fish embryos in long-term tests is related to low levels of dissolved oil constituents has been dismissed by industry researchers seeking alternative hypotheses at each toxicological level, including 1) spilled *Exxon Valdez* oil is not the source of toxic PAH (Page et al. 1996, 1999, 2002b; Wooley 2002; Brannon et al. 2006), 2) the observed embryotoxicity could not be caused by dissolved oil because a) effects require embryos to be in physical contact with particulate oil (Pearson et al. 1999; Brannon et al. 2006), b) the observed toxicity was caused by laboratory conditions peculiar to the exposure system such as the buildup of metabolic wastes (Pearson 2002; Brannon et al. 2006), oil degradation by bacterial metabolism (Neff et al. 2000; Page et al. 2002a; Brannon et al. 2006), or an artifact of experimental weathering (Page et al. 2002a; Brannon et al. 2006) and c) effects are not consistent with recognized narcotic action of oil (Page et al. 2002a), and 3) if real, the observed toxicity to herring embryos in 1989 could not be related to the subsequent population collapse four years later (Pearson et al. 1999).

The alternative sources of hydrocarbons in PWS suggested by some (Page et al. 1996, 1999, 2002b; Wooley 2002) neither explain the post-*Exxon Valdez* toxicity record nor diminish the importance of the Exxon spill. These include hydrocarbons from historical activity, oil spills caused by the 1964 earthquake, and a subtidal natural background that is apparently coal (Van Kooten et al. 2002), not Katalla seep oil as hypothesized by Page et al. (1996). Seep oil was undetectable in mussels 9 km from its source, <10% of the distance from Katalla to PWS (Short et al. 1999). Limited distribution (<0.2% of the shoreline was contaminated by historical activity; Boehm et al. 2004), long residence time (>25 y), and inert forms (coal) explain why hydrocarbon toxicity due to these sources was not evident at the time of the *Exxon Valdez* oil spill. Rather, *Exxon Valdez* oil was by far the most abundant and biologically active source of

PAH in PWS at the time of the spill and for many years thereafter because it persisted in intertidal sediment, a source of chronic exposure to various organisms such as mussels, pink salmon embryos, sea otters, harlequin ducks, and pigeon guillemots (Esler et al. 2000; Carls et al. 2001, 2004; Bodkin et al. 2002; Golet et al. 2002; Short et al. 2004). Some 15% of PWS shoreline was oiled by the *Exxon Valdez* spill and the amount of biologically available oil declined with time as the oil weathered, was removed, or became insulated from the environment by burial or other processes (Carls et al. 2001, 2004; Boehm et al. 2005). After 10 y *Exxon Valdez* oil concentrations in mussels had typically merged with background concentrations (Carls et al. 1999). Previously spilled oil, such as the Monterey oil spilled by the earthquake 25 y before the Exxon spill undoubtedly became unavailable to marine organisms by the same natural processes.

Alternative explanations to PAH toxicity include oil coating (Pearson et al. 1999), ammonia (Pearson 2002), and microbial metabolites (Neff et al. 2000; Page et al. 2002a; Brannon et al. 2006). The hypothesis that oil must adhere to developing eggs to cause negative effects, (Pearson et al 1999, Brannon et al. 2006) conflicts with a large body of literature that demonstrates significant oil toxicity in the absence of coating (e.g., Moore and Dwyer 1974; Neff and Anderson 1981; Grimmer 1983; Carls et al. 1999; Heintz et al. 1999; Kiparissis et al. 2003; Brand et al. 2001). Recently, Brannon et al. (2006) suggested that the toxicity of oiled-rock column effluent (the technique used by Marty et al. 1997a, Carls et al. 1999, and Heintz et al. 1999) is due to particulate oil. At most, particulate oil is a minor factor in such assays and although extensively examined, occurrence of particulate oil was not detected in (or on) eggs (59 samples) and does not explain adverse embryonic responses (Appendix 2). Pearson (2002) determined that ammonia and sulfide concentrations increase in oiled rock columns when the water is stagnant, but neither are detectable when water flows for a day or more. Water was never stagnant in any of the oiled rock column assays, eliminating ammonia and sulfides as potential toxic factors. Inferred from Pearson (2002), oxygen was at or near saturation in oiled rock column assays, consistent with our observations. At most, microbial metabolites explain <3% of the toxicity observed in oiled-rock column assays (Middaugh et al. 1996, 1998, 2002; Shelton et al. 1999; Carls et al. 2002; Appendix 3). Microbial degradation was not detected in the aliphatic hydrocarbon data from these experiments (Marty et al. 1997a; Heintz et al. 1999, 2000; Carls et al. 1999, 2005) even though straight-chain aliphatics are the most easily degraded of the hydrocarbons present. Finally, metabolites can be substantially less toxic (4 to 3000 times) than parent compounds (Hamdoun et al. 2002; Sepic et al. 2003).

Inconsistent with the claim of limited PAH toxicity, increased PAH contribution to toxicity was demonstrated by Neff et al. (2000) as oil was experimentally weathered. One oil was acutely toxic to silverside minnows (*Menidia beryllina*), mysid (*Americamysis bahia*), and shrimp (*Penaeus vannamei*) at <10 parts per billion total hydrocarbons (monoaromatics + PAH + phenols). Results of this experiment were subsequently misquoted by Page et al. (2002a) who asserted that toxicity declined with weathering and that PAH explained proportionately less of the total toxicity. Both of these statements are incorrect; toxicity consistently increased with weathering and the contribution of PAHs to toxicity increased with weathering. The results of Neff et al. (2000) are consistent with those of Carls et al. (1999) who also observed toxicity increased with weathering and attributed this increase to proportionately greater concentrations of higher molecular weight PAH. Weathered aqueous PAH are more toxic than unweathered counterparts because they contain proportionately more high molecular weight compounds.

Also controversial was whether the toxic effects of *Exxon Valdez* oil were sufficient to affect the herring population. Pearson et al. (1995; 1999) argued that the population was not affected; predicted and actual spawning biomass was consistent from 1990 to 1992, the 1984 year class remained dominant, and a strong 1988 year class developed. Carls et al. (2002) argued that oil toxicity damaged a significant proportion of the 1989 year class and probably reduced recruitment into the spawning population but observed the latter could not be quantified because recruitment of this year class was also low in other Alaskan herring stocks.

Review of contemporary toxicity research

Impacts of PAHs on fish embryos

Contemporary research continues to find that PAH are highly toxic, comparable to the toxicity observed earlier in Pacific herring and pink salmon (0.4 to 18 µg/L total PAH; Marty et al. 1997a; Carls et al. 1999; Heintz et al. 1999, 2000). Fathead minnows (*Pimephales promelas*) exposed to PAH leaching from oil sands died at <23 µg/L TPAH (Colavecchia et al. 2004). Japanese medaka (*Oryzias latipes*) hatch length was reduced at 2.2 µg/L when exposed to base neutral PAH extracts of oil-sand tailings (Rhodes et al. 2005). The lowest observed effective concentration causing edema was 22 µg/L. Swimming ability of Pacific herring larvae exposed to water-accommodated fractions of Alaska North Slope crude oil was impaired at 32 µg/L; when exposed to sunlight the effective concentration dropped to 14 µg/L (Barron et al. 2003). Herring embryos exposed to 17 µg/L developed edema (Barron et al. 2003). Emergent pink salmon growth was significantly depressed 6 months after exposure of embryos to <0.94 µg/L total PAH, a concentration less than that required to significantly induce cytochrome P4501A (Carls et al. 2005). Stress-induced morphologic lesions were observed in pink salmon (*Oncorhynchus gorbuscha*) fry exposed 10 d to 25 to 54 µg/L TPAH of the water-soluble fraction of Alaska North Slope crude oil (Brand et al. 2001).

Recently, industry researchers Brannon et al. (2006) strongly confirmed results of oiled-rock column assays, ascites in pink salmon embryos exposed to 7.8 µg/L TPAH (in contact with water only), and death at 16 µg/L. Despite this evidence, the authors concluded that dose can only be expressed as whole oil on rock or TPAH on rock, based on visual observation of oil droplets in the effluent and the assumption that this particulate oil was responsible for toxicity. Rather, the chemical evidence presented by Brannon et al. (2006) unambiguously demonstrated differential loss of smaller PAH molecules, a process inconsistent with substantive particulate movement. What is known is that aqueous TPAH concentrations, from which Brannon et al. (2006) did not exclude particulate oil, were about 2 to 3 orders of magnitude smaller than TPAH concentrations on rock, severely constraining the hydrocarbon concentrations experienced by the embryos, regardless of oil phase (particulate or dissolved). The assumption of Brannon et al. (2006) that particulate oil explains the results of the oiled rock column assays of Heintz et al. (1999) and colleagues is in error: chemical evidence (absence of highly insoluble phytane) demonstrates that no embryos in any oiled rock column assay were contaminated by particulate oil (Marty et al. 1997a; Heintz et al. 1999, 2000; Carls et al. 1999, 2005; Appendix 2). In contrast to the particulate toxicity conjecture, response to dissolved PAH has been demonstrated with retene in a partition-controlled assay (Kiparissis et al. 2003) and in many other pure compound tests such as those assembled by Di Toro et al. (2000). Thus, despite these issues, the actual results of Brannon et al. (2006) are in fundamental agreement with ours and other contemporary researchers.

Contemporary petroleum toxicity research is underpinned by the observation that dissolved oil constituents are toxic. For example, the model developed by Di Toro et al (2000) is derived from a large median lethal database containing multiple chemicals and species. The model is applied to PAH analysis using octanol-water partition coefficients; nowhere is particulate oil discussed or considered in this model. The same is true for the hazard index model developed by industry researcher Neff et al. (2005); soluble PAH are the predictors of toxicity. Although the short-term acute bioassay data used to parameterize these models (together with acute to chronic estimates) should be replaced with long-term chronic assay data, the basic premise is sound; PAH in solution are toxic. Despite reliance on acute data, Di Toro et al. (2005) estimated PAH toxicity was a few parts-per-billion (μg/L), consistent the previously cited literature.

Weathering

Weathering influences aromatic composition and composition controls toxicity, therefore total aromatic concentration alone does not adequately explain toxicity. Thus, earlier studies focused on monoaromatic hydrocarbons generally suggested toxic response occurred at parts-per-million concentrations (e.g., see summary by Rice et al. 1977). However, progressively higher molecular weight aromatics, including PAHs, are increasingly toxic (e.g., Anderson et al. 1974; Rice et al. 1977; Moore and Dwyer 1974; Hutchinson et al. 1980; Black et al. 1983; Neff 1985, 2002), partially explaining why PAHs and weathered PAHs are more toxic than generally understood in earlier work. In addition, marine organisms are progressively less likely to respond rapidly as molecular weight increases (NRC 1985); the effects of higher molecular weight PAHs (such as cell damage, mutagenesis, teratogenesis, and cancer (Neff 1985) require prolonged observation to discern compared to the narcotic response elicited by the lower molecular weight monoaromatic hydrocarbons.

Mechanisms of toxicity at the cellular level

Historically, PAHs have been treated as a common class of compounds and considered to either be narcotics (Di Toro et al. 2000, French-Mccay 2002, Barron et al. 2004) or to have dioxin-like toxicity mediated by activation of the aryl hydrocarbon receptor (AHR) (Bols et al. 1999, Billiard et al. 2002). Recent work demonstrates that the mechanisms of PAH toxicity are highly variable.

Acute, narcotic mechanism. At high concentrations (part-per-million), narcotic action is apparent (Di Toro et al. 2000). Narcotic toxicity is characterized by depression of the central nervous system and rapidly reversible effects (Barron 2002). Narcotic chemicals are not reactive in acute exposures and do not interact with specific receptors in an organism in acute exposures (type I narcotics), rather the mechanism is completely non-specific and potency is dependent on hydrophobicity (Verhaar et al. 1992). "Baseline narcosis is characterized by progressive lethargy, unconsciousness, and death without any specific sustained symptoms such as hyperventilation, erratic or convulsive swimming, or hemorrhage" (Veith & Broderius 1990). Because the toxic modes of action for type I narcotics are similar, they are considered additive (Verhaar et al. 1992, Di Toro et al. 2000). In the scheme proposed by Verhaar et al. (1992), the acute toxicity of less inert (type II or polar) narcotics is slightly greater than toxicity predicted by type I narcotics; increasingly reactive chemicals are classified as type III or type IV and effect concentrations for these chemicals can be 5 to 10^4 times lower than predicted by type I narcotic toxicity.

Reactive mechanisms. Reactive mechanisms cause physiological changes and may be very site specific, or may be generalized, such as when enzymatically produced oxygen free radicals react with proteins or DNA. Activation of AHR, cytoplasmic receptors (AHR1 or AHR2), initiates transcript of a battery of genes including CYP1A (there are at least two forms in fish, CYP1A1 and CYP1a2) that convert PAHs to water-soluble metabolites (Wassenberg & Di Giulio 2004, Incardona et al. 2006). For some PAHs (e.g., benzo[a]pyrene), as with the halogenated planar hydrocarbon dioxin (tetrachlorodibenzo-*p*-dioxin), these metabolites (such as epoxides, quinones, and peroxides) can cause cell damage (Livingstone 1991, Wassenberg & Di Giulio 2004). Reactive metabolites can covalently link with DNA to form mutagenic adducts, compromising replication fidelity and resulting in mutations (Perlow & Broyde 2001). The toxicity of dioxin is mediated by AHR and may be caused by CYP1A activity, demonstrated with knockout studies in mice where either AHR or CYP1A were rendered inoperative (Wassenberg & Di Giulio 2004).

The PAHs interact with specific receptors in fish embryos, thus at sub-narcotic doses at least three non-narcotic mechanisms prevail. Some of these require AHR and CYP1A activation, others do not (Incardona et al. 2004, Wassenberg & Di Giulio 2004, Incardona et al. 2005, Incardona et al. 2006). Cardiac damage caused by tricyclic PAHs are independent of the AHR, and properly functioning AHR pathways provide modest protection against these molecules (Incardona et al. 2004, Incardona et al. 2005). In contrast, some tetracyclic PAHs (pyrene and benz[a]anthracene) cause toxicity through the AHR pathway but the mechanisms of AHR-dependent toxicity vary (Incardona et al. 2006). Effects of benz[a]anthracene on cardiac function and morphogenesis are AHR2-dependent and CYP1A-independent (Wassenberg & Di Giulio 2004, Incardona et al. 2006). For pyrene, products from hepatic metabolism apparently circulate to cause damage or systemic effects result from altered liver function secondary to CYP1A-mediated hepatotoxicity (Incardona et al. 2006).

In addition, PAHs are likely to interfere with steroid metabolism because CYP1A is involved in the oxidative metabolism of endogenous compounds such as arachidonic acid, prostaglandins, and steroids (Wassenberg & Di Giulio 2004). Thus, induction of CYP1A by PAHs can suppress steroids; 3-methylcholanthrene and β-naphthoflavone, for example, reduced vitellogenisis in juvenile rainbow trout (Navas & Segner 2000). Effects of steroid suppression by PAHs in fish embryos is apparently unknown; however, embryo-larval exposure to estradiol can induce vitellogenin production and bias sex-ratios toward females (Edmunds et al. 2000, Koger et al. 2000, Brion et al. 2004), demonstrating hormones can be influenced with discernable results during embryonic development. Other PAHs toxicity mechanisms, including cancer, immune suppression, and genetic damage are discussed separately.

Multiple PAHs toxicity mechanisms suggest that additive toxicity models are not appropriate predictors of toxicity (Wassenberg & Di Giulio 2004, Incardona et al. 2006). This is the most likely reason the toxic units approach of Di Toro et al. (2000) failed to accurately predict PAHs toxicity in Pacific herring and pink salmon assays (Carls et al. 1999, Heintz et al. 1999, Barron et al. 2004). Although toxic equivalency may be used to predict carcinogenic potential (which is mediated by AHR activation and CYP1A metabolism), this approach fails to predict early life stage toxicity in fish. This type of model predicts that chryene is more toxic than pyrene, yet despite robust CYP1A induction, chrysene has no discernable impact through at least the first 5 d post-fertilization in zebrafish (Incardona et al. 2006). The specific tissues impacted by AHR activation are apparently more relevant to toxicity than the overall levels of pathway activation. Patterns of CYP1A induction are tissue-specific and may depend on active

circulation. Incardona et al. (2006) conclude that these data do not support models that assume lipids are non-specific targets for PAHs, resulting in narcotic-like toxicity (Di Toro & McGrath 2000, Di Toro et al. 2000). Rather current models of PAH toxicity in fish are greatly oversimplified; the biological effects of PAHs cannot be predicted simply by quantitative measures of AHR activity or a compound's hydrophobicity. Individual PAHs are pharmacologically active compounds with distinct and specific cellular targets, thus should be considered as individual compounds or subfamilies of compounds with specific activities (Incardona et al. 2006).

Sensitivity of fish embryos to PAHs

The biological complexity of embryonic development further contributes to PAH toxicity, resulting in adverse effects at part-per-billion concentrations, and explaining the general observation that early life stages are highly vulnerable to PAHs and other pollutants, more so than later life stages (e.g., Moore & Dwyer 1974). The toxicity of PAHs begins with interference with subcellular processes. Embryonic toxicity occurs when these subcellular processes are affected, causing effects on short and long (or delayed) time scales at concentrations that may be very low compared to effective dose levels in later life stages. The embryonic development process is very complicated and very dependent on what happened in previous developmental stages. In adult systems, cellular damage can be repaired, or at worst, functioning cells in affected organs can compensate for cell loss. The loss of a single, rapidly differentiating cell in an embryo may be considerably more important. Damage may disrupt gradient-driven cell migration and differentiation, potentially affecting many organ systems, leading to immediate, secondary, and delayed effects (Carls et al. 2005). Cellular damage may possibly be passed to daughter cells, also negatively affecting organ systems. Simply slowing the development of some differentiating cells with respect to others or interference with normal intracellular hormonal signaling may be sufficient to cause developmental problems. Random damage throughout the development process may result in different macroscopic abnormalities; alternatively, damage to critical organs can cause secondary damage in other tissues. For example, cardiac conduction was impeded by exposure of zebrafish embryos exposed to dibenzothiophenes and phenanthrenes, resulting in secondary effects on cardiac morphogenesis, kidney development, neural tube structure, and formation of the craniofacial skeleton (Incardona et al. 2004). The relative importance of primary and secondary effects on embryo development remains open for further study. Changing metabolic profiles as various enzymes are activated or repressed may also influence embryonic sensitivity during development (Miller et al. 1996). Permanent, multiple defects are likely to have lasting consequences, such as poorer growth and marine survival (Heintz et al. 2000).

Tools originally developed for genetic study provide one important means of understanding the specific mechanisms of PAH damage in developing embryos; conversely, perhaps PAH toxicity will provide a tool that leads to fundamental progress in understanding embryonic development. The damage caused by PAHs, expressed phenotypically in embryos, can be compared to mutant phenotypes, such as the silent heart mutant in zebrafish to infer specific pathways of damage (Incardona et al. 2004). Even more specifically, anti-sense oligonucleotides (morpholinos) can be experimentally injected into embryos to block specific gene products, thus allowing parallel experimentation with PAH toxicity and genetic manipulation in embryos. This approach has lead to considerable recent understanding of PAH

toxicity mechanisms (Incardona et al. 2004, Incardona et al. 2005, Incardona et al. 2006) as outlined in this section.

Abnormalities in fish embryos

Exposure to PAHs causes embryonic abnormalities in many fish species; compared to other effects, abnormalities are the most visible and most documented responses. For example, Incardona et al. (Incardona et al. 2004, Incardona et al. 2005) demonstrated that zebrafish exposed to PAHs develop characteristic abnormalities including edema, cardiac dysfunction, and spinal curvature (Fig. 5). These are the same abnormalities observed in the petroleum assays of pink salmon, rainbow trout (Fig. 6), Pacific herring (Fig. 4), mummichogs, and fathead minnows (Marty et al. 1997a, Carls et al. 1999, Couillard 2002, Colavecchia et al. 2004, Sundberg et al. 2005). Alaska North Slope crude oil also caused intercranial hemorrhaging in zebrafish, an effect not observed in pure compound tests (Incardona et al. 2005) and not observable in herring embryos because blood does not become pigmented as early in development. Hemorrhages were observed in mummichogs, fathead minnows, and rainbow trout (Couillard 2002, Colavecchia et al. 2004, Sundberg et al. 2005). Dibenzothiophene and phenanthrene influence cardiac conduction, affecting subsequent embryo development (Incardona et al. 2004). Blood circulation was impaired by about 65 µg/L total PAHs from Alaska North Slope crude oil; assays to determine minimum effective concentrations were not completed (Incardona et al. 2005). Median effective concentrations of retene (7-isopropyl-1-methylphenanthrene) causing blue sac disease in Japanese medaka was 10 µg/L (Kiparissis et al. 2003). Blue sac disease is a syndrome consistent with response to other PAHs, including CYP1A induction, yolk-sac edema, hemorrhaging, craniofacial deformities, and mortality (Billiard et al. 1999). (To avoid confusion with pathogen-caused disease, we suggest the term 'blue-sac disease' be discontinued in the literature and replaced by 'edema' or 'ascites.') Many different kinds of substances can produce the same kinds of embryo abnormalities, though the actual mechanisms and phenotypic expression vary (Weis & Weis 1987, Incardona et al. 2004).

Phototoxicity

Ultraviolet light exposure of transparent embryos with PAH-contaminated tissue can significantly increase PAH toxicity. Some species deposit eggs or larvae into very shallow habitats; these early life stages are often very transparent to reduce predation. However, this survival strategy may make such species more vulnerable to photoenhanced toxicity when PAHs sequestered in tissues are activated by ultraviolet light (radiation between 280 and 400 nm). For example, PAH toxicity increased 1.5 to 48 times in herring larvae briefly exposed to sunlight (about 2.5 h/d for 2 d; Barron et al. 2003). The environmental relevance of this phenomenon is questioned by some (e.g., McDonald & Chapman 2002) who argue that phototoxicity is ameliorated by physical, chemical, and biotic factors, i.e., attenuation of ultraviolet radiation in the water column and by animal pigmentation. Others counter that damaging intensities of ultraviolet light can sufficiently penetrate water to meaningfully contribute to embryo toxicity (Barron & Ka'Aihue 2001, Diamond et al. 2006). Conclusions in both of the latter studies were based on actual solar radiation measurement. Phototoxic components in crude oil include 3- to 5-ring PAHs and heterocycles (e.g., dibenzothiophene). Because the potential for damage in the natural environment can be underestimated if based only on laboratory assays completed in the absence of ultraviolet light (the general case), photoenhanced toxicity should be considered as a risk factor for relatively translucent animals that inhabit the photic zone and intertidal areas.

Microbes and metabolites

Microbial action may contribute to PAH toxicity (Middaugh et al. 1996, Middaugh et al. 1998, Shelton et al. 1999, Hamdoun et al. 2002, Middaugh et al. 2002), though effects may primarily be indirect rather than caused by increased metabolite toxicity. In most of the cited papers (Middaugh et al. 1996, Middaugh et al. 1998, Shelton et al. 1999, Middaugh et al. 2002), the authors conclude microbial metabolites are more toxic than parent PAHs, yet each assay was confounded by increased total PAH concentration in microbially-amended treatments. As a result of microbial activity, concentration of the water-soluble fraction of oil increased, probably as a result of surfactant secretion, thus increasing the surface area of the weathered oil and allowing more compounds to dissolve. Aqueous hydrocarbon concentration explains the majority of the toxicity reported by Shelton et al.(Shelton et al. 1999); survival of grass shrimp was correlated with the water-soluble fraction concentration ($r^2 = 0.72$, $P = 0.007$; our analysis, Appendix 3) and the type of hydrocarbons degraded, alkane or aromatic, did not appear to influence the resultant toxicity. The response of inland silversides (*Menidia beryllina*; Middaugh et al. 2002) was almost entirely explained by changes in the water-soluble fraction concentration ($0.96 \leq r^2 \leq 0.98$; our analysis). In the study by Hamdoun et al. (2002), soluble hydrocarbon concentrations as a result of microbial action were also higher (14 times) than in oil-water incubated without microbes; they concluded that non-degraded water-soluble fractions were significantly more toxic (about 10 times) than biodegraded water soluble fractions of crude oil because of increased concentration. Microbial growth was promoted in each of these tests by static conditions, warm water temperature, and nutrient amendment. In contrast, significant microbial growth was unlikely in the cold-water oiled rock column assays previously cited that demonstrate high PAH toxicity to fish embryos (Marty et al. 1997a, Carls et al. 1999, Heintz et al. 1999, Heintz et al. 2000, Carls et al. 2005), indicating that microbially mediated transfer of hydrocarbons to water is not crucial to PAH toxicity (Carls et al. 2002). Rather, microbial action may be an important contributor to toxicity only in limited environmental circumstances, such as natural oil seeps (Hamdoun et al. 2002) and the primary mechanism may be to increase bioavailability to other organisms by increasing the rate PAHs enter solution.

Immune function

Although immune function has been extensively in humans and other mammals, less effort has been directed towards fish and we are aware of only one publication concerning immunotoxicity, PAHs, and fish embryos. In that study, rainbow trout embryos were exposed for 30 minutes to 0.5 mg/L aflatoxin B_1 (a PAH associated with hepatic carcinogenesis and immunomodulation in vertebrates and transformed into a biologically active epoxide by cytochrome P450 enzymes) (Ottinger & Kaattari 2000). In the following two years, leukocyte proliferation and immunoglobulin production were greater in previously exposed fish when stimulated with mitogens than in control fish, suggesting that PAHs may pose a significant long-term health threat to fish (Ottinger & Kaattari 2000).

Based on life stages older than embryos, exposure to immunotoxins can either boost or depress immune function, depending on the compound, dose, time of exposure, and experimental procedures (Smith et al. 1999, Reynaud & Deschaux 2005). PAH-induced changes in immune function have been reported in many animals, including fish, mollusks, earthworms, birds, and mammals (including humans) (Trust et al. 1994, Smith et al. 1999, Arkoosh & Collier 2002, Carlson et al. 2002a, Ma & Ma 2002, Rodriguez et al. 2002, Komiyama et al. 2003, Wootton et

al. 2003, Auffret et al. 2004). Increased immune function might explain hormesis, a commonly observed experimental situation where small doses of a toxin are apparently beneficial (Calabrese 2005). PAHs may mimic the intracellular pathway to lymphocyte activation (Davila et al. 1995). 3-Methylcholanthrene inhibited lymphocyte proliferation and stimulated peripheral blood leukocyte proliferation in parallel with CYP1A induction in carp (*Cyprinus carpio*) (Reynaud & Deschaux 2005). Immunotoxic response in tilapia (*Oreochromis niloticus*) to benzo(a)pyrene parallels that in rodents (Smith et al. 1999). Immune function in Japanese medaka and chinook salmon (*O. tshawytscha*) was reduced by PAH exposure and exposed fish were more susceptible to disease (Arkoosh & Collier 2002, Carlson et al. 2002b). Chemicals such as PAHs that cause immunosuppression have the potential to adversely impact populations (Arkoosh & Collier 2002, Reynaud & Deschaux 2005). The big question is, how long can immune systems remain compromised?

Immune deficiencies increase the susceptibility of offspring to infection and may persist for long periods, ≥18 mo in mouse offspring (Rodriguez et al. 2002, Jedrychowski et al. 2005). We are unaware of any reports concerning the persistence of immunosuppression in fish and recommend that this be tested, particularly in light of the resultant long-term alteration in leukocyte proliferation and immunoglobulin production when trout embryos were exposed to aflatoxin B_1. Protracted immune damage suggests increased susceptibility to disease; this was apparent in newborn and infant humans exposed during gestation (Jedrychowski et al. 2005).

Cancer

Cancer can be a long-term outcome of exposure to PAHs, which are known carcinogens, cause oxidative DNA damage, and result in DNA adducts. For the most part, higher molecular weight PAHs (4- through 7-ring), such as benzo[a]pyrene are responsible for this damage (Grimmer & Misfeld 1983, Neff 2002). Considerable time can lapse between cause and effect. For example, Wales et al. (Wales et al. 1978) found that a single 1-hr exposure of rainbow trout (*O. mykiss*) embryos to the precarcinogen aflatoxin B_1 induced a significant number of liver tumors in fish 9-12 months later. Neoplasms were observed 2 and 3 years after brown bullhead (*Ictalurus nebulosus*) exposure to PAH-contaminated sediment (Baumann & Harshbarger 1998). DNA replication fidelity can be compromised by damaged DNA, resulting in mutations (Perlow & Broyde 2001). These are clearly significant long-term problems with the potential to negatively affect fish populations, yet we are unaware of any such reports in fish exposed to *Exxon Valdez* oil, the most studied spill in the world. Failure to focus research on this issue is a possible reason for this. Alternatively, relatively few carcinogenic PAHs (> 3 rings) are present in a crude oil such as *Exxon Valdez* oil (about 1 to 3% of the total PAH). These large PAHs are more insoluble than PAHs with fewer rings, thus aqueous concentrations of molecules such as chrysenes are consistently low in field and experimental research with *Exxon Valdez* oil. The total incidence of summed concentrations > 0.1 µg/L of PAHs with >3 rings was 5% in experimental studies (n = 131) and 14% in water samples from Prince William Sound (n = 236; Short et al. 1996).

Genetics

The longest conceivable potential impact of PAHs on marine organisms may be genetic alteration leading to reduced fitness in subsequent generations. There are at least four possibilities, 1) that somatic DNA damage or mutations reduce the fitness of the exposed generation, thus impacting reproduction and immediate offspring, 2) that genetic diversity is

reduced by exposure to PAHs, 3) that toxin(s) apply selection pressure, causing populations to adapt, and 4) that DNA damage or mutations in reproductive tissue are passed to subsequent generations.

Somatic damage can clearly lead to abnormalities, reduced fitness, population effects, and extinction but the contribution of genetic damage to this scenario is unclear. For example, oil-induced mutations at a mutational hotspot (K-*ras*) were observed in pink salmon (Roy et al. 1999). This gene is of potential phenotypic importance; the K-*ras* protein is involved in inter- and intracellular messaging and in the regulation of cell division and differentiation. However, the functional importance of this damage and the potential for intergenerational transmission remains unknown. Chromosomal aberrations caused by exposure to PAHs have been observed in a diversity of organisms (Fig. 8) (Carls et al. 1999; Krishnamurthi et al. 2003), including humans (Bocskay et al. 2005). Chromosomal aberrations, measured by comet analysis, were observed in rats after consumption of fuel oil-contaminated mussels but growth (15 to 30 d) was not affected (Lemiere et al. 2005).

Genetic diversity can be reduced by exposure to PAHs. Meiobenthic populations in sediment contaminated by offshore oil platforms lost mitochondrial DNA diversity; harpacticoid copepods had smaller egg sizes, fewer juveniles and females, and decreased larval survival (Carr et al. 1996, Montagna & Harper 1996, Street & Montagna 1996). In a follow-up study, Street et al. (1998) demonstrated that genetic diversity in harpacticoid populations (*N. lacustris*) can be reduced by exposure to phenanthrene-contaminated sediment. Haplotype diversity decreased when reproductive output decreased; surviving females were apparently more tolerant and conferred this resistance to their offspring.

Adaptive selection pressure may explain why some organisms become more resistant to PAHs. Mummichogs from a PAH-contaminated section of the Elizabeth River, Virginia, USA, are genetically distinct from those at other sites on the river and are more tolerant of PAH exposure (Ownby et al. 2002; Mulvey et al. 2003). Fathead minnow populations continuously exposed to sublethal fluoranthene concentrations developed increased resistance to the toxicant, apparently the result of differential egg production by susceptible and tolerant fish (Diamond et al. 1995). Such genetically-based adaptation is likely to be long-lived, but the ability to adapt to other stressors may be impaired. Such competitive disadvantages have been demonstrated in plants (Hickey & McNeilly 1975, Wilson 1988).

Genetic transmission of damaged or mutated DNA caused by spills such as the *Exxon Valdez* oil spill seems generally unlikely, particularly if phenotypic expression is detrimental. Rather, adaptation is more likely to result from selective advantages conferred on tolerant fish. Genetic effects may be self-limiting; natural selection will remove lethal mutations or limit them to low frequencies (Cronin & Bickham 1998).

Ecosystem

The effects of PAHs in natural ecosystems may be compounded by other stressors such as limited food supply, predators, temperature, salinity, light, and other xenobiotics. As previously discussed, the interaction of the ultraviolet portion of sunlight and PAHs can dramatically increase toxicity, increasing risk for transparent life forms in shallow habitats. Other stresses may be more difficult to avoid. For example, little food is available to Pacific herring over winter, requiring them to utilize stored fat supplies. Individuals with insufficient energy reserves perish (Paul et al. 1998) and utilization of lipid resources mobilizes PAHs sequestered in this fat. Combined starvation and PAH stress have been inadequately studied (Hylland et al. 1996,

Richardson et al. 2004). Predator-prey interaction can be modified by pollutants; substandard prey are more vulnerable to predation, for example (Kruzynski & Birtwell 1994, Mesa et al. 1994). Predation success can also be reduced; darter goby (*Gobionellus boleosoma*) consumed fewer copepods after 24 h exposure to diesel-contaminated sediment (Gregg et al. 1997). The combined effects of toxicant and predator-induced stress not only affect survival but can have disadvantageous sublethal consequences. Swimming ability of herring larvae exposed to PAHs as embryos is reduced (Carls et al. 1999), thereby reducing the ability to avoid predators or capture food, hence decreasing survival potential. The combined stress of PAHs and disease causes mortality in fish, e.g., Pacific herring (Carls et al. 1998) and rainbow trout (Springman et al. 2005). For example, only rainbow trout exposed to infectious hematopoietic necrosis virus died, not those exposed only to β-naphthoflavone, demonstrating the contaminant effect was indirect (Springman et al. 2005).

Prediction of ecosystem effects from laboratory data clearly can be complicated by multiple factors, some acting to increase effects, others antagonistically. The latter effect was noted in brown trout (*Salmo trutta*); embryonic response to ammonia plus a PCP and PAH mixture was less than response to either ammonia or PCP/PAH alone (Luckenbach et al. 2003). We recommend continued multi-factor experimentation to elucidate such relationships and improve predictive models.

Population

Population level effects provide the ultimate measure of meaningful response to contaminants because it integrates all effects from all mechanisms across all life stages. Through a variety of mechanisms, human activity has accelerated the rate of species extinction worldwide (Mora et al. 2007), elevating the importance of this topic.

Few studies have attempted life cycle tests starting with embryonic exposure to PAHs. For many species, such research would be too lengthy and not logistically feasible. However, the consequences of pink salmon embryo exposure to PAHs were studied after the *Exxon Valdez* oil spill in Prince William Sound, Alaska, both in natural and experimental settings. Mortality of pink salmon embryos spawned in oiled intertial streams was elevated (compared to that in reference streams) and modeling indicated that 2 million fewer adult salmon returned (Bue et al. 1996, Geiger et al. 1996). Later, controlled laboratory exposures confirmed low PAH doses could mimic the elevated embryo mortalities at a few part-per-billion aqueous total PAH concentrations (Heintz et al. 1999, Heintz et al. 2000, Carls et al. 2005). In follow-up experiments, thousands of visually normal marked fry produced from control and PAH-exposed embryos were released into the north Pacific environment, to feed, grow, avoid predators, mature, and return to their natal stream 1.3 years later. Returning adults were sorted according to their exposure history. At 19 parts per billion total PAH, 40% fewer adults returned compared to marked controls; at 5 parts per billion total PAH, 20% fewer adults returned (Fig. 3). The outcome of this experiment, which has been verified in three different brood years, is important because it confirmed that part-per-billion total PAH doses can result in fewer returning adult fish, even when damage was not visible at the time of release. This is good evidence that embryonic exposure to low PAH doses can have a population effect.

Experimenting across many generations to mimic chronic population effects is generally not practical but modeling can address this complexity. Fish populations where embryos are chronically exposed to PAHs can be driven to extinction. Modeling demonstrates that the probability of pink salmon population extinction within a stream system increases with increased

PAH concentration (Heintz in press). Density-dependence compensated for reduced productivity at low exposure concentrations but the probability of extinction increased as toxic concentrations increased because random environmental variation overcame this compensatory mechanism. Under these conditions, pink salmon population extinction is possible at 18 μg/L (11% probability within 35 generations); extinction is virtually certain at 30 μg/L (Heintz in press). Modeling suggests population effects are more likely for fish with short life spans, rapid maturation, frequent spawning, and parental guarding (such as the round goby, *Neogobius melanostomus*) than anadromous salmonids (Spromberg & Birge 2005).

Over long periods, even no-observed effect concentrations can increase the likelihood of population extinction (Snell & Serra 2000) as contaminants will always take place with the concurrent pressures of natural selection (climate, predators, prey availability). If PAHs cause immunosuppression in a fraction of the population, the entire population may be more vulnerable. Arkoosh et al. (1998a) have demonstrated that juvenile Chinook salmon in a contaminated estuary are immunosuppressed and that mortality exceeds that in juveniles from clean locations when both are challenged with the marine pathogen *Vibrio anguillarum*. Increased susceptibility thus may alter the relationship among host, pathogen, and environment sufficiently to affect the population (Arkoosh et al. 1998a,b). Although specific interactions among disease, pollutants, and population impacts is unknown (Arkoosh et al. 1998b), reductions of fish populations in many urban estuaries have occurred for some time and PAH exposures are likely to be part of the problem (e.g., Arkoosh et al. 1998b, Johnson et al. 1998, Keiter et al. 2006).

Discussion

Interpreting the complex results of hydrocarbon toxicity testing across experiments is challenging (Appendix 4), a problem that can be compounded by researcher assumptions. Contributing to the complexity of PAH toxicity research are a wide variety of dosing methods, assay procedures, responses, life stages, and species. For example, acute assays are better suited to test rapidly acting toxins that cause mortality; chronic assays typically measure a broader range of responses over longer observation periods. A narcotic toxicity model that seeks to account for many of these variables for the purpose of establishing water quality guidelines (Di Toro et al. 2000) makes one of these fundamental assumptions, that PAH toxicity is narcotic and short-term acute lethality assays appropriately describe organism response. This model fails to adequately explain the PAH toxicity observed in oiled-rock column assays (e.g., Carls et al. 1999; Heintz et al. 1999) as summarized by Barron et al. (2004). The failure is not an experimental failure, but rather an assumption failure: exclusive use of 4 day acute toxicity data to build the narcotic model completely misses non-narcotic and latent responses (Zhao and Newman 2004) caused by PAH with specific modes of action (Appendix 5). The idea that different chemicals have different modes of action is not new (e.g., Weis and Weis 1987). Clearly toxicity research must encompass the entire range of possible organism responses to be relevant. Exposure duration, intensity, and latent effects that are manifest after exposure must be observed in assays or ecologically relevant information is lost (Zhao and Newman 2004). Environmental law based on inadequate research or faulty assumptions is unlikely to adequately protect.

Implications for PWS herring

The high toxicity of PAH to fish embryos continues to support the original conclusions of Natural Damage Assessment researchers that the *Exxon Valdez* oil spill damaged exposed Pacific herring embryos in 1989, the year of the spill (Brown et al. 1996; Hose et al. 1996; McGurk and Brown 1996; Norcross et al. 1996; Marty et al. 1997b; Carls et al. 2002). At the time of the previous herring review by Carls et al. (2002), the estimated PAH toxic range was 0.4 to 18 µg/L (Marty et al. 1997a; Carls et al. 1999; Heintz et al. 1999, 2000). Estimates reported in the last few years closely overlap the previous range, <0.94 µg/L to 32 µg/L (Billiard et al. 1999; Barron et al. 2003; Kiparissis et al. 2003; Rhodes et al. 2005; Carls et al. 2005). This new research provides solid evidence that particular investigators, techniques, or species are not responsible for unusually low toxicity estimates. Rather, observation of both lethal and sublethal responses over sufficient periods of time identify PAH as highly toxic to fish embryos. The best available evidence supports the hypothesis that Pacific herring embryos were damaged by *Exxon Valdez* oil in the year of the spill.

The potential for *Exxon Valdez* oil to cause immunosuppression in Pacific herring is supported by contemporary research. Immunosuppression in adult herring was observed experimentally by Carls et al. (1998) and is a common effect in a wide variety of animals. The proportion of the population sufficiently exposed to elicit immunosuppression is also unknown. How long immunosuppression might persist in wild herring populations was and is unknown. Unfortunately no disease-related data were collected between 1989 and when then population collapsed in 1993. However, there was no evidence that population immunity was compromised by oil. Rather, spawning biomass increased through 1992. Immune function in embryos and larvae was never measured in wild PWS herring. The plausibility of these immunocompromised life stages surviving to maturity and recruitment is unlikely. Viral hemorrhagic septicemia outbreaks occur in age 0 Pacific herring when stressed by confinement; resultant mortality be >50% (Kocan et al. 2001). In contrast, few age 1 herring die under similar confinement, suggesting immunity and that disease stress is typically encountered early in life. Natural selection probably weeds out impaired individuals well before recruitment. Winter starvation events may remove weak fish each year. The best available estimate is that oil-induced immunosuppression was not a major contributor to the 1993 collapse.

Pacific herring habitat did not retain oil, thus reducing the likelihood of oil-related effects in years after the spill. In PWS, water was the principal exposure route for herring (all life stages) to *Exxon Valdez* oil (Carls et al. 2002). Aqueous PAH concentrations peaked shortly after the spill and declined during or shortly after herring egg incubation (Neff and Stubblefield 1995; Short and Harris 1996; Carls et al. 2002). Unlike the situation for pink salmon, oil reservoirs did not persist in herring habitat. Overlap between spawning grounds and oiled shoreline was limited, sharply reducing the potential for embryonic damage in subsequent years (Pearson et al. 1999; Carls et al. 2002). Oil stranded on intertidal shoreline in PWS slowly dissipated with time and became increasingly less biologically available (Carls et al. 2001, 2004). Although a portion of this oil dissipated into the water column, the dilution factor represented by the immense volume of water filling the PWS basin was responsible for maintaining low, background level aqueous PAH concentrations. Aqueous PAH concentrations declined to background before autumn 1989 and remained there (Neff and Stubblefield 1995). Thus, long-term exposure of herring and to oil was unlikely. By 1990, most data indicated that residual oil impacts (PAH uptake and physiological responses) were undetectable in embryos

and larvae (Carls et al. 2002). Strong recruitment of the 1988 year-class (in 1991) signaled the post-spill recovery of the herring population in PWS.

Zooplankton biomass, the presumptive food supply of Pacific herring in PWS, declined after the spill but there is little possibility that oil was causal. Zooplankton biomass peaked in 1984 and reached a minimum in 1992 before rebounding (Brown, personal communication). The general zooplankton decline was in progress prior to 1989, strongly suggesting natural causes were responsible. Celewycz and Wertheimer (1996) were unable to find oil-related reductions in zooplankton biomass, density, or diversity in either 1989 or 1990. Rather, the density of opportunistic harpacticoid copepods increased in oiled areas, probably due to organic enrichment (Celewycz and Wertheimer 1996; Laur and Haldorson 1996; Wertheimer et al. 1996). Negative impacts were reported for oil-sensitive amphipods within and adjacent to eelgrass beds (Jewett et al. 1999). Copepods and euphausiids are principal prey for herring (Hart 1973) and although amphipods are also consumed, we suspect oil-caused changes in amphipod abundance were too limited to explain declines in herring body mass.

High Pacific herring abundance, poor nutrition and subsequent susceptibility to disease provide one possible natural explanation for the 1993 population collapse, one that does not require a mechanism to explain delay between a toxic event and response (Pearson et al. 1999; Carls et al. 2002; Brown personal communication). Pacific herring size-at-age and zooplankton biomass are apparently related, suggesting that natural changes were responsible for the population collapse in 1993. Declines in size at age lagged behind declines in zooplankton biomass, suggesting a classic predator-prey relationship. The nutritionally stressed population was vulnerable to increased disease, a well-known phenomenon. By comparison, competing hypotheses that require long term oil-caused immunosuppression and continuous disease in segments of the herring population seem contrived. See Chapters 2 and 5 for further discussion.

In conclusion, there are no known direct or indirect oil-related explanations for the herring population collapse in 1993 and there are no known oil-related explanations for failure of the population to rebound. The only factor that cannot be ruled out is long-term (multi-year) immunosuppression, an unlikely, but understudied possibility. This conclusion is consistent with that of Pearson et al. (1999) and Carls et al (2002), who suggested factors not related to oil (population size, disease, and nutrition) were the most likely factors for the collapse. Thus, despite the direct toxic damage observed in the first year of the spill, oil-related toxicity does not explain the population collapse 4 y later and continued recruitment failure.

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Chapter 2

Evidence that the *Exxon Valdez* oil spill did not cause the 1993 disease epidemic in the Pacific herring population of Prince William Sound, Alaska

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Abstract

Several lines of evidence support the conclusion that the *Exxon Valdez* oil spill had significant but minor population affects in 1989 that were no longer detectable after 1990. In retrospect, the conclusion best supported by the evidence is that strong recruitment of the 1988 year-class marked population recovery in 1991 from the toxic effects of the spill. By 1993 that recruitment—plus the addition of 1 to 2% of the population because of failure to conduct the fishery in 1989—helped precipitate a catastrophic disease outbreak. Epidemiological analysis identifies three significant risk factors for the 1993 population crash: 1) relatively large biomass from 1988 – 1992, augmented by the lack of a fishery in 1989 (i.e., susceptible host); 2) relatively low zooplankton production in 1991 and 1992 (i.e., environmental conditions contributing to poor overwinter condition); and 3) the presence of VHSV and filamentous bacteria. The effects of that outbreak continue to cycle through the population, preventing recovery 13 years later.

Keywords – Pacific herring, *Clupea pallasi*, Prince William Sound, *Exxon Valdez* oil spill, disease epidemic

Introduction

The Pacific herring population of Prince William Sound, Alaska, supported productive commercial fisheries throughout much of the 20th century. As the Pacific herring population grew and markets expanded in the 1980s and early 1990s, the 5 commercial fisheries harvested an average annual ex-vessel value of about $8.3 million. Commercial harvest of Pacific herring was prohibited in 1989 as a result of the March 24, 1989 *Exxon Valdez* oil spill. Commercial harvest of Pacific herring resumed from 1990 – 1992, but ended in early 1993 when a severe disease outbreak killed 50 to75% of the population (Meyers et al. 1994). Through 2006, commercial harvest was limited to brief re-openings from fall 1996 – fall 1998, but the population never recovered to pre-crash levels. Preliminary surveys provide evidence that the 2006 population is the lowest since the oil spill.

Worldwide, disease outbreaks are frequently reported in fish populations, and reports of these outbreaks are increasing (Harvell et al. 1999; Sherman 2000). Disease involves poorly understood interactions between three major variables: the host, the pathogen, and the environment (Hedrick 1998); the mere presence of a pathogen is not equivalent to disease. For example, viral hemorrhagic septicemia virus (VHSV) and *Ichthyophonus hoferi* have been identified in several different species along the west coast of the United States and Canada

(Meyers and Winton 1995; Hedrick et al. 2003), but we have little information on why these pathogens cause a disease outbreak in a given population in any given year.

The Pacific herring population of Prince William Sound has been the subject of the most comprehensive disease study ever conducted on a fish population. For nine years (1994 – 2002), 230 – 400 adult Pacific herring were sampled each year and subjected to complete necropsy that included virus isolation from individual fish and complete histopathology of 10 organs. The main diseases significant at the population level were caused by (1) VHSV and filamentous bacteria, and (2), *Ichthyophonus hoferi*. Risk factors for a VHSV outbreak include acute exposure to weathered crude oil (Carls et al. 1998), abundant young fish (3-year-olds) recruiting into the spawning population, and depletion of fat stores at the end of the winter (Marty et al. 2003). VHSV was isolated from spring samples in 7 of 9 years (range = 1 – 15% prevalence). By comparison, all 710 samples collected in the fall from 1995 – 2001 were negative for VHSV. Prevalence of *I. hoferi* consistently increases with age (Marty et al. 2003), but reasons for the abnormal increase in *I. hoferi* prevalence from fall 2000 through spring 2001 are unknown. Fish probably die from VHSV-ulcers in late winter and from *I. hoferi* during the summer.

The proximity of the 1989 *Exxon Valdez* Oil Spill to the 1993 disease outbreak leads to the question: was the 1993 population crash and failure to recover a result of the *Exxon Valdez* Oil Spill, or was the crash a result of population and environmental factors independent of toxicity from *Exxon Valdez* oil? The purpose of this report is to present evidence based on standard epidemiological principals that natural interactions of the host (Pacific herring), environment (food availability in 1992), and pathogens (viral hemorrhagic septicemia virus and filamentous bacteria) are sufficient and compelling reasons to explain the 1993 population crash. Further, despite broad study of Prince William Sound Pacific herring from 1989 to 1992, there is no conclusive evidence of oil-induced damage to Pacific herring beyond 1990.

Discussion

Evidence of toxicity from *Exxon Valdez* oil

Evidence for exposure and toxicity of *Exxon Valdez* oil in Pacific herring has been reviewed for larvae and juveniles (Brown et al. 1996), adults (Marty et al. 1999), and with respect to the 1993 disease outbreak (Carls et al. 2002). All life stages were exposed to oil 1989, but evidence of exposure to oil after 1989 is limited to eggs in a few highly contaminated beaches in 1990. Therefore, significant population-level oil exposure ceased by late 1989 or early 1990. The link to the 1993 crash requires that some type of permanent or repressed damage be carried in the population from 1990, not to be expressed until early 1993.

Was the 1993 disease outbreak a result of oil-induced immunosuppression?

While it is tempting to attribute the disease outbreak to oil-induced immunosuppression, the minimum 3-year lag between last exposure (1990) and disease outbreak (1993) is unprecedented in the scientific literature. Fish tissues including eggs readily metabolize crude oil, and trophic-transfer bioaccumulation of crude oil fractions is not significant. No case has ever been reported in which exposure to toxins like crude oil results in significant population level decline 3 years after toxicant exposure ends. Indeed, no such case has ever been reported with more than a 1-year lag from exposure to population decline. Pacific herring recovering from VHSV infections are immune to re-exposure, and a carrier state for VHSV has not been documented (Kocan et al. 2001). A preliminary study provided some evidence that in 1992 reproductive success of Pacific herring from the 1988 year-class spawning in previously oiled

areas was less than in previously un-oiled areas; however, histopathology of these fish provided evidence that lesions related to poor reproductive success were not related to previous oiling of the spawning sites (Kocan et al. 1996). Further, the 1993 disease outbreak affected all year classes equally, independent of their age at the time of the oil spill (Marty et al. 1998). By 1995, there was no evidence of significant reproductive effects in Pacific herring born before the oil spill (Johnson et al. 1997).

Why did the Prince William Sound population crash and continue to have disease problems, while all other Pacific herring populations have no disease problems?

Population decline related to disease is not unique to the Prince William Sound population. During February and March of 1942, "several thousands of tons" of Pacific herring were found dead along the southeast coast of Vancouver Island, British Columbia, Canada (Tester 1942). The epidemic near Vancouver Island involved the dominant 1938 year-class (4-yr-olds) that probably was at about the same level of maturity as the dominant 1988 year-class (5-yr-olds) in Prince William Sound in 1993. The description by Tester (1942) is very similar to the 1993 VHSV-ulcer outbreak in PWS. Puget Sound used to support several commercial Pacific herring fisheries, but biomass in many of these fisheries has declined in the past 30 years, and inhibition of maturation of Pacific herring in some populations has been attributed to increased mortality from *I. hoferi* infection (Hershberger et al. 2002). Some Pacific herring stocks in British Columbia are currently depressed, but the populations have not been analyzed for the role of disease. Major population crashes like the one in Prince William Sound are sporadic and nearly impossible to predict, but they are not unprecedented and they do occur independent of crude oil spills.

Was the 1993 disease outbreak a result of oil-induced alteration in food supply?

No evidence has been presented to support the hypothesis that oil toxicity decreased primary production (photosynthesis and algae) and their consumers (e.g., copepods), particularly in 1992, the year before the disease outbreak and the year when fish needed to store energy to get through the winter of 1992-1993.

Evidence for host and environmental variables independent of *Exxon Valdez* oil

The best population biomass estimates by the Alaska Department of Fish and Game in the early 1990s predicted near-record biomass returns in 1993 after relatively high biomass from 1988 – 1992 (Fig. 2.1). Based on the basic epidemiological principle that the risk of an infectious disease outbreak increases with population size, the population was at increased risk in any of the years from 1989 – 1993. This conclusion is essentially in agreement with study supported by Exxon (Pearson et al. 1999).

Was the 1993 disease outbreak a result of limited food availability in 1992?

Based on zooplankton data and our current understanding of disease outbreaks in Pacific herring, 1993 was the year at greatest risk for a catastrophic disease outbreak. Limited data on zooplankton availability provides evidence that both 1991 and 1992 were relatively poor years for zooplankton production, but 1992 zooplankton production was less than any other year (Fig. 2.2). These data are consistent with fish lacking sufficient food resources to build energy stores to get through the winter. Although we lack data on energy stores of fish in 1993, data from moderate VHSV-ulcer outbreaks in 1994 and 1998 support the hypothesis that disease occurs

when a large proportion of the population has complete depletion of abdominal fat stores (Marty et al. 2003).

Did failure to conduct the fishery in 1989 contribute to the 1993 disease outbreak?

Based on analysis of available data, failure to conduct the fishery 1989 increased the risk of an infectious disease outbreak because of increased population size through the early 1990s, including 1992. All evidence published in the scientific literature supports the conclusion that the *Exxon Valdez* Oil Spill killed fewer adult Pacific herring in 1989 than would have been harvested by the commercial fisheries. Therefore, survivors of the fish that were not harvested probably were 1-2% of the stressed population entering the winter of 1992-1993. The 1989 harvest quota for all Pacific herring commercial fisheries in Prince William Sound was 16,775 metric tons (source: Alaska Department of Fish and Game). If we ignore the possibility that some of these fish were killed by exposure to *Exxon Valdez* oil or a related VHSV-ulcer outbreak in 1989, an estimate from harvest ratios and natural mortality yields as much as 2500 metric tons of fish were still alive in late 1992 (Table 2.1). This number can be adjusted downward in a linear ratio based on the estimate for oil-related adult mortality in 1989. For example, 10% of adult Pacific herring sampled from oiled sites in 1989 had hepatic necrosis (Marty et al. 1999), and this lesion has been associated with VHSV expression and mortality in controlled laboratory studies (Kocan et al. 1997; Carls et al. 1998). A reasonable estimate of oil-VHSV-ulcer mortality in 1989 is half of the fishing quota. This leads to an estimate of about 1250 metric tons of older fish still contributing to population biomass in late 1992.

Which was more stressful to the fish: the 1989 oil spill or the 1993 disease outbreak?

Based on analysis of pigmented macrophage aggregates in the livers of the 1988 and 1994 year-classes, the 1993 disease outbreak resulted in significant cellular membrane damage whereas the 1989 oil spill was undetectable. Pigmented macrophage aggregates are irregularly spherical structures that normally occur in the liver, kidney, and spleen of fish (Fournie et al. 2001; Agius and Roberts 2003). They increase with age, toxicant exposure, disease, starvation, and nutritional imbalances. The volume of Pigmented macrophage aggregates in the livers of Pacific herring from Prince William Sound were compared from the 1988 year-class (1992 – 1999; n = 574) and the 1994 year-class (1996 – 2002; n = 578). In both the 1988 and 1994 year classes, mean volume of pigmented macrophage aggregates was consistently low in young fish, increased relatively quickly over a two-year period as each year class entered the spawning population, and then increased slowly as the year classes aged (Fig. 2.3). However, the magnitude of the increase with first spawning was twice a large in the 1988 year-class as in the 1994 year-class, consistent with the severe 1993 disease outbreak experienced by the 1988 but not the 1994 year-class (see Fig. 2.3, age 6, spring).

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Table 2.1. Calculation of estimated biomass of Pacific herring not fished in 1989 that were still alive before the fishery in 1993. Calculations are based on the ratio of actual harvest reported by the Alaska Department of Fish and Game, the prespawning total biomass estimates in Fig. 2.1, and a constant natural mortality of 25% (calculated after fishing mortality). All numbers other than years are in metric tons.

Year	Prespawning survivors	Harvest	Prespawning total biomass	Survivor's fishing mortality[a]	Postfishing survivor biomass[b]	Survivor natural mortality[c]
1989	16775[d]	0	107510	0	16775	4194
1990	12581	13069	94972	1731	10850	2712
1991	8137	20222	90602	1816	6321	1580
1992	4741	26479	94046	1335	3406	852
1993	2555					

[a]1990 example: (13069/94976) * (12581) = 1731

[b]1990 example: 12581 – 1731 = 10850

[c]1990 example: 10850 * 0.25 = 2712

[d]Harvest quota set by the Alaska Department of Fish and Game and not fished; all other numbers in this column are calculated by subtracting fishing mortality and survivor mortality from prespawning survivors in the previous year. 1991 example: 12581 – 1731 – 2712 = 8137

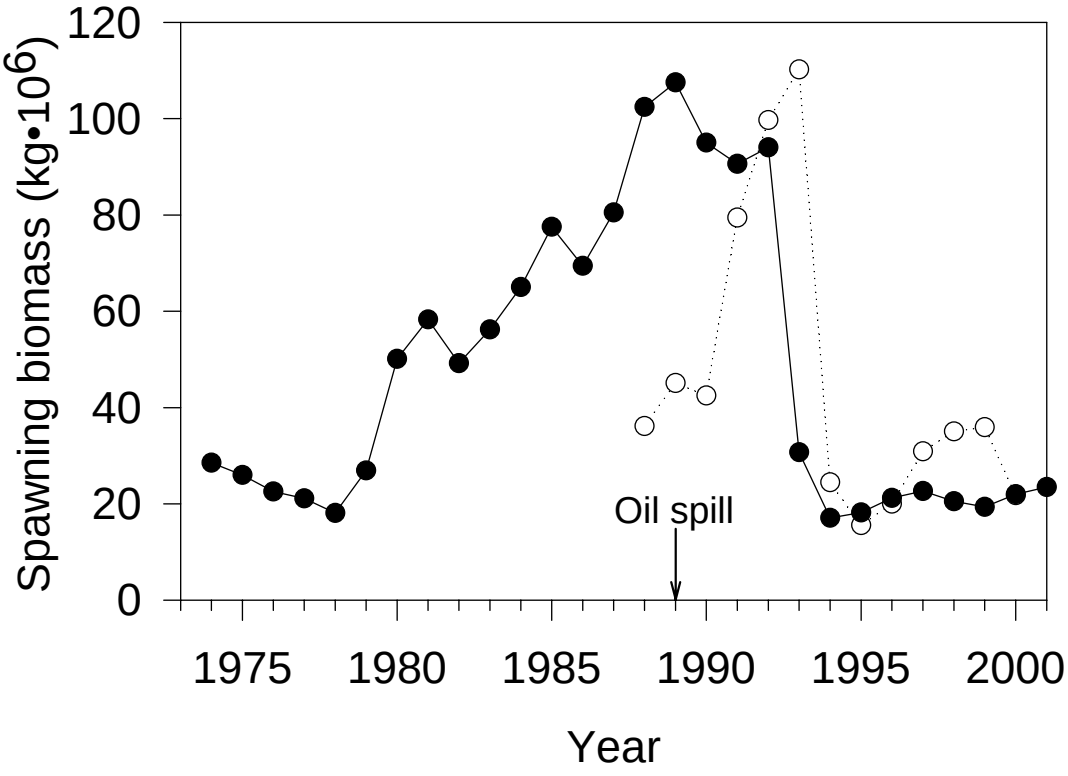

Fig. 2.1. Biomass estimates of mature Pacific herring in Prince William Sound, Alaska. Unexploited spawning biomass projected in the year before spawning (○; source, Alaska Department of Fish and Game, Cordova, Alaska) and calculated after spawning (best estimate, ●) using an age structured assessment model modified by a disease index after 1993 (Marty et al. 2003).

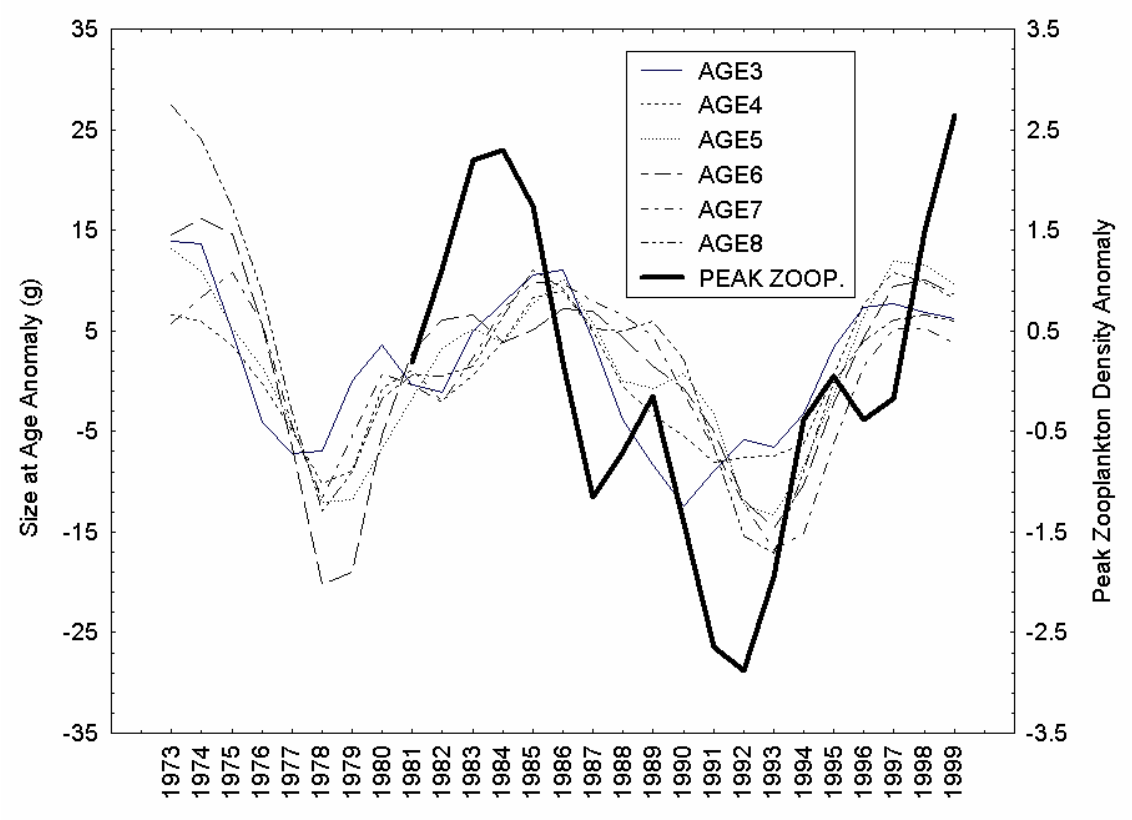

Fig. 2.2. A time series of weight-at-age from the Alaska Department of Fish and Game, and the peak zooplankton biomass from the plankton watch program sampling in southwestern Prince William Sound (graph provided by Evelyn Brown, Institute of Marine Science, University of Alaska Fairbanks, Fairbanks, AK 99775-7220).

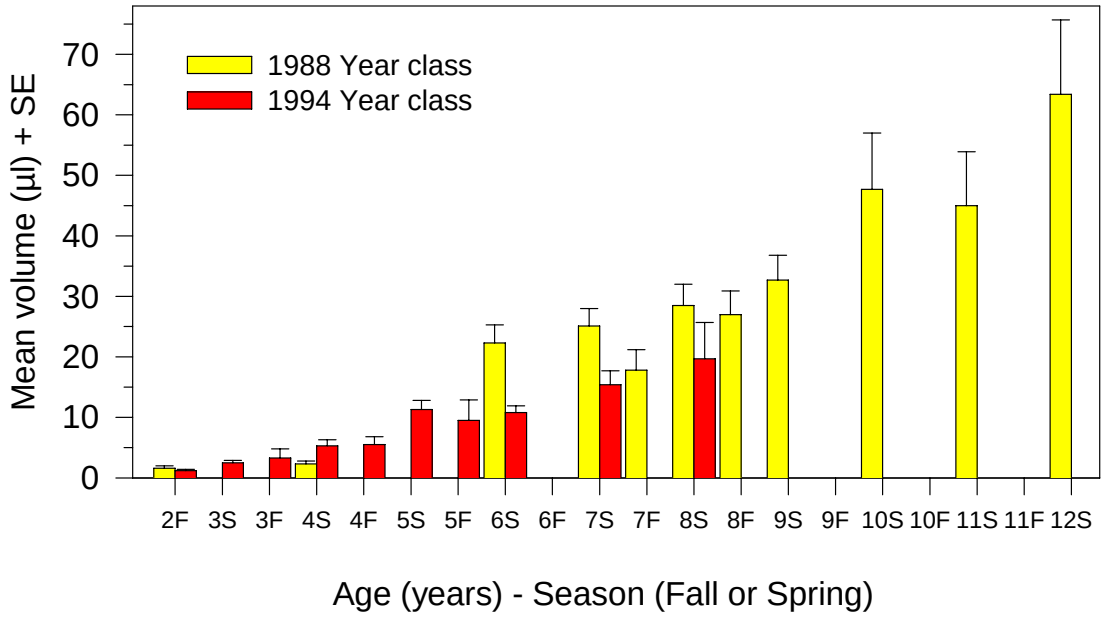

Fig. 2.3. Volume of pigmented macrophage aggregates in livers of Pacific herring sampled from Prince William Sound, Alaska, from 1990 to 2002.

Chapter 3

Is Recent Pacific Herring Recruitment in Prince William Sound, Alaska, Unusually Low Compared to Recruitment Elsewhere on the West Coast of North America?

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Abstract

Following a population crash in 1993, Prince William Sound herring experienced very low recruitment from the 1995 through 1998 year classes. This run of low recruitments was unique on the Pacific coast. However, four-year to six-year runs of low recruitment have occurred at other times in other herring populations. The recruitment events of the 1990s broke down what had been an extremely strong correlation between PWS and Sitka recruitments. Prince William Sound has experienced 3 modest recruitment events since the 1993 crash (the 1993, 1994, and 1999 year classes), but biomass has yet to increase above low levels. Strong recruitment from the lowest biomass levels has not been observed at PWS or Prince Rupert, but five of the ten examined herring populations (Togiak, Sitka, Craig, Queen Charlotte Islands, and West Coast of Vancouver Island) have generated extremely strong recruitment events from the lowest biomass levels. While the low recruitments from the 1995 to 1998 year classes are within the range of natural variability, recovery of PWS herring will require further recruitment events, combined with increased adult survival from disease and other sources.

Keywords: Pacific herring, recruitment, population crash, Prince William Sound

Introduction

In the spring of 1993, reports of unusual herring behavior and conditions surfaced among fishermen awaiting an expected large harvest in the Prince William Sound (PWS) Pacific herring (*Clupea pallasi*) sac roe fishery. Test fishing then began showing fish with lesions, and unusual schooling patterns; both fishermen and biologists flying aerial surveys noted a dramatically less than expected showing of herring schools.

Subsequent studies revealed that viral hemorrhagic septicemia virus (VHSV), associated ulcers, and the fungus-like organism *Ichthyophonus hoferi* cause the major diseases in Pacific herring, and that VHSV and associated ulcers probably contributed most to the population decline in 1993 (Meyers et al. 1994; Marty et al. 1998). PWS Pacific herring fisheries were abruptly curtailed in 1993, and were never opened in 1994 or 1995. Herring abundance has remained at relatively low levels. Only two small harvests have been allowed since the crash of 1992-1993, in 1997 and 1998. Debate continues among scientists about how much of the unusual condition of PWS herring can be attributed to the 1989 Exxon Valdez oil spill (Marty et al. 1998, Pearson et al. 1999).

Concurrent with the increased prevalence of the two pathogens during the 1990s, recruitment to the herring population appeared to be unusually low. This paper examines the historical and contemporary information about herring recruitment in PWS, contrasting it to recruitment patterns elsewhere on the west coast of North America, to determine whether the low

post oil-spill recruitment patterns are unique to PWS, or whether they are within the expected range of natural variability.

Methods

The longest possible time series of recruitment and spawning biomass were synthesized from available sources. Williams and Quinn (2000) had previously distilled the available historic herring stock assessment information into long time series of recruitment, with year class strength in some cases dating back to 1917. However, the most recent year class analyzed by Williams and Quinn (2000) was 1993. Contemporary stock assessment documents and consultation with Pacific coast herring stock assessment scientists were used to extend Williams and Quinn's (2000) information through the 2001 year class. Alaska reference populations included for contrast with PWS are Togiak (Bristol Bay), Sitka, Craig, and Southeast Alaska combined. To the south, recruitment and spawning biomass information was compiled for Prince Rupert, Queen Charlotte Islands, Central B.C. Coast, Strait of Georgia, and the West Coast of Vancouver Island in British Columbia, and for Cherry Point in northern Puget Sound. San Francisco Bay herring information was also reviewed, but is not included in these recruitment comparisons.

The historic time series of recruitments is examined for patterns and trends graphically and using a Wald-Wolfowitz runs test (Bradley 1968) for non-randomness in the sign of deviations from the median of the time series. Anomalies in the time series are compared among areas using normalized deviates of log recruitment within each time series. "Strong" year classes are defined as those in the upper 15^{th} percentile, corresponding to a normalized deviate exceeding 1. This definition generally agrees with common perception of the large year classes that occasionally occur north of Vancouver Island. Similarly, "weak" year classes are defined as the lower 15^{th} percentile, corresponding to normalized deviates less than –1.

Recruitment information is then cast into spawner-recruit form, using the time series of spawning biomass for each area. Because of the strong time-series correlations in these spawner-recruit datasets, it would be inappropriate and misleading to reduce the spawner-recruit pairs to a "Ricker" or other simplistic mathematical model to describe the relationship of spawners to recruits. More complex models incorporating environmental data and properly treating the time-series nature of these data are possible, but are beyond the scope of this initial analysis. Recruitment patterns plainly evident in the graphical analysis of the suite of areas are not so subject to misleading interpretation and are sufficient for the conclusions that need to be drawn regarding recent Prince William Sound herring recruitments.

Prince William Sound

The time series of spawning biomass (1980 to 2004) and resulting age 3 recruits for PWS was drawn from Hulson et al. (2006). The herring population model in their age-structured approach derives the best fit of abundance time series to all the available stock assessment information: a time series of age compositions, spawn deposition surveys, aerial milt surveys, and hydroacoustic surveys. The incorporation of hydroacoustic information is a recent addition to the PWS stock assessment approach, but ended up having little effect on the abundance time series, even over a broad range of weights for the acoustic information (Hulson et al. 2006). This recruitment analysis uses spawning biomass and age 3 year class strength from Hulson et al. (2006) where hydroacoustic information is included in the stock assessment model with a weight (lambda) of 0.5. To assemble the longest possible time series, age 3 recruits for the 1925-55 and 1970-76 year classes were taken from Williams and Quinn (2000), while Hulson et al. (2006) were used as the source for the 1977- 2001 year classes. There is no stock assessment information available for the 1956 through 1969 year classes in PWS.

Togiak

An age-structured model (Funk et al. 1992) has been used for annual stock assessments in the Togiak district of Bristol Bay for over a decade. The model synthesizes test and commercial catch age compositions, catch, and aerial survey biomass estimates. Spawning biomass and recruitment time series were drawn from 2004/2005 stock assessments (West 2005, Lowell Fair, Alaska Department of Fish and Game, Anchorage, personal communication). Herring recruits are tracked beginning at age 4 in the Togiak stock assessments. The time series in these contemporary assessments spans 1977 to 2005, or the 1973 through 2001 year classes. The 1969-1972 year class strengths were taken from Williams and Quinn (2000).

Southeast Alaska

Age-structured assessment models are used for the major spawning locations in Southeast Alaska. In this analysis, assessments for the two largest spawning aggregations, Sitka and Craig, are used, spanning the years 1971 through 2005, corresponding to the 1968 through 2002 year classes. 1971 through 1997 data was taken from Carlile et al. (1999), 1998-2003 from Dressel et al. (2005), 2004 from Pritchett (2005), and 2005 from Davidson et al. 2005). These contemporary time series were combined with older historic time series from Williams and Quinn (2000), dating back to 1917.

British Columbia

Long time series of data on age composition, catch, and abundance are integrated into stock assessment models for the principal spawning locations in British Columbia, dating back to the 1948 year classes (Schweigert 2004). These data completely replaced the historical time series given in Williams and Quinn (2000). When specified, the age of recruitment is taken to be two years in British Columbia.

Cherry Point (Washington)

Abundance is estimated annually for herring spawning near Cherry Point, though a formal stock assessment model is not used. Point estimates of abundance are derived from spawn deposition surveys and acoustic surveys. Cherry Point herring are unique in coastal Washington because of their extremely late spawning season. They are also genetically distinct from other Washington herring, as well as from British Columbia herring (Stick 2005). The Cherry Point time series extends back to 1973, with recruitment at age 2, so that the data series encompasses the 1971 through 2002 year classes.

San Francisco Bay (California)

San Francisco Bay herring were tentatively included in this research pending a review of stock assessment information. Age-structured assessment models were used in a preliminary peer review of the San Francisco Bay herring management (Dewees and Leet, 2003), but age compositions and age-structured models are not yet routinely used for California herring assessment. Also, the age composition of the catch has shifted towards younger individuals over time, likely as a result of changes in fishery selectivity (Dewees and Leet, 2003). At present there are essentially no individuals aged 6 years or older in the catch, while in earlier years these ages made up over 50% of the catch (Dewees and Leet, 2003). Because the fishery may have been targeting younger fish in recent years, the view of year class strengths could be distorted. The San Francisco Bay herring population has been reduced to a level of roughly 20% of the unfished level and is presently at or near the lowest abundance observed since the early 1970s. High fishery exploitation rates may also have affected the stock productivity. Because these factors are not comparable to other west coast herring populations, San Francisco Bay herring were not

considered further in this analysis.

Results

The range between the weakest and strongest year classes spans two to three orders of magnitude at all areas (e.g. from 9 to 1,338 million recruits in PWS; Table 3.1). For the more northerly areas, strong recruitment events tended to occur in a single year or a pair of back-to-back strong year classes, separated by a run of years with low recruitment (Figs. 3.1 and 3.2). For the most southerly areas (Strait of Georgia and Cherry Point), stronger recruitments tended to occur over a 10-15 year series of adjacent years, with no intervening runs of low recruitment. The Strait of Georgia and Cherry Point herring in particular, almost completely lack the very strong isolated recruitment "spikes" that characterize herring from the more northerly regions. Because of these patterns and the different life histories, Strait of Georgia and Cherry Point herring were not compared further to the more northerly herring populations like PWS.

Togiak

For Togiak, the northern-most herring population examined, herring have had strong year classes about every 10 years between 1969 and 2001 year classes (Fig. 3.1). In the Bering Sea, herring are larger (likely an adaptation to long-distance migration), and have longer reproductive lifespans (approximately 12 years) than in the Gulf of Alaska (approximately 7 years). Togiak's two strongest year classes (1977 and 1978) were generated from the two lowest observed spawning biomasses (Fig. 3.3). The 1993 to 2001 spawner-recruit pairs are approximately in the middle of the joint distributions (solid red circles of Fig. 3.3).

PWS

In PWS, strong year classes generally occurred at shorter intervals (4-6 years), until recently, perhaps in conjunction with the shorter lifespans of Gulf of Alaska herring. The last strong year class observed was spawned in 1988. Subsequently, recruitments fell to lower levels, with the 1995 through 1998 year classes being some of the smallest on record. However, there have been 3 "modest" recruitment events observed since the 1993 crash: the 1993, 1994, and 1999 year classes, with estimated sizes of 151, 193, and 224 million recruits.

In terms of spawner-recruit, the recent year classes (1993-2001) are in the far lower-left of the spawner-recruit space (Fig. 3.3). No large year classes have been observed from spawning biomasses in the lower range of spawning biomass in PWS, although this pattern is not uncommon in Pacific herring. The four largest year classes assessed (1980, 1981, 1984, and 1988) were spawned from biomasses double to four times the recent biomass.

Southeast Alaska

At Sitka, the 1993-2001 spawner-recruit pairs are spread over a very broad range of spawning biomass, with mostly moderate recruitment throughout the time period (Fig. 3.3). However, very poor recruitment resulted from the largest year class (2001) on record. The largest year classes (1984 and 1988) occur in the middle of the spawning biomass range.

Like Togiak, Craig has produced its two largest year classes from the two smallest spawning biomasses (1984 and 1985), and has the 1993-2001 spawner-recruit pairs in the lower middle of the spawner-recruit space. The Southeast Alaska areas do not show extremely low abundance of the 1995 through 1998 year classes, as in PWS.

A longer time series of year class strengths was generated by Williams and Quinn (2000) using data from the older herring reduction fisheries in Southeast Alaska and PWS, dating back to 1917 for Southeast Alaska and 1925 for PWS. These older data were not area-specific or as high-quality as the modern data, but this Southeast Alaska pooled data displays similar patterns

to PWS through the 1988 year class (Fig. 3.1).

British Columbia

Herring spawning along the northern and central coasts of British Columbia show the same episodic near four-year cycle as in PWS and Southeast Alaska (Figs. 3.1 and 3.2). However, unlike PWS and Southeast Alaska, the spiked recruitment trends continued up to the present time. Occasional single year classes were weak between 1995 and 1998, but this rare occurrence was not unusual, and there was no indication of a four-year span of low recruitment, as occurred in PWS.

Herring in southern British Columbia (Strait of Georgia and West Coast of Vancouver Island have very different recruitment patterns (Fig. 3.2), perhaps resulting from different oceanographic conditions and life history strategies. Herring recruitment appears cyclic on a much longer scale, and there are runs of strong back-to-back year classes, not individual strong year "spikes", as in the more northerly areas.

At Prince Rupert, the largest recruitment events are produced from the largest year classes, with 1993-2001 year classes around the medians of the spawner-recruit space. The Queen Charlotte Islands has a very strong year class (1952) produced from low biomass. The 1993-2001 observations occur at the lower left of the spawner-recruit space. The Central B.C. coast has a single very strong year class (1952) near the median spawning biomass. Like Sitka, the 1993-2001 recruitments are spread over the larger biomass observations. The Strait of Georgia spawner-recruit observations (Fig. 3.4) look remarkably similar to Prince Rupert (Fig. 3.3), except that the 1993 to 2001 observations are near the larger end of the spawning biomass. For the west coast of Vancouver Island, 1993-2001 spawner-recruit pairs are spread over lower third of the spawning biomass range. Three moderate year classes (>400 million recruits) occurred during this time period.

Cherry Point (Washington)

Cherry Point herring also do not exhibit single-year spikes of strong year classes, similar to the adjacent southern British Columbia herring in the Strait of Georgia. However, recent genetic studies have suggested that the Cherry Point herring stock is genetically distinct from other Washington and British Columbia stocks (Beacham et al. 2002, Small et al. 2004). Cherry Point recruitment has fallen to extremely low levels since 1993, similar to PWS. The 1993-2001 spawner recruit observations are in the lower left of the spawner recruit space at Cherry Point (Fig. 3.4). Coupled with lowered adult survival (Stick 2005), Cherry Point herring are constrained to very low abundance levels.

PWS-Sitka Recruitment Synchrony

Recruitment patterns are remarkably similar between PWS and Southeast Alaska (older, longer time series) and particularly Sitka, where area-specific data is available after 1964 (Fig. 3.1). Both Sitka and PWS had nearly identical 4-year cycles of recruitment from the 1976 year class through the 1988 year class. Using the better quality assessment data beginning in 1980, the correlation between these two data sets is extremely high (r^2=0.95) up until the PWS population crash of 1993 (Fig. 3.5). In fact, the simplest possible model of y=x is reasonable and most parsimonious for these data. Orthogonal residuals from a geometric fit of this simple model indicate the extent to which the 1995-1998 recruitments diverged between PWS and Sitka after the period of high correlation (Fig. 3.6).

Recruitment Anomalies

The recruitment time series normalized to historical medians all displayed fewer than expected runs of positive and negative values. For Southeast Alaska ($p < 0.01$) and, to a lesser

extent, PWS ($p < 0.06$) these longer runs tested as significantly nonrandom, but this outcome might reasonably be expected by chance given that 11 time series were examined. Normalized deviates of log recruitment (Figs. 3.7 and 3.8) demonstrate that the four-year (1995-1998) negative recruitment anomaly is clearly unique to PWS, but is not unique in time or geography. In Southeast Alaska, there was one six-year negative recruitment anomaly (1958-1963), one five-year (1917-1921), and, in the area-specific data, one five-year (1965-1969) at Sitka. Although only PWS displays the strong negative recruitment anomaly from 1995-1998, no Alaska areas examined have shown very strong positive recruitments since 1990.

Discussion

Concern over fluctuating herring populations in PWS is not new (e.g. Rounsefell and Dahlgren 1931). However, the magnitude and duration of the current low herring population event in PWS is unprecedented in historical times. In PWS, year class strength held up with at least occasional strong year classes through the early 1990s. It was adult mortality that caused the catastrophic population decline observed in 1993. On the heels of the adult mortality event, a low recruitment phenomenon also occurred in the late 1990s, contributing to the very low current population level.

For Prince William Sound, the Queen Charlotte Islands, and Cherry Point, the 1993-2001 spawner-recruit information occurs almost entirely at the lower-left of the spawner-recruit space. In all other areas, the majority of the 1993-2001 spawner-recruit pairs are near the medians of the respective ranges or higher. If there is a linkage in productivity affecting recruitment among these widely disparate herring populations, it is not a simple matter of geography.

If herring exhibit density-dependent recruitment, a critical element of herring life history strategy would be to obtain a significant recruitment event within a reproductive lifespan, so that a strong year class can replace itself. When this fails to happen, it can be viewed as a knife-edge process which may trap a population at low levels for extended periods, especially for species which may require threshold abundance levels for social behavior leading up to spawning, as seems reasonable for Pacific herring. It may be predicted that herring populations with longer reproductive lifespans (e.g. Bering Sea) should exhibit longer runs of recruitment failures without risk to the population than areas with shorter lifespans.

Periods of lowered survival continue to occur in PWS herring, such that stock assessment models now incorporate explicit external information about disease (Quinn et al. 2001, Hulson et al. 2006). When elevated adult mortality is coupled with a dangerously low run of recruitments as occurred in PWS from 1995 through 1998, the population could be at risk of catastrophic failure. Fortunately for PWS, this run of low recruitment ended with the modestly strong recruitment event of the 1999 year class, before the duration of low recruitment had exceeded the reproductive lifespan of the remaining herring. Recovery of PWS herring will require further strong recruitment events, combined with increased adult survival from disease and other sources.

Conclusions

1. PWS was the only herring population examined to have a four-year run of low recruitments from the 1995 through 1998 year classes
2. Four-year to six-year runs of low recruitment have occurred at other times in other herring populations, so that recent recruitment in PWS is within the range of natural variability.
3. Prince William Sound has experienced 3 modest recruitment events since the 1993 crash (the 1993, 1994, and 1999 year classes), but biomass has yet to increase above low levels.

Recovery of PWS herring will require further recruitment events, combined with increased adult survival from disease and other sources.

4. Strong recruitment from the lowest biomass levels has not been observed at PWS or Prince Rupert, but five of the ten examined herring populations (Togiak, Sitka, Craig, Queen Charlotte Islands, and West Coast of Vancouver Island) have generated extremely strong recruitment events from the lowest biomass levels.

5. Recruitment patterns and life histories of Strait of Georgia and Cherry Point herring are not comparable to more northerly herring populations like PWS.

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Table 3.1. Recruiting year class strength (millions) for herring stocks along the west coast of North America, for the 1917-2001 year classes. Boxed values have been updated from Williams and Quinn (2000) from recent stock assessments.

Year Class	Togiak	Prince William Sound	Sitka	Southeast Alaska	Craig	Prince Rupert	Queen Charlotte	Central Coast	Vancouver Island	Strait of Georgia	Cherry Point
1917				8							
1918				45							
1919				19							
1920				40							
1921				55							
1922				171							
1923				238							
1924				164							
1925		122		2080							
1926		301		908							
1927		210		296							
1928		247		856							
1929		189		266							
1930		1338		2125							
1931		116		172							
1932		104		161							
1933		83		219							
1934		908		581							
1935		782		167							
1936		145		45							
1937		194		24							
1938		220		77							
1939		31		283							
1940		131		192							
1941		220		536							
1942		126		225							
1943		462		869							
1944		104		153							
1945		113		149							
1946		64		305							
1947		155		151							
1948		20		80		470	148	323	291	1131	
1949		18		103		270	76	129	303	1149	
1950		202		211		386	159	233	444	1211	
1951		22		93		581	954	1113	772	1921	
1952		428		1718		166	167	91	326	1097	

Year Class	Togiak	Prince William Sound	Sitka	Southeast Alaska	Craig	Prince Rupert	Queen Charlotte	Central Coast	Vancouver Island	Strait of Georgia	Cherry Point
1953		9		113		375	131	100	580	520	
1954		22		73		119	65	195	450	577	
1955		38		73		230	62	279	552	548	
1956				603		453	190	285	682	1026	
1957				1010		122	39	88	419	720	
1958				94		747	171	165	242	369	
1959				70		371	191	460	595	1089	
1960				87		188	365	299	307	967	
1961				23		520	105	242	389	909	
1962				16		71	338	106	137	586	
1963				118		57	7	193	123	169	
1964			442	442		64	21	153	113	190	
1965			54	54		35	30	28	56	41	
1966			28	28		36	38	22	80	74	
1967			14	197	184	150	73	90	324	236	
1968			20	132	37	111	155	98	614	262	
1969	4		17	161	13	54	192	124	467	209	
1970	13	354	118	310	43	210	421	202	565	252	
1971	1	130	14	202	18	134	341	122	606	427	0.2
1972	81	117	17	66	11	119	323	176	1011	605	0.5
1973	214	110	3	21	6	67	82	71	429	388	0.2
1974	239	82	85	145	5	111	145	57	283	795	6
1975	21	146	123	161	3	58	143	59	501	574	14
1976	51	890	575	640	11	56	73	41	166	298	1
1977	847	231	134	1186	567	389	1163	354	332	465	4
1978	744	128	18	222	89	77	80	70	242	380	40
1979	294	135	75	396	211	96	39	71	124	330	6
1980	84	426	546	766	78	138	35	34	92	248	16
1981	189	348	203	672	211	372	224	28	155	199	25
1982	38	97	42	207	44	67	92	103	316	260	24
1983	159	101	158	341	89	71	24	44	335	407	24
1984	138	1065	1000	1742	589	247	51	88	132	258	31
1985	42	97	115	450	256	195	396	485	762	699	13
1986	78	74	7	160	78	103	115	35	142	204	15
1987	392	85	34	121	33	65	46	31	179	600	34
1988	276	981	1041	1442	144	241	19	102	108	283	27
1989	130	91	61	263	67	267	139	468	338	805	11
1990	143	93	27	156	56	59	12	56	174	571	24
1991	152	49	45	103	22	27	8	100	129	601	55
1992	115	140	247	432	78	68	19	29	61	275	74

Year Class	Togiak	Prince William Sound	Sitka	Southeast Alaska	Craig	Prince Rupert	Queen Charlotte	Central Coast	Vancouver Island	Strait of Georgia	Cherry Point
1993	203	151	189	702	222	269	68	86	83	611	20
1994	41	193	390	478	89	111	81	340	447	869	9
1995	53	75	351	473	122	164	217	288	121	922	4
1996	254	17	99	176	78	44	13	63	65	455	13
1997	383	23	247	391	144	123	40	113	59	722	4
1998	107	18	241	330	89	169	38	48	133	977	5
1999	15	224	137	204	67	104	39	149	248	1502	6
2000	12	68	197	419	222	450	115	364	322	1456	11
2001	10	38	8	8		47	8	57	142	725	14
Mean:	167.3	220.6	187.4	358.3	116.9	190.6	153.3	169.4	316	614	17.1
Min:	0.9	9.4	2.9	7.6	2.5	26.5	6.7	21.9	56	40.7	0.2
Max:	846.6	1337.8	1041.1	2124.8	588.9	746.8	1162.8	1113.2	1011.1	1920.6	73.7

Figure 3.1. Time series of recruitment for herring spawning from Togiak (Bristol Bay) through Prince Rupert, British Columbia.

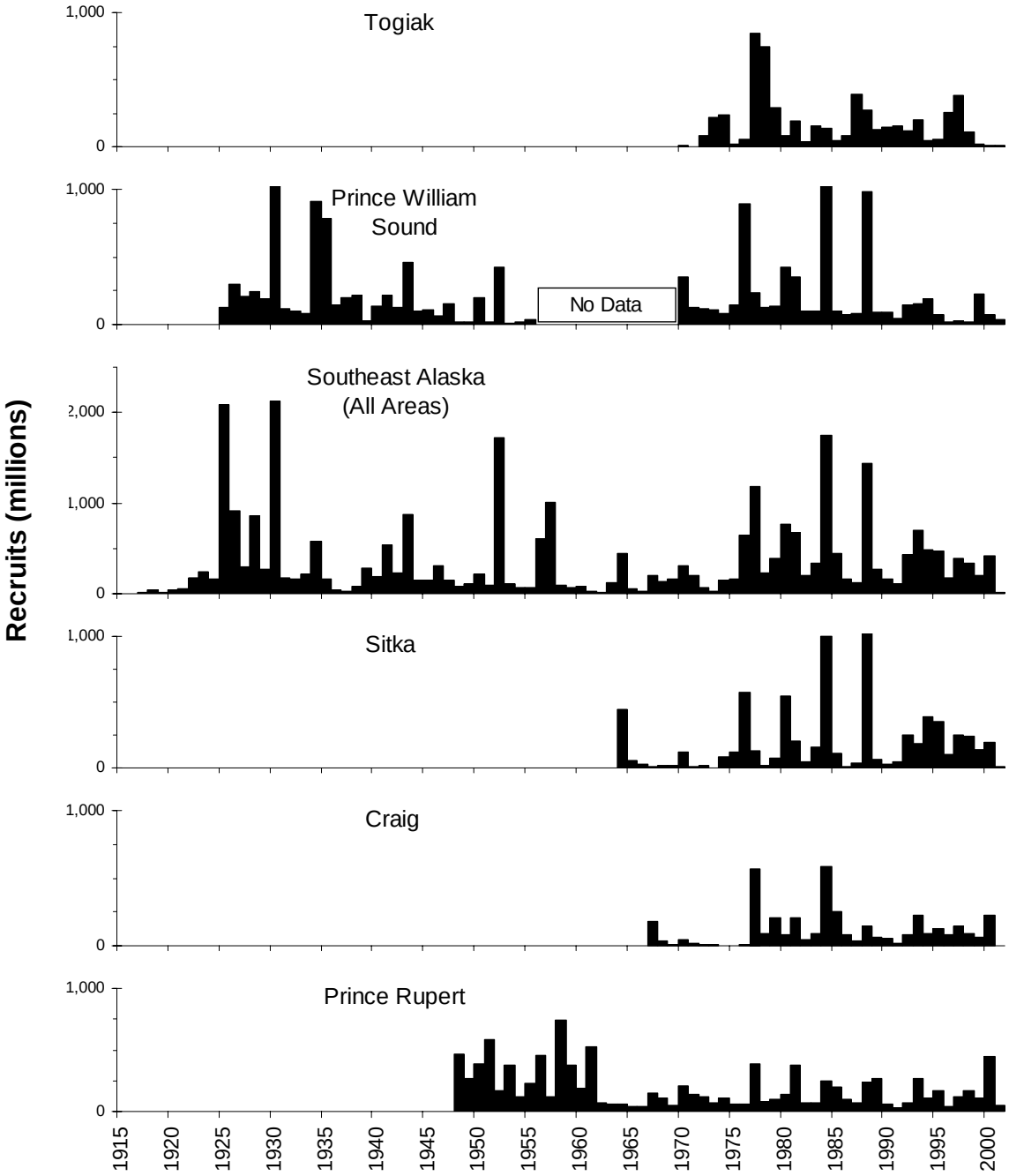


Figure 3.2. Time series of recruitment for herring spawning from the Queen Charlotte Islands, British Columbia, through Cherry Point, Washington.

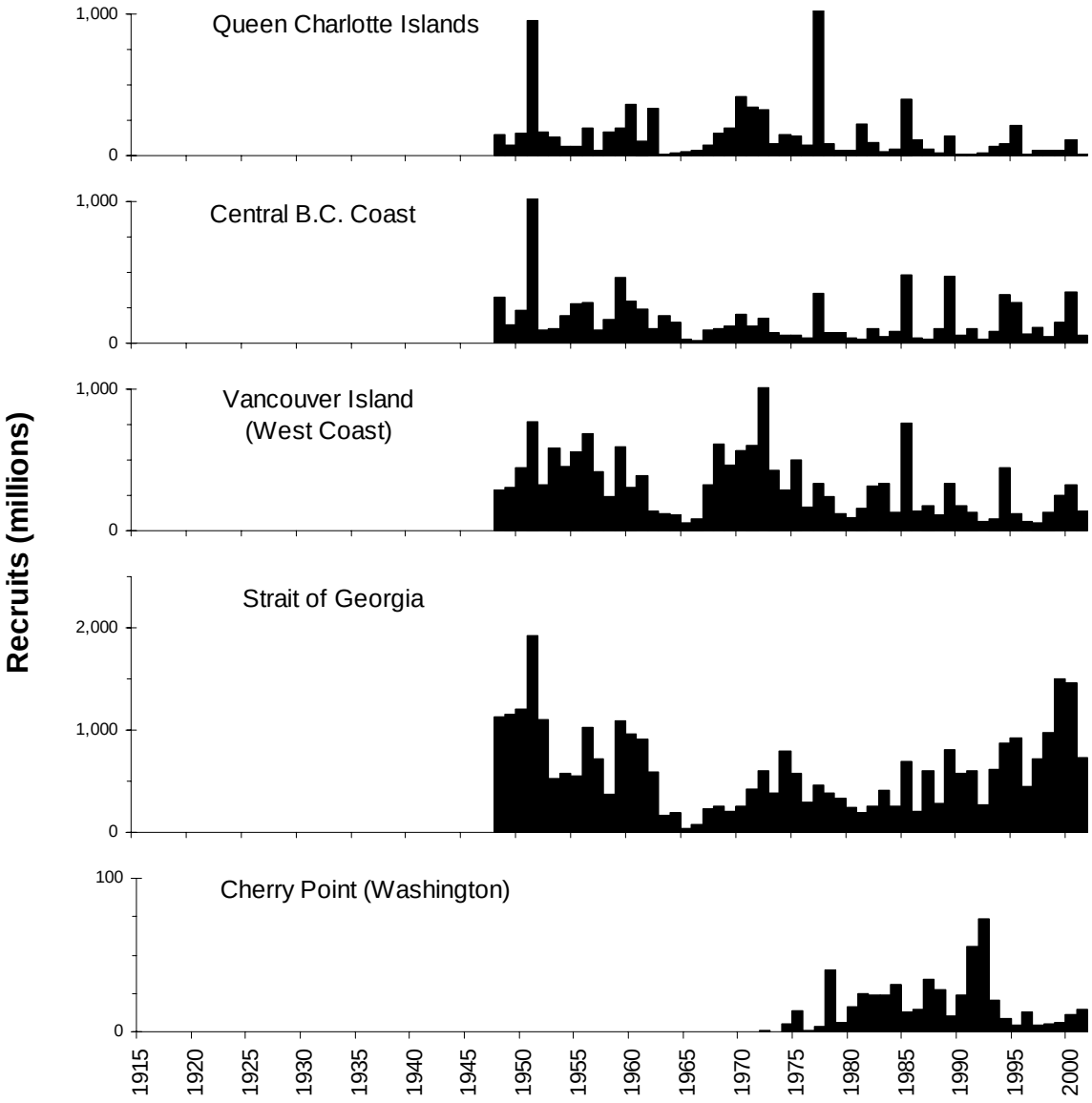


Figure 3.3. Herring spawner-recruit information for Alaska and northern British Columbia. Red filled circles indicate the 1993-2001 year class spawner-recruit pairs. Solid black squares indicate median values.

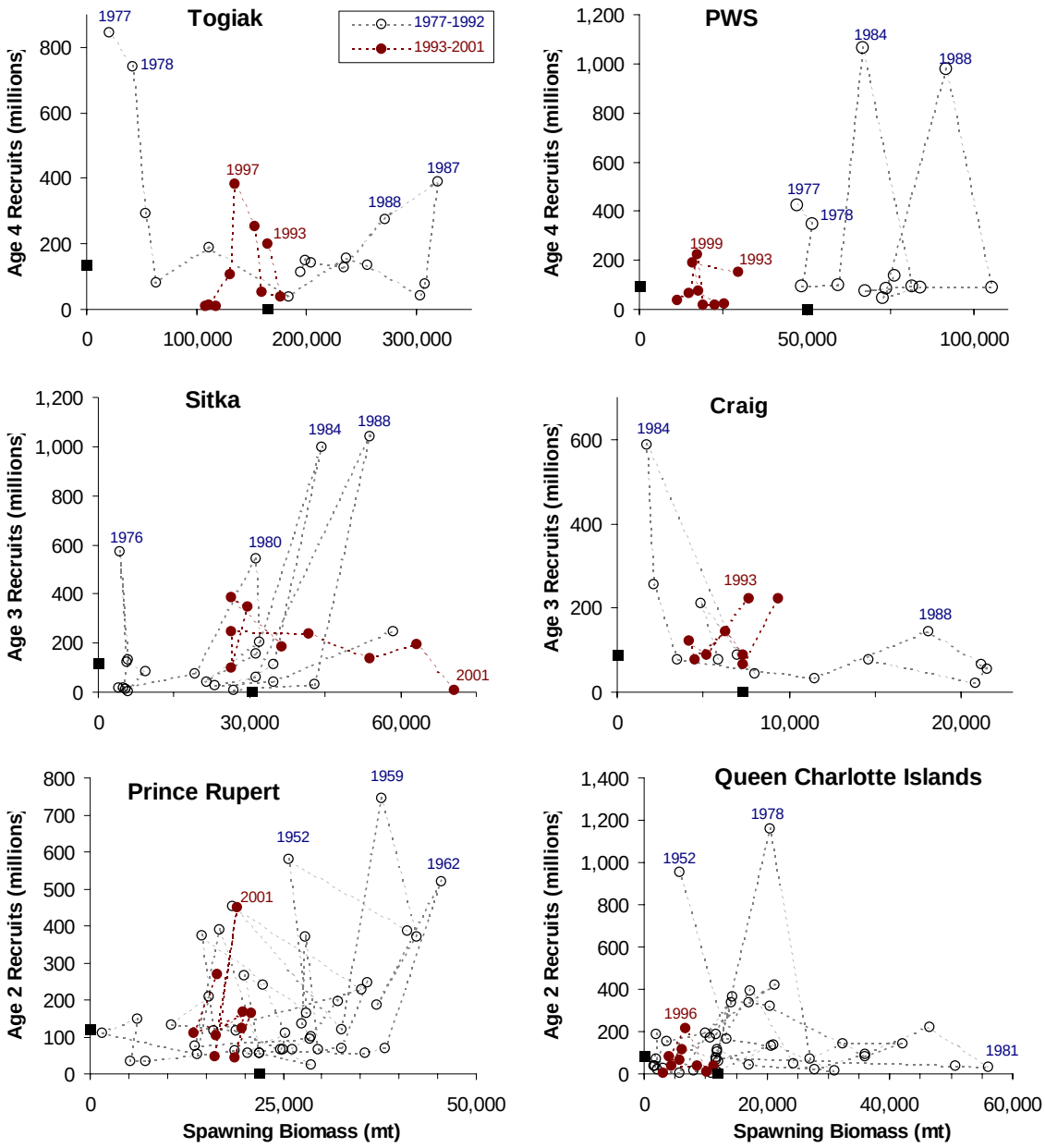


Figure 3.4. Herring spawner-recruit information for central British Columbia to northern Puget Sound (Cherry Point). Red filled circles indicate the 1993-2001 year class spawner-recruit pairs. Solid black squares indicate median values.

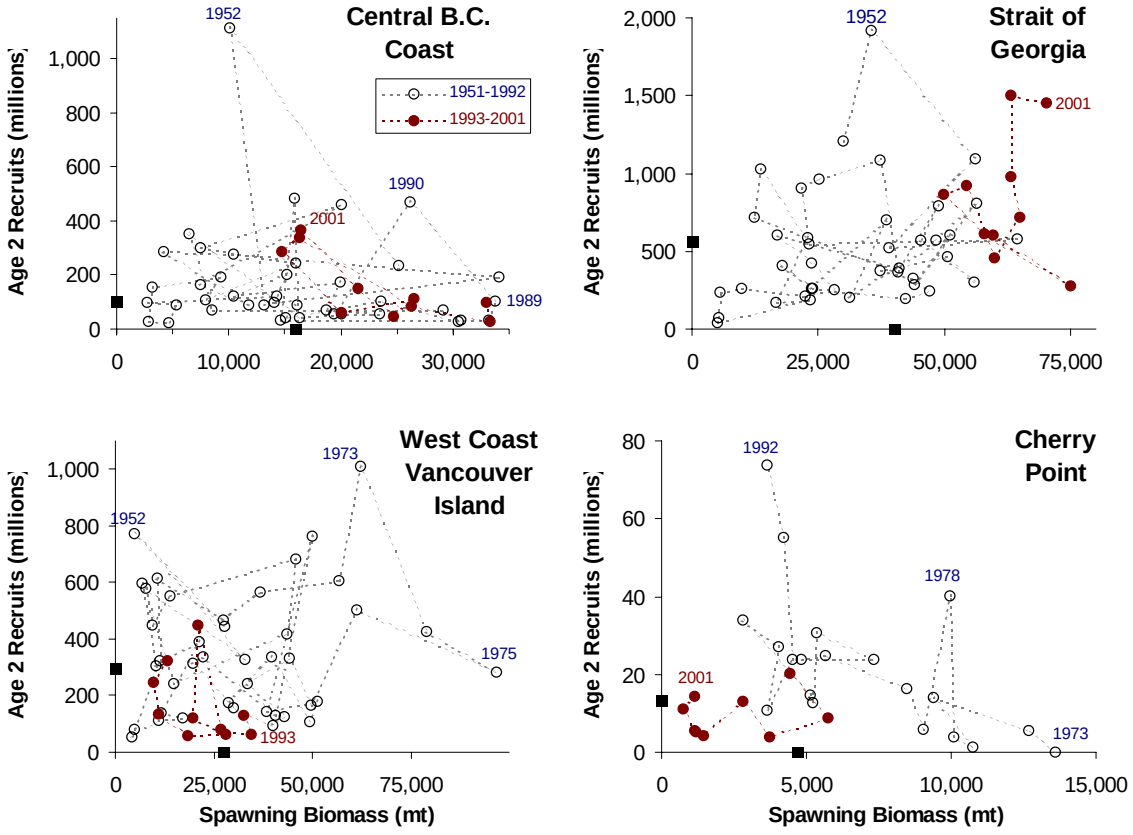


Figure 3.5. Correlation between Sitka and PWS recruitment, 1980 to 2001, showing a simple model of y=x.

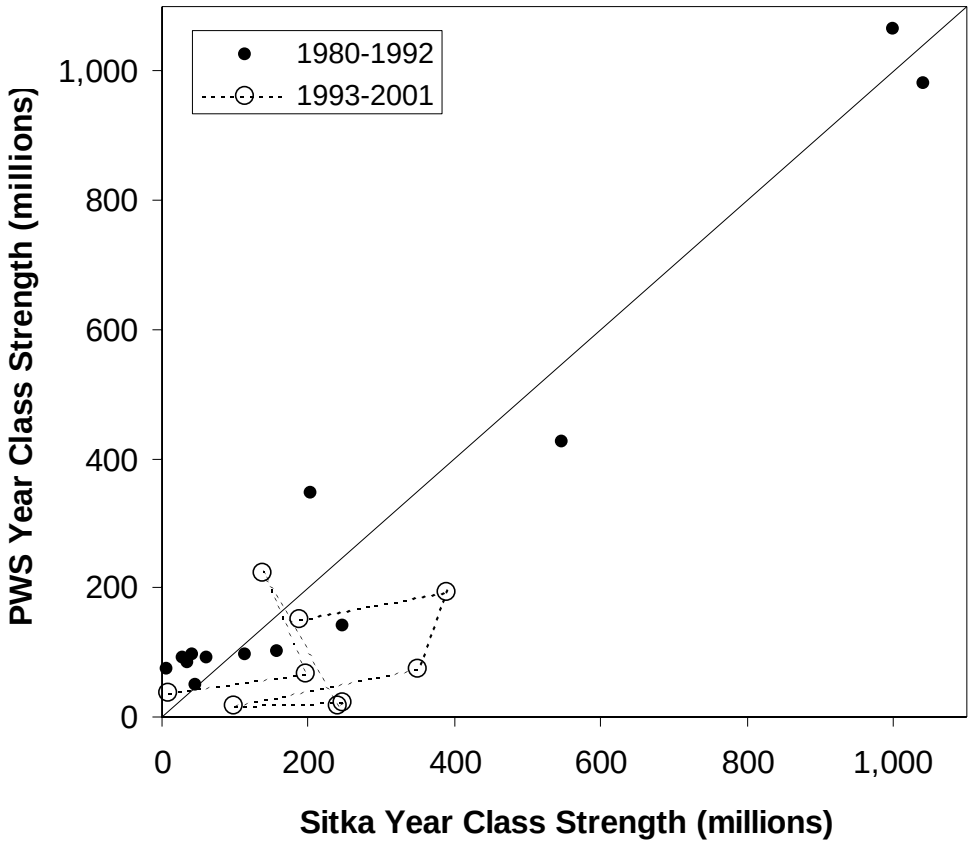


Figure 3.6. Orthogonal residuals from a geometric fit of the simple model y=x relating PWS to Sitka year class strengths for 1980-2001.

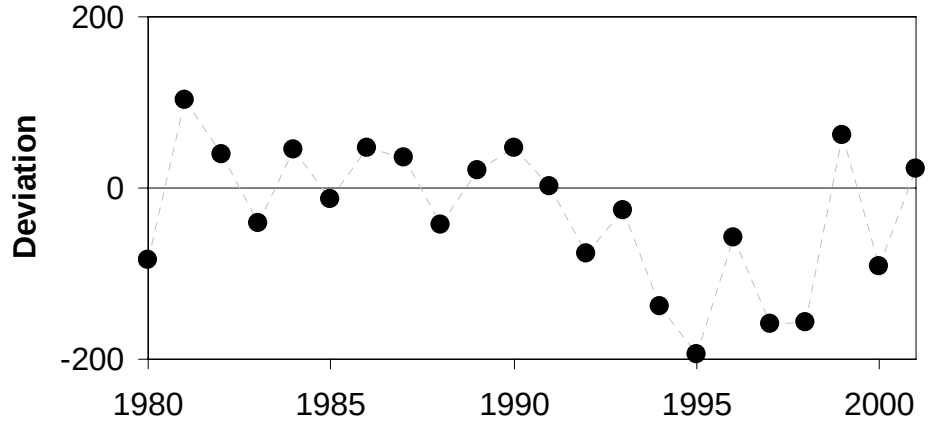


Figure 3.7. Recruitment anomalies (as normalized deviates of log recruitment) for herring spawning from Togiak (Bristol Bay) through Prince Rupert, British Columbia.

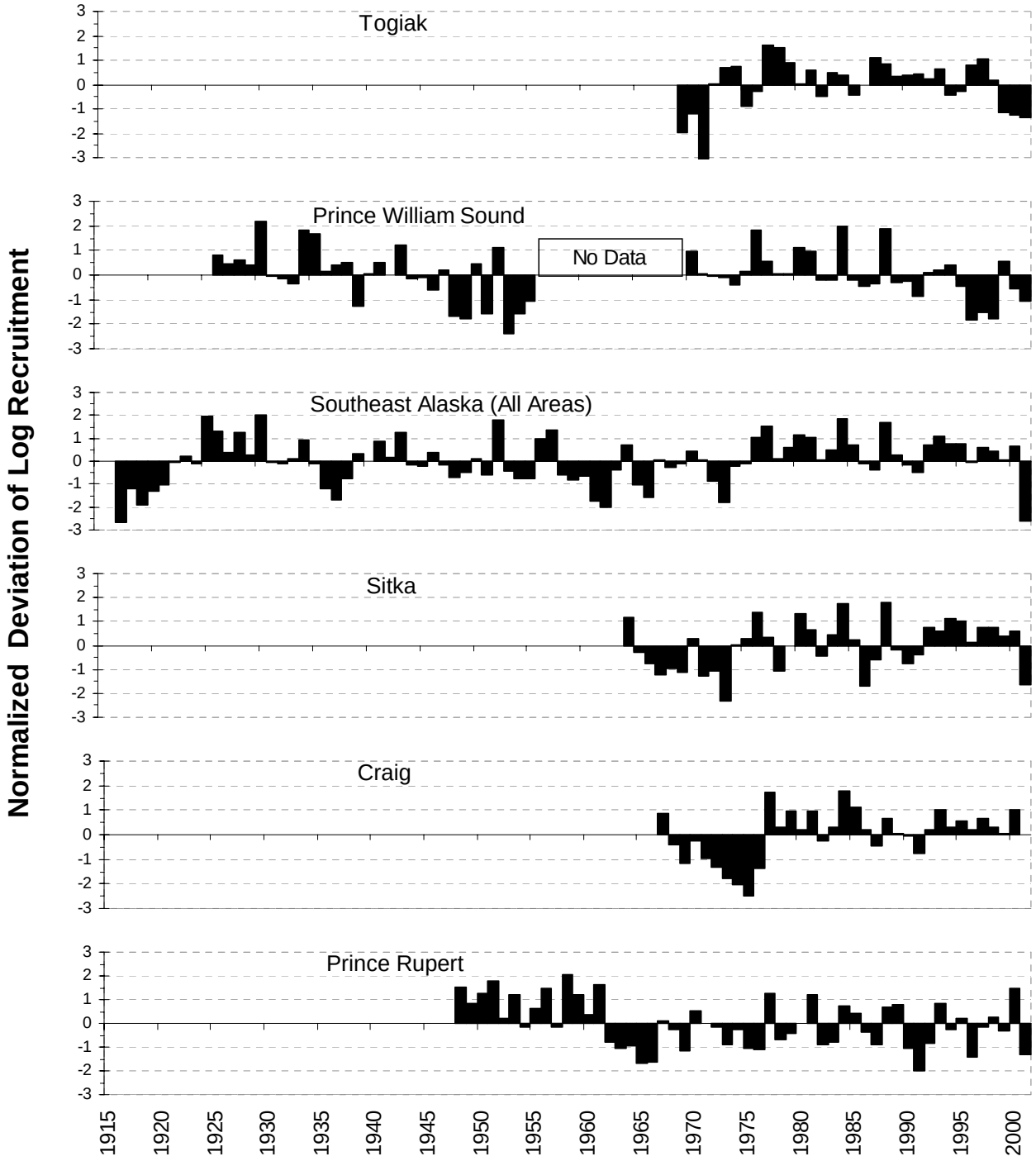
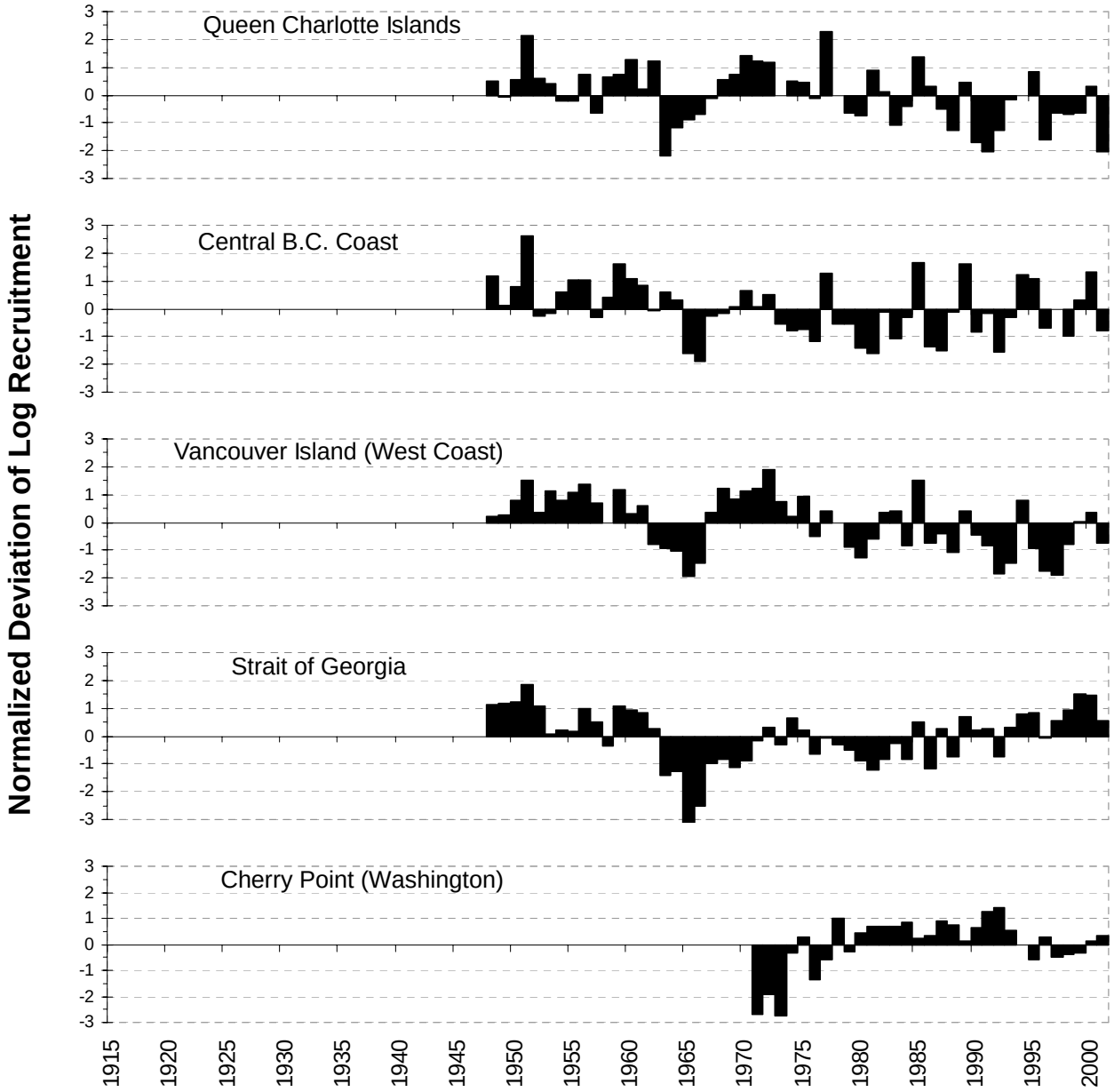


Figure 3.8. Recruitment anomalies (as normalized deviates of log recruitment) for herring spawning from the Queen Charlotte Islands, British Columbia, to Cherry Point, Washington.



Chapter 4

Data conflicts in fishery models: Incorporating hydroacoustic data into the Prince William Sound Pacific herring assessment model

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Abstract

A feature of integrated age-structured assessment models is multiple datasets with weighting terms. We illustrate the difficulties that data conflicts present using the Pacific herring population in Prince William Sound (PWS), Alaska. After the 1989 *Exxon Valdez* oil spill, the Pacific herring (*Clupea pallasi*) population of PWS remained fairly stable for three years but crashed over the winter of 1992-1993. Systematic collection of disease information after 1993 and its incorporation into the age-structured assessment model identified more recent disease outbreaks. Other researchers back-calculated estimates from hydroacoustic abundance surveys started in 1993 and concluded that the age-structured assessment model overestimated herring biomass in 1990-1992; they also concluded that significant population decline began in 1989. To expose data conflicts, we incorporate the hydroacoustic survey information directly into the age-structured model. In this way, the substantial uncertainty about population parameters from 1989 to 1992 due to data conflicts is quantified. Consequently, the magnitude of declines for that period depends on the weights chosen for the various datasets, particularly mile-days of milt.

Keywords: age-structured assessment; hydroacoustic survey; Pacific herring; Prince William Sound

Introduction

Age-structured fishery assessment models that integrate multiple data sources are ubiquitous worldwide (Quinn ad Deriso, 1999), but data conflicts often arise due to associated measurement and process errors. A common conflict is between fishery catch-per-unit-effort and a survey index of abundance (NRC, 1998; Booth and Quinn, 2006). As stock assessments incorporate a wider variety of data (spawning, reproduction, size-structured), conflicts are becoming more prominent.

The Prince William Sound (PWS), Alaska, population of Pacific herring (*Clupea pallasi*) provides an excellent example of data conflicts and how they might be resolved. Pacific herring in PWS are an important forage species for marine fish and wildlife and a valuable commercial resource (Thomas and Thorne, 2003). During the winter of 1992-1993, the biomass of adult Pacific herring in PWS apparently crashed. Over 110 000 mt (metric tonnes) were predicted to be available in 1993 by an age-structured assessment (ASA) model, but only about 30 000 mt actually returned to spawn (Quinn *et al.*, 2001). The Alaska Department of Fish and Game

(ADF&G) closed the 1993 fishery and it has remained closed except for small fisheries in 1997 and 1998. A disease epidemic was likely responsible for the unexpected large natural mortality, and the North American strain of viral hemorrhagic septicemia virus (VHSV) was isolated from Pacific herring sampled from PWS in 1993 (Meyers *et al.*, 1994). When population decline continued a systematic and unique time series commenced in 1994 that documented the significance of three major diseases or pathogens in PWS Pacific herring: ulcers related to filamentous bacteria, VHSV, and the mesomycetozoan *Ichthyophonus hoferi* (Quinn *et al.*, 2001; Marty *et al.*, 2003). On a population scale, VHSV and ulcers apparently contribute more to mortality of young Pacific herring (ages 4 and below), whereas *I. hoferi* contributes more to mortality when fish are old (ages 5 and above) (Marty *et al.*, 2003). The ASA model with disease information fitted the data better than the original model with constant mortality over the entire time period and accounted for perceived mortality events in the population in 1992-1993 and again in 1999 (Quinn *et al.*, 2001; Marty *et al.*, 2003).

The Prince William Sound Science Center (PWSSC) initiated hydroacoustic surveys in the fall of 1993 to determine the condition of the herring stock as a consequence of the unexpected biomass decline (Thomas and Thorne, 2003). Acoustic surveys conducted each year thereafter generated over a decade's worth of data. From a back-calculation of a regression relationship between their hydroacoustic biomass estimates and a measure of spawning activity (miles of milt production), Thomas and Thorne (2003) extrapolated herring biomass back into the 1980s and obtained consistently lower values than the ASA model during 1990 – 1992. They concluded that the ASA model overestimated biomass in those years and recommended that, in its place, ADF&G use only the most recent hydroacoustic survey point estimate to set harvest quotas for the PWS fisheries in the spring just prior to harvest.

The goal of our study is to revisit the conclusions of Thomas and Thorne (2003) with an alternative analytical approach. Rather than abandon the ASA model and ignore several datasets contained within it, we integrate the hydroacoustic data set into the ASA model. The objectives of this analysis are to: (1) examine the effects of acoustic survey index on the estimates of herring spawning biomass from the ASA model, (2) evaluate the value of this new acoustic dataset as an important index in the model, and (3) determine whether the acoustic index should be treated as a relative or absolute indicator of herring biomass.

Methods

Data

The ASA model integrates various data sources relevant to Pacific herring in PWS from 1980-2004. Herring data sets include the age distribution of catch for the purse seine fishery (n = 88), spawning age composition (n = 158), estimates of total egg deposition from diver surveys (n = 10), and mile-days of milt from annual aerial surveys (n = 25). Other data sources include weight-at-age, fecundity-at-age, total seine catch, and other fisheries' catch-at-age.

Disease indices for ulcers, VHSV, and *I. hoferi* are available for the years 1994 to 2004 (Table 1). The indices are stratified by age (3-4, 5-9) and originated from disease prevalence values adjusted to reflect the significance of each component (Marty *et al.*, 2003).

We recompiled hydroacoustic biomass estimates from 1995-2004 (Table 2), using data collected by PWSSC (Thomas and Thorne, 2003) and ADF&G. In 1995 and 1996, the estimates were taken directly from PWSSC through unpublished contract reports to ADF&G. The first year that ADF&G had acoustic gear and conducted their own surveys was 1997 (Biosonics 70 kHz single beam). From 1997 to 2004 the estimates were taken from a combination of PWSSC

and ADF&G surveys with the exception of 2000, in which ADF&G did not conduct a survey and only PWSSC data were used. In previous assessment analyses, the dataset was used as a constraint on the minimum value of spawning biomass rather than as an index. We used only the spring values for consistency; there were no spring hydroacoustic surveys before 1995.

Model

To investigate whether the ASA model is inferior to using only the hydroacoustic information, it is necessary to present the full structure of the model. The ASA model is a standard implementation of an age-structured assessment model (Quinn and Deriso, 1999, chapter 8), modified to include the impact of disease on mortality and abundance (Quinn *et al.*, 2001; Marty *et al.*, 2003). The observations are compared with model estimates in a least-squares setting to obtain parameter estimates of recruitment, maturity, seine selectivity, a milt calibration coefficient, and disease coefficients (explained below). Notation is given in Table 3.

The recruitment and maturity parameters are utilized to obtain three population matrices: pre-fishery total abundance, pre-fishery spawning biomass, and natural spawning population (by ages 3 to 9+ and years 1980-2004). Pre-fishery total abundance $N_{a,t}$ derives from recruitment parameters: abundances (in millions of fish) for age-3 from 1980-2004, and ages ≥ 4 in 1980. It is given by:

$$N_{a+1,t+1} = \left[\left(\left(N_{a,t} - \left(C^s_t \Theta^s_{a,t} + C^g_{a,t} + P_k C^p_{a,t}\right)\right) S^{1/2}_{a,t}\right) - C^{f/b}_{a,t}\right] S^{1/2}_{a,t+1} \quad (1)$$

in which a cohort at age a and year t becomes one year older the next year. The population decreases due to four commercial fishery catches (s-seine, g-gillnet, p-pound utilization, f/b-food and bait) and natural mortality (expressed in terms of natural survival S). Natural survival S is written as a function of age and year to accommodate disease prevalence. A half-year convention to survival is applied, because the seine, gillnet, and pound utilization fisheries occur in the spring and the food and bait fishery occurs in the fall. Mortality in the pound fishery is the number of fish impounded $C^p_{a,t}$ times the proportion P_k of impounded herring killed. ADF&G sets P_k to 0.75 based on previous studies.

The seine catch is expressed as total catch C^s_t multiplied by age composition $\Theta^s_{a,t}$, corresponding to data sources. The seine age-composition is assumed to be measured with error due to sampling variability and ageing error. Vulnerability parameters are used to estimate the proportion of fish in the pre-fishery total population matrix that are caught with respect to age. Vulnerability is assumed to be a logistic function:

$$V_a = \frac{1}{1 + e^{-\omega(a-\xi)}} \quad (2)$$

in which ω, ξ are estimated parameters.

Maturity parameters are used to transform the pre-fishery total abundance matrix into both the pre-fishery and post-fishery spawning biomass matrices. Maturity of PWS herring is not directly measured, but rather estimated from spawning age composition. Maturity is assumed to be a logistic function

$$mat_{a,t} = \frac{1}{1 + e^{-\delta_i(a-\varepsilon_i)}} \quad (3)$$

in which δ_i and ε_i are estimated parameters. The subscript i is present because analysis of residuals suggested that a change in maturity occurred before and after 1997. Thus, different

parameters were estimated for these two time periods. Maturity at age-5 and older was set to 1, which also implies that all of these herring are present on the spawning grounds.
For a given age class and year, pre-fishery spawning biomass is:

$$PB_{a,t} = w_{a,t} mat_{a,t} N_{a,t} \quad (4)$$

such that $w_{a,t}$ is the observed weight-at-age in year t and $mat_{a,t}$ is the maturity at age a in year t. Similarly, the abundance of the natural spawning population after the spring fisheries is:

$$SN_{a,t} = mat_{a,t}\left(N_{a,t} - \left(\Theta^{s}_{a,t} C^{s}_{a,t} + C^{g}_{a,t} + P_k C^{p}_{a,t}\right)\right) \quad (5)$$

The spawning biomass in a given year is obtained by multiplying the spawning abundance by the weight-at-age and summing the values across the age classes. Equivalently:

$$SB_t = \sum_a w_{a,t} SN_{a,t} \quad (6)$$

In Quinn *et al.* (2001) and Marty *et al.* (2003), it was assumed that natural survival $S_{a,t}$ decreases linearly as a function of disease prevalence variables $\{x_{it}\}$. In this paper, natural mortality rather than survival is assumed to be a linear function of these disease prevalence variables, because the model fits the data better (results not shown) and because parameter estimation becomes more stable by avoiding negative survival values. Expressed mathematically,

$$M_{a,t} = M_0 + \sum_i \beta_i x_{it} \quad (7)$$

in which M_0 is background natural mortality due to other natural phenomena (predation, in particular) and β_i is a disease coefficient that scales the disease index to a mortality value. This equation is applicable only in the years 1994-2004, years for which there are disease indices. A separate natural mortality parameter is estimated for the last half-year in 1992 and the whole year in 1993 to accommodate the population crash. Prior to this year, natural mortality is set to 0.25 by convention, because there is too much uncertainty in the early data to estimate a stable natural mortality for ages 8 and younger (Quinn *et al.*, 2001). In all years, natural mortality for the plus age class (ages 9 and older) is estimated using a multiplier on natural mortality at earlier ages.

The half-year survival used in (1) is then

$$S^{1/2}_{a,t} = \exp(-M_{a,t}/2) \quad (8)$$

.

Objective functions

The least squares setting of the age-structured model follows from consideration of the statistical distributions of the various datasets: seine age composition, spawning age composition, mile-days of milt, and egg deposition. Each dataset is assumed to follow a normal distribution, sometimes after transformation.
Seine age composition is the observed proportion of seine catch with respect to age, related to vulnerability and abundance by

$$\hat{\Theta}^{s}_{a,t} = \frac{V_a N_{a,t}}{\sum_a V_a N_{a,t}} \quad (9)$$

The residual sum of squares (RSS_i) for seine age composition is

$$RSS_s = \sum_t \sum_a \left(\Theta^{s}_{a,t} - \hat{\Theta}^{s}_{a,t}\right)^2 \quad (10)$$

Spawning age composition, the proportion of the population that has reached sexual maturity and is available on the spawning grounds, from the model is estimated as the ratio of spawning abundance at age to total spawning abundance after the spring fisheries from (5)

$$\hat{\Theta}_{a,t}^{SP} = \frac{SN_{a,t}}{\sum_{a} SN_{a,t}} \quad (11)$$

.

Its residual sum of squares is

$$RSS_{SP} = \sum_{t}\sum_{a}\left(\Theta_{a,t}^{SP} - \hat{\Theta}_{a,t}^{SP}\right)^2 \quad (12)$$

.

The mile-days of milt $\left(\widetilde{M}_t\right)$ and egg deposition $\left(\widetilde{E}_t\right)$ are modeled with a log-normal statistical distribution. Thus, the residual sum of squares compares the logarithms of the observed and estimated values. Both of these datasets utilize the natural spawning population matrix to estimate values since spawning occurs subsequent to the seine, gillnet, and pound fisheries, and both mile-days of milt and egg deposition are indicators of the spawning activity. Estimates for mile-days of milt utilize a milt calibration coefficient (ψ) and are obtained by

$$\hat{\widetilde{M}}_t = \frac{(1-\%F_t)SB_t}{\psi} \quad (13)$$

In which $1-\%F_t$ is the percentage of male spawners. Basically, male spawning biomass is divided by the calibration coefficient to estimate the accumulated amount of milt during spawning. The residual sum of squares is obtained by

$$RSS_M = \sum_{t}\left(\ln\widetilde{M}_t - \ln\hat{\widetilde{M}}_t\right)^2 \quad (14)$$

The estimate of egg deposition is simply female spawning abundance multiplied by fecundity f, or

$$\hat{\widetilde{E}}_t = \%F_t\sum_{a} f_{a,t}SN_{a,t} \quad (15)$$

Fecundity data are available in all years in which the egg deposition survey was conducted. Like the mile-days of milt, the residual sum of squares is

$$RSS_E = \sum_{t}\left(\ln\widetilde{E}_t - \ln\hat{\widetilde{E}}_t\right)^2 \quad (16)$$

We assume that biomass from the hydroacoustic survey in the spring is an index of the total estimated pre-fishery biomass. The estimated hydroacoustic biomass values from the model are then:

$$\hat{H}_t = B_t e^{\gamma} \quad (17)$$

in which γ is the estimate of the logarithm of the hydroacoustic survey calibration coefficient and B_t is the pre-fishery estimated biomass:

$$B_t = \sum_{a} N_{a,t}w_{a,t} \quad (18)$$

If the log calibration coefficient □ is equal to 0, then the hydroacoustic survey provides an absolute estimate of abundance ($e^0 = 1$). If the log calibration coefficient is less than 0, then the hydroacoustic survey provides a relative index of abundance (less than pre-fishery abundance).

Like the other population quantities, we assume that the hydroacoustic biomasses follow a log-normal distribution, so that the residual sum of squares is given by:

$$RSS_{\mathrm{H}} = \sum_t (\ln H_t - \ln \hat{H}_t)^2 \quad (19)$$

A Ricker spawner-recruit relationship was introduced into the model to stabilize recruitment parameters. This relationship models the recruitment $\{R_t = N_{3,t+3}\}$ in year t+3 as a dome-shaped function of spawning biomass in year t, or

$$R_t = \alpha e^{-\tau SB_t + \varepsilon_t} \quad (20)$$

with lognormal process error □ allowed, with parameters α, τ estimated by the model. Consequently, a residual sum of squares term between the model's estimates of recruitment to the estimated Ricker curve on a log scale is introduced. This term prevents recruitment estimates from going to zero or negative values. The residual sum of squares term is:

$$RSS_R = \sum_t \left(\ln(R_t) - \ln(\alpha) - \tau SB_t\right)^2 \quad (21)$$

Finally, the objective function to be minimized is the total weighted sum of squares

$$RSS_{tot} = \sum_i \lambda_i RSS_i \quad (22)$$

.

in which λ_i is a weighting term for each dataset. The weighting term influences the model's fit to the respective dataset, with a lower weighting having less influence in the objective function. The baseline weighting scheme is $\lambda_S = 1$, $\lambda_{SP} = 1$, $\lambda_M = 0.5$, $\lambda_E = 0.5$ (S-Seine age composition, SP-Spawning age composition, M-Mile-days of milt, E-Egg deposition) based on previous work (Quinn *et al.*, 2001; Marty *et al.*, 2003). The weight λ_R was chosen to be 0.03 so that recruitment estimates did not converge to 0 but that spawner-recruit relationship allowed sufficient stochasticity to account for environmental anomalies. As the focus of this study was the incorporation of the hydroacoustic index, the weighting λ_H (H-Hydroacoustic) was varied from 0.1 to 10.

Bootstrap

A bootstrap procedure (Quinn *et al.*, 2001) is used to obtain standard errors for estimated parameters. Bootstrap replicates for age composition data (seine, spawning) are generated from the multinomial distribution. For population indices (egg production, mile-days of milt, hydroacoustic data), residuals (on a logarithmic scale) are resampled with replacement and added to estimated values from the original model. The number of replications is 1000, the recommended level for calculating confidence intervals. Because it is not possible to estimate background mortality M_0 from other sources than disease, the resulting variance estimates are conditional on the fixed value of 0.25 and, therefore, are underestimates of the total uncertainty.

Model Scenarios

In our analysis, a sensitivity analysis with several modeling scenarios was performed to examine the robustness of the model to datasets, parameters, and weighting schemes. We focused on the change to influential quantities, including recruitment estimates, survival estimates, the maturities of age-3 and age-4, the unweighted RSS's for all datasets, the total RSS, and the estimated spawning biomasses from 1980-2004. Following is a description for each of the scenarios considered.

M0: The base model without hydroacoustic data, described in section 2.2.
M1: The hydroacoustic dataset is added to M0.
M2: Same as M1, except that the maturities of age-3 and age-4 fish are assumed constant from 1980-2004.
M3: Same as M1, except that hydroacoustic survey biomass is treated as an index of spawning biomass, rather than total biomass.
M4: Same as M1, except that an alternative set of weights ($\lambda_S = .25$, $\lambda_{SP} = 1$, $\lambda_E = 0.25$, $\lambda_M = 2$) is used [from previous ADF&G herring assessments (Moffitt, unpublished)].
M5: Same as M1, except that natural mortality from 1989-1992 is estimated. There are two versions: M5a uses the M1 weighting scheme, while M5b uses M4 weighting scheme.
M6: Same as M5, except that natural mortality from 1989-1992 is stratified into two age groups, ages 3-4 and 5-8.

Parsimony

The most parsimonious model was chosen based on AICc (Akaike Information Criterion, corrected) comparisons outlined in Burnham and Anderson (1998). AICc values can only be compared for models with the same datasets and weighting scenario. In order to calculate AICc, the likelihood must be calculated first from the equation

$$(23)\quad \ln L = \sum_i -\frac{1}{2}\left[n_i \ln(2\pi\sigma_i^2) + \frac{RSS_i}{\sigma_i^2} \right]$$

(Quinn and Deriso, 1999, p. 170, eq. [4.55]), in which σ_i^2 is the unexplained variance and n_i is sample size. Each weighting term λ_i is the variance of the first dataset (seine age composition) relative to the variance of the ith dataset, or $\lambda_i = \sigma_1^2 / \sigma_i^2$. The maximum log likelihood can be shown to be

$$(24)\quad \max \ln L = \sum_i -\frac{n_i}{2}\left[\ln(2\pi\hat{\sigma}_1^2 / \lambda_i) + 1\right], \text{ in which}$$
$$\hat{\sigma}_1^2 = \sum \lambda_i RSS_i / \sum n_i, \text{ and } \hat{\sigma}_i^2 = \hat{\sigma}_1^2 / \lambda_i$$

Thus the maximum likelihood corresponds to the minimum weighted residual sums of squares. The Akaike information criterion (AIC) and its corrected version for small sample sizes (AICc) are then obtained from

$$(25)\quad AIC = -2\ln L + 2p$$
$$AICc = AIC + 2p(p+1)/(n-p-1)$$

where p is the number of estimated parameters and n is the combined sample size. When comparing between models, the lowest AICc value corresponds with the most parsimonious model. Differences in AICc below 4 are considered statistically insignificant.

Results

The pattern of age-3 recruitment from base model M0 appears cyclic with a period of 4-5 years up to 1993 (Fig. 1). Thereafter, recruitment was generally lower with an absence of strong recruitment events. Trends in spawning biomass include an increase until 1989 to greater than 100 000 mt, a slight decrease until 1992, and then a large decrease in 1993 with low abundance to the end of the time series, not increasing past 30 000 mt.

Estimates of population biomass from model M1 ran a continuum from similar results to M0 (at the lowest hydroacoustic weight λ_H=0.1) to the most different (at the highest hydroacoustic weight λ_H=10). The estimates of the hydroacoustic biomass from M1 followed the observed hydroacoustic survey biomass closely, with marginally better fit at higher λ_H (Fig. 2).

The recruitment pattern from the various M1 scenarios was not affected much by the choice of λ_H (Fig. 1, λ_H = 0.5). Before 1993, there were negligible deviations in recruitment relative to M0. After 1993, recruitment estimates were only slightly larger compared to M0, no matter what the hydroacoustic weight. Compared to M0, the largest increase in recruitment occurred in 1997. The estimated increase in recruitment was not more than 100 million fish for hydroacoustic weights less than or equal to 2, and about 200 million fish with a hydroacoustic weight of 10. These deviations pale in comparison to about 800 million less fish for strong recruitment events after 1993 (i.e., 1997 and 2002, Fig.1), as compared to strong recruitment before 1993 (i.e., 1987 and 1991, Fig.1).

Overall, M1 had little effect on total spawning biomass estimates as compared to M0, with deviations being positive on occasion. Further, M1's results remained well within bootstrap confidence intervals from M0 for all years. The bootstrap analysis of M1 revealed reasonably precise estimates (Fig. 3).

The natural mortality estimates for age groups 3-4 and 5-9 from M1 were very similar to estimates from M0 for all choices of λ_H (Fig. 4a). Natural mortality estimates for age group 3-4 differed the most in 1994 among the various hydroacoustic weights, and then remained extremely stable for the remainder of the time series. The greatest difference in the 1994 mortality value for age group 3-4 corresponded to only a 10% change in survival (Fig. 4a). Natural mortality estimates for age group 5-9 were more different than for age group 3-4 when comparing M1 to M0. While the same general trend occurred as in M0, there was a steady increase in mortality for each year in the time series. The largest disparity was in 2001, where M1 (at λ_H = 10) produced a survival estimate 40% less than M0 (Fig. 4b).

In M0, maturities for ages 3 and 4 shifted in 1997 from lower to higher values (Table 4). As the hydroacoustic weight increased in M1, the shift in maturity disappeared and the maturity value went down (Table 4). The effect of λ_H on maturity was larger after 1997 than before.

Unsurprisingly, the unweighted RSS_H for M1 decreased as λ_H increased (Fig. 5), because the greater weight made the nonlinear procedure fit the hydroacoustic data better. In contrast, a nearly exponential degradation in fit to spawning age composition (increasing RSS_{SP}) occurred as λ_H increased (Fig. 5). At the same time, linear degradation in the fits to the seine age composition (increasing RSS_S) and egg survey (increasing RSS_E) occurred (Fig. 5). As λ_H increased up to 2, the fit to mile-days of milt improved slightly (decreasing RSS_M), and then deteriorated slightly as the weight increased past 2 (Fig. 5).

The mile-days of milt dataset spans the entire timeframe of the model and is one of two data sets that measure reproductive output of herring in PWS. Both observed and estimated values in M1 from years of overlap (1995-2004) for the hydroacoustic index and mile-days of milt display the same trends (Fig. 6, at λ_H = 0.5, choice of weight discussed below), and the two estimates are significantly correlated (Pearson r = 0.79, P = 0.007).

The maturity estimates for age-3 and age-4 from the constant maturity model M2 for all hydroacoustic weights were within the range of those from M1 with pre- and post-1997 maturity parameters. As λ_H increased in M2, the maturity estimates decreased slightly for each age group (Table 4). Comparison of AICc values between M1 and M2 with the same weighting scheme showed that M1 (AICc = −285.9) was the better model as compared to M2 (AICc = −283.9),

although the difference was not large. The effect on recruitment estimates from M2 was negligible as compared to M1 (less than 10 million fish). There were no significant effects on natural mortality of age group 3-4, but there were increases of the order of 5% in natural mortality of age group 5-9 relative to those estimated by M1.

Comparing scenario M3 results to those of M1 reveals that using the hydroacoustic index as an index of spawning biomass instead of total biomass has no significant effect on any important estimates in the model, particularly estimates of total and spawning biomass. Further, the alternative weighting scheme in M4 produced estimates that were consistent with M1. Therefore, parameter estimation is robust for the set of parameters used in M1 and M4.

Estimated natural survival across all ages from 1989-1992 was 87% for M5a with the baseline weighting scheme and 66% for M5b with the ADF&G weighting scheme, compared to the fixed value of 78% from M1 or M4; confidence intervals are wide (Table 5). Estimated natural survival for ages 3-4 was 92% for M6a with the baseline weighting scheme and 100% for M6b with the ADF&G weighting scheme; these estimates are unrealistically high and also have wide confidence intervals (Table 5). Therefore, M6 is eliminated on biological grounds. For the baseline weighting scheme, the AICc value for M5a was only 0.7 lower than M1, suggesting a lack of evidence to support any differences in natural mortality during 1989-1992 (Table 5). For the ADF&G weighting scheme, the AICc value for M5b was 5.0 lower than M4, suggesting that there was higher mortality during 1989-1992 than the baseline survival of 78% (Table 5). Therefore, there remains uncertainty in whether mortality was higher than baseline during 1989-1992, because the results depend on the weighting used for the reproductive index of mile-days of milt or egg deposition.

The results of our analysis suggest that there is a clear conflict between two groups of datasets (milt – hydroacoustic versus age composition – egg survey) during the period 1989 to 1992. To investigate the conflict between the hydroacoustic data and the spawning age composition, the estimates of recruitment from M1 were compared to the observed spawning age composition. The topographic representation of the spawning age composition indicates periods of strong year classes as they age (represented by the peaks that move from the bottom left to top right, e.g., the peak starting in 1987, Fig. 7). The spawning age composition data is not an absolute indicator of abundance. However, the dominance of a year class in the spawning proportion points relatively toward larger abundance of certain year-classes. The estimates of initial abundance ($\{N_{3,t}\}$ for $\lambda_H = 0.5$) revealed large year-classes in 1984 and 1988, prior to the Exxon Valdez oil spill (age-3 in 1987 and 1991 respectfully, Fig. 7). These large year-classes are also remarkable in the spawning age composition represented by the peaks in the topographic plot that follow the arrows in the chart. After 1989 there were several large cohorts, albeit much smaller than pre-Exxon Valdez oil spill, that also followed trends in the spawning age composition. The conflict between the two data sets could be from the initial abundance estimates in 2002 (region enclosed by the ellipse). The hydroacoustic index did not let the 2002 cohort reach the abundance that the spawning age composition data required, thus possibly causing a conflict between these two datasets.

Discussion

The hydroacoustic index has little influence on estimates of spawning biomass in the ASA model. Modest effects do occur, such as slight increases in the recruitment estimates $\{N_{3,t}\}$, slight increases in the natural mortality values for ages ≥5, and a convergence of pre and post-1997 maturity values for ages 3 and 4.

How are these minor effects reconciled in the hydroacoustic-augmented ASA model? It appears that the hydroacoustic model produces similar spawning biomasses by introducing more age-3 recruits, while at the same time removing older age classes. The model is also decreasing the overall maturity of age-3 recruits and age-4 fish so that the increase in younger cohort abundance does not translate into an increase in spawning biomass.

The addition of the hydroacoustic index increases the overall robustness of the ASA model. The unweighted RSS for mile-days of milt changes little as a function of hydroacoustic weight λ_H. This result agrees with Thomas and Thorne (2003) that the acoustic survey's estimated biomass is highly correlated with mile-days of milt ($r^2 = 0.75$, from Thomas and Thorne 2003). However, estimated maturities of age-3 and 4 fish were different for pre- and post-1997 time periods with lower values of λ_H but converged to a constant value at higher values of λ_H, implying that it remains unresolved whether there was a shift of maturity accompanying the persistence of disease. Within the ASA model there is almost a redundancy in these two data sets with virtually identical trends; but the incorporation of both leads to quantification of the uncertainty in the population's dynamics. Thus, future assessment of PWS herring through an ASA approach should include hydroacoustic data. Although, the resolution of the discrepancy in maturity estimates requires that a field study of maturity be conducted for PWS herring.

An important question is how should this dataset be weighted with respect to others? If the relative variability and bias of all datasets were the same, then using weights of 1 would be reasonable. We have no *a priori* information to suggest that variability and bias of the datasets is appreciably different. Furthermore, the amount of relative change in parameter estimates went up with weights of 5 or 10. Because the high correlation between mile-days of milt and hydroacoustic biomass essentially doubles the contribution of these data sources when both are in the model we suggest using $\lambda_H = 0.5$ for robustness of results.

Estimates of population biomass based on hind-casting of mile-days of milt are extremely unreliable before 1993, primarily because the range in miles of spawning from 1988-1993 is almost ten times the range when hydroacoustic data are available from 1993-2002. Given the positive correlation coefficient between mile-days of milt and an absolute estimate of biomass from 1993-2002, the method of hind-casting before 1993 would only be appropriate if the range of magnitude of the independent variable in the longer time series was within the range from 1993-2002 and if the regression relationship was invariant over the longer time period.

We repeated the hind-cast procedure with our hydroacoustic and mile-days of milt datasets and found large disparities in biomass estimates compared to those determined by Thomas and Thorne (2003). The calculated values of biomass from Thomas and Thorne's regression ($B = 697M$, where B is calculated biomass, and M is mile-days of milt, Thomas and Thorne 2003) and a regression from our data ($B = 554M$, $r^2 = 0.62$, $P < 10^{-5}$, Fig. 8) do not agree. According to their back calculation, they concluded that the population showed a general increase to a peak in 1988 of about 100 000 mt. However, from our regression the estimate in 1988 (mile-days = 236.9) is in excess of 130 000 mt and using their regression the peak was more than 160 000 mt.

A possible explanation for this discrepancy could be from the use of miles of milt rather than mile-days of milt in the back-calculation by Thomas and Thorne (2003). As a measure of male spawning activity, one can use either miles of milt production or mile-days of milt production. Historical analysis from the ASA has shown that mile-days of milt data are more useful for estimating spawning biomass than the miles of spawning. We found that the peak in

1988 was about 100 000 mt using the miles of milt ($B = 720Mi$, B-biomass, Mi-miles of spawn, $r^2 = 0.62$, $P < 10^{-5}$, Fig. 8) and the estimates of biomass are lower than the ASA for most of the pre-1993 time series, which follows the results of Thomas and Thorne (2003). Further, from 1995-2004 the correlation between hydroacoustic biomass and miles of milt is virtually identical to the correlation between hydroacoustic biomass and mile-days of milt. Despite the nearly indistinguishable correlations between hydroacoustic biomass and the two measures of milt production, the regressions give highly variable biomass estimates beyond the range of the hydroacoustic time series (Fig. 8). Thus, there is no significant basis to choose one of the regressions over the other.

Not only does hind-casting fail to provide reliable estimates outside the range of hydroacoustic data, but using only mile-days of milt during years with robust hydroacoustic data (1995-2004) results in large departures between the observed and estimated values. Indeed, even in a period of greater certainty (as compared to pre-1995, Fig. 8), the observed survey biomass falls outside the 95% confidence 20% of the time (2000, 2003, Fig. 9), and is either not included or contained in the extreme tails of the confidence intervals 60% of the time (1995, 1997, 1998, 2000, 2001, 2003, Fig. 9). Therefore, the model presented by Thomas and Thorne (2003) fails to properly estimate observed survey values within the range of known data. With such failure within the range of known data, it can only be concluded that the model will also break down in a range that is ten times as variable.

The largest data conflict exposed in this analysis is between the spawning age composition dataset and the hydroacoustic surveys. The magnitude of this conflict depends on the choice of weight given to either dataset's likelihood component in the objective function. Sensitivity analysis showed that this conflict was not resolved by treating the hydroacoustic biomass as an index of spawning biomass instead of total biomass. Other datasets also have conflicts with the hydroacoustic data, such as the egg survey (Fig. 5).

A key issue of PWS herring dynamics is when did the decline in PWS herring actually begin? Results from Thomas and Thorne (2003) suggested that the rapid decline in herring biomass began in 1989, the year of the oil spill, as opposed to 1993, the year that the decline was recognized by fisheries managers. Population trends during this time from other data sources are more complicated. Within the ASA model, there are two datasets that contribute strongly to estimates of spawning biomass around 1989: mile-days of milt and the egg deposition survey. Thomas and Thorne found that the egg deposition estimates were in general agreement with their mile-days extrapolation except for 1990-1992. The conflict between the two datasets in 1990-1992 is reflected by a decrease in mile-days of milt starting in 1988 (trend enclosed by the solid ellipse, Fig. 10), while the egg survey does not display a decrease until 1993 (enclosed by the dashed ellipse, Fig. 10). The ASA fitted estimates of these two variables (with $\lambda_H = 0.5$, mile-days solid line, egg deposition dashed line, Fig. 10) synthesized from all the datasets, show that the estimated decline in both mile-days of milt and egg deposition do not occur until after 1992. There is some evidence of a decline in the population in 1989; however, this decline is not as substantial as the decline after 1992. An alternative explanation is that natural mortality of herring increased in 1989 and remained elevated until the crash of 1993 that had even higher natural mortality. This hypothesis remains viable under the ADF&G weighting scheme. Though we prefer the baseline data scheme because of its more equal weighting of datasets, there is no hard evidence to choose one scheme over the other.

Thomas and Thorne (2003) suggested the ASA model's limitations led to overestimation of herring abundance from 1989-1993, resulting in large mortality from over-harvesting. These

limitations included the ASA dependence on the egg deposition dataset and assumptions of constant mortality over the subsequent year. We have shown the ASA is not just dependent on the egg deposition, but on all datasets relevant to PWS herring and such synthesis of historical information lends stability to parameter estimation and avoids errors due to rapidly collected data from a single source. Their second suggested limitation is incorrect. The ASA model does not need to hold mortality constant for any age group of fish after 1989 and we have shown that estimating mortality for most cases results in highly unstable parameter estimates. Rather, natural mortality is inferred from disease information and projected based on understanding of the progress of disease over an 11 year period. Nevertheless, it is true that a change in disease or biomass condition after the previous year could lead to inaccuracies in the spawning biomass used for making management decisions. How large these inaccuracies could be is not clear.

The main strength of the ASA model is that it integrates numerous data sources to estimate key population parameters of the PWS herring stock. The model has been useful for understanding population dynamics in showing that multiple disease events and low recruitment have adversely affected the population since 1993. It has been useful in fishery management, because spawning biomass is used to decide whether to open the fishery and how much fish can be caught. By comparison, Thomas and Thorne (2003) argued that the acoustic survey alone should be used as the primary management tool. One advantage of their approach is that large changes in the population early during the winter would not be reflected in the ASA model, which uses data from previous years. A disadvantage of this approach is that the robustness of the ASA model in synthesizing datasets and exposing data conflicts would be disregarded. Further, ignoring over a decade of modeling and research into variables affecting the PWS herring population does not seem precautionary (O'Riordan, 1992; Dovers and Handmer, 1995). There is a straightforward middle ground, in which the ASA model (including historical hydroacoustic data) would continue to be used as a pre-season tool, but that results from the current year's hydroacoustic survey could be examined for the possibility of emergency action if there appears to be a major change in the population. This protocol has been followed by ADF&G since the late 1990s.

Each of the datasets used in the PWS assessment model has limitations. In theory, the most accurate assessment of the spawning population should come from the egg deposition survey (J. Schweigert, pers. comm.). However, these data have measurement errors due to the difficulty of detecting and locating all of the spawning events. Further, the accuracy of the deposition estimate can depend on predation, dispersal, and loss due to surf and wave action on the spawning beds (Bishop and Green, 2001; Rooper *et al.*, 1998). The collection of miles of milt data depends largely on weather conditions impacting the ability of aerial detection of spawning sites. This estimate can also be affected by spawning that occurs in deep water where the milt is not observable (J. Schweigert, pers. comm.). The hydroacoustic biomass estimate is contingent on all of the spawning fish being in the survey area. The accuracy of this method will be affected by the mobility of the fish schools, the temporal effects of age-dependent spawning, and the choice of target strength for herring whose size-at-age is variable over time. The age composition data is dependent on the selectivity of the gear and the timing of the survey since older fish tend to spawn prior to younger fish (J. Schweigert, pers. comm.).

Frequently, the magnitude of these measurement errors cannot be determined by sampling or experiments. Statistical theory does not provide an *a priori* method to estimate the weights of multiple datasets. When data conflicts arise, assessment results often depend on the choice of weighting that the researcher must make. This study illustrates that data conflicts

cannot always be resolved, but they can be explicitly identified so that the uncertainty in estimation is exposed.

Acknowledgments

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Tables

Table 4.1. Disease prevalence indices of VHSV and *Ichthyophonus hoferi* in PWS Pacific herring.

	ages 3-4	ages 5-9
Year	VHSV-ulcer	*I. hoferi*
1994	1.04%	7.47%
1995	0.14%	11.98%
1996	0.00%	11.35%
1997	9.40%	10.24%
1998	0.60%	15.19%
1999	1.05%	11.22%
2000	0.00%	11.15%
2001	0.01%	20.00%
2002	0.14%	15.79%
2003	0.01%	30.30%
2004	0.05%	16.42%

Table 4.2. Hydroacoustic estimates of herring biomass in metric tons. Only the spring values were used due to lack of data on fall acoustic surveys.

YEAR	Spring (mt)
1995	13 284
1996	23 000
1997	40 002
1998	17 655
1999	20 324
2000	7281
2001	6384
2002	10 700
2003	27 100
2004	19 098

Table 4.3. Notation used in the formulae for the age-structured assessment model.

Observed quantities:

Notation	Description
$C^i_{a,t}$	catch in the i th fishery at age-a in year-t
P_k	proportion of impounded herring killed
$w_{a,t}$	weight-at-age in year-t
$\Theta^i_{a,t}$	age composition in the i th dataset at age-a in year-t
$f_{a,t}$	fecundity at age-a in year-t
$x_{i,t}$	disease prevalence variables
H_t	hydroacoustic survey biomass in year-t
$\widetilde{E}_t$	egg deposition survey in year-t (in trillions of eggs)
$\widetilde{M}_t$	mile-days of spawn in year-t
$\%F_t$	percent of females present in the spawning population

Quantities derived from estimated parameters:

Notation	Description
$N_{3,t}$	age-3 recruits in year-t
$N_{a,1980}$	initial abundance for all ages
V_a	gear vulnerability of age-a with parameters ω and ξ
$mat_{a,t}$	maturity at age-a in year-t with parameters δ and ε
$M_{a,t}$	mortality with disease parameter β
$S_{a,t}$	survival of age-a in year-t
$\hat{\Theta}^i_{a,t}$	estimated age composition
$\hat{\widetilde{E}}_t$	estimated egg deposition in year-t
$\hat{\widetilde{M}}_t$	estimated mile-days of milt in year-t with parameter ψ
$\hat{H}_t$	estimated hydroacoustic biomass in year-t with parameter γ
R_t	Ricker estimates of recruits in year-t with parameters α and τ

Population Quantities

Notation	Description
$N_{a,t}$	total abundance at age-a in year-t in millions of fish
$PB_{a,t}$	pre-fishery spawning biomass at age-a in year-t
$SN_{a,t}$	natural spawning population after spring fisheries at age-a in year t
SB_t	total spawning biomass in year-t (after spring fisheries)
B_t	pre-fishery biomass in year-t

Table 4.4. Maturity estimates of age-3 and age-4 fish from M1 at different weights (standard errors shown in parenthesis). The estimates from M2 are in the right column.

	Maturity of Age-3		
	M1 1980-1996	M1 1997-2004	M2 1980-2004
λ = 0.1	0.282 (0.056)	0.599 (0.15)	0.340 (0.065)
λ = 0.5	0.270 (0.059)	0.505 (0.12)	0.318 (0.065)
λ = 1	0.266 (0.054)	0.472 (0.13)	0.308 (0.064)
λ = 2	0.266 (0.054)	0.449 (0.12)	0.297 (0.046)
λ = 5	0.252 (0.06)	0.367 (0.14)	0.281 (0.066)
λ = 10	0.241 (0.067)	0.334 (0.17)	0.269 (0.062)
	Maturity of Age-4		
	M1 1980-1996	M1 1997-2004	M2 1980-2004
λ = 0.1	0.781 (0.13)	0.983 (0.14)	0.818 (0.12)
λ = 0.5	0.751 (0.13)	0.836 (0.16)	0.771 (0.12)
λ = 1	0.740 (0.12)	0.778 (0.17)	0.739 (0.13)
λ = 2	0.731 (0.12)	0.737 (0.17)	0.706 (0.13)
λ = 5	0.688 (0.13)	0.647 (0.22)	0.662 (0.12)
λ = 10	0.657 (0.13)	0.581 (0.27)	0.626 (0.13)

Table 4.5. Estimates of natural survival during 1989-1991, 95% confidence intervals, and AICc values from 3 model configurations [fixed at 78% (Model 1 or 4), estimated for all ages (Model 5), stratified by ages 3-4, 5-8 (Model 6)] and two weighting schemes [baseline (Models 1, 5a, 5b) and ADF&G (Models 4, 5b, 6b)].

Estimated natural survival			
M1	78%	M4	78%
M5a	87%	M5b	66%
M6a, 3-4	92%	M6b, 3-4	100%
M6a, 5-8	87%	M6b, 5-8	63%

95% confidence intervals			
M1	NA	M4	NA
M5a	(64%,100%)	M5b	(50%,75%)
M6a, 3-4	(43%,100%)	M6b, 3-4	(42%,100%)
M6a, 5-8	(42%,100%)	M6b, 5-8	(47%,76%)

AICc[a]			
M1	-292.4	M4	-159.2
M5a	-293.1	M5b	-164.2
M6a	-290.1	M6b	-165.3

[a] AICc for M0 is not presented because it can not be properly compared to other models which include the hydroacoustic dataset in the objective function.

Figures

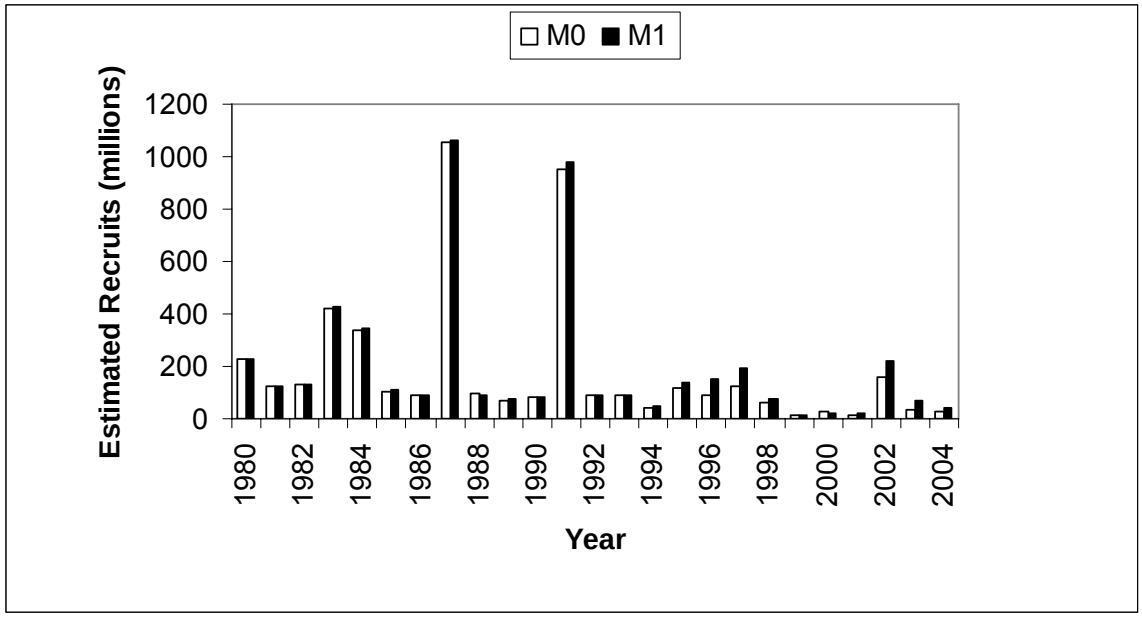

Fig. 4.1. Age-3 recruitment estimates (in millions of fish) from M0 and M1 at $\lambda_H = 0.5$.

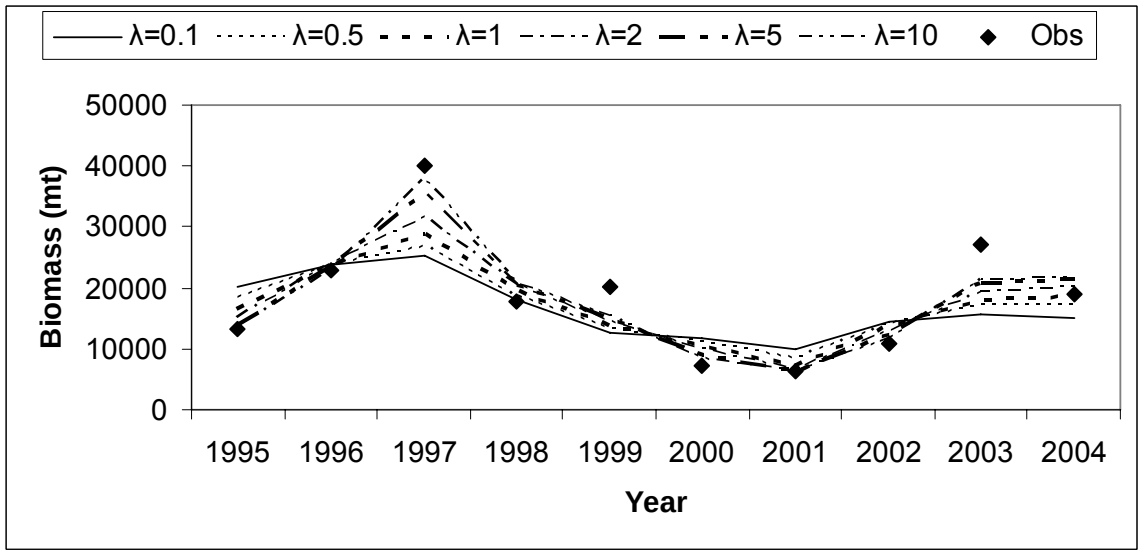

Fig. 4.2. Estimated hydroacoustic biomass (following eq. 6) from M1 over all weighting scenarios compared to observed values (diamond points) for the pre-fishery (spring) hydroacoustic biomass.

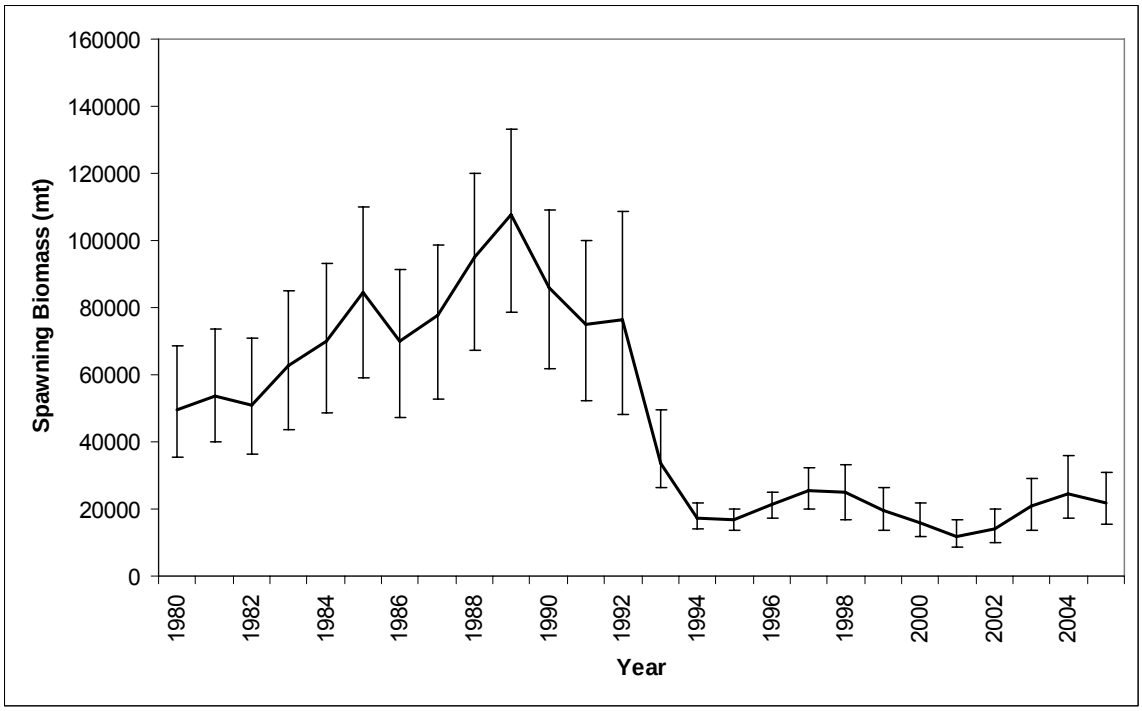

Fig. 4.3. Bootstrap of estimated spawning biomass with 95% confidence intervals from M1 (λ_H = 0.5).

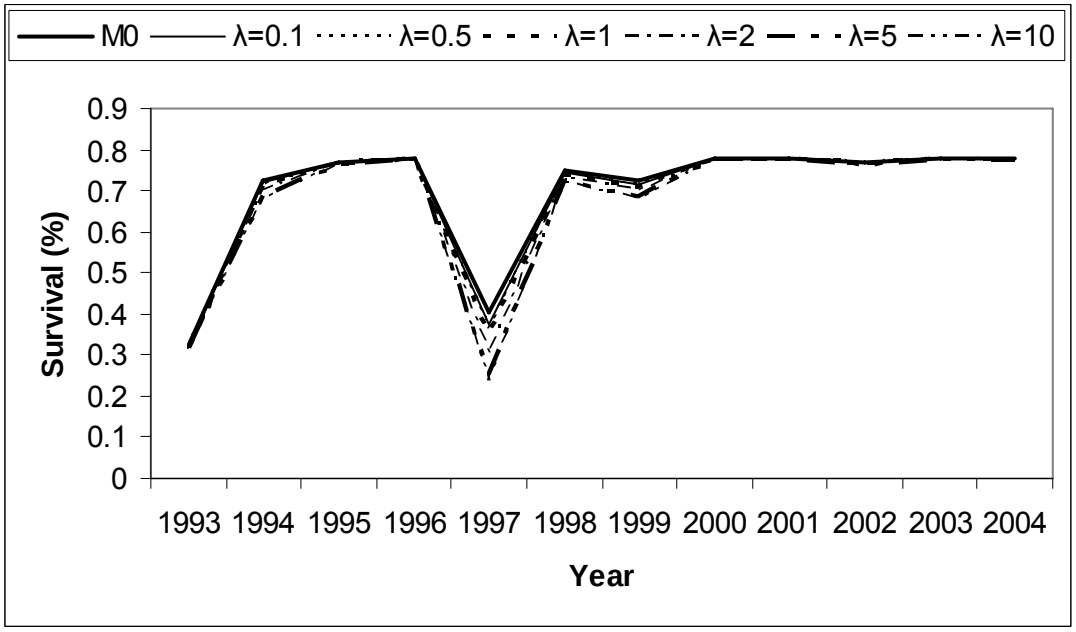

Fig. 4.4a. 1993-2004 survival estimates for ages 3 and 4 from M1**.**

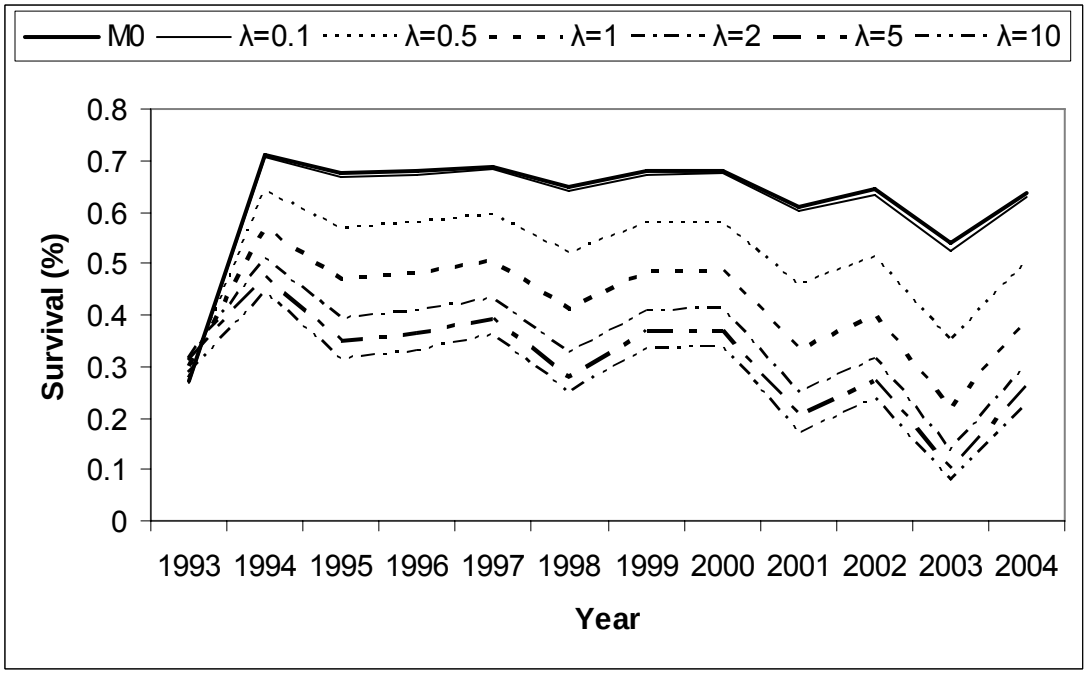

Fig. 4.4b. 1993-2004 survival estimates for ages 5-8 from M1.

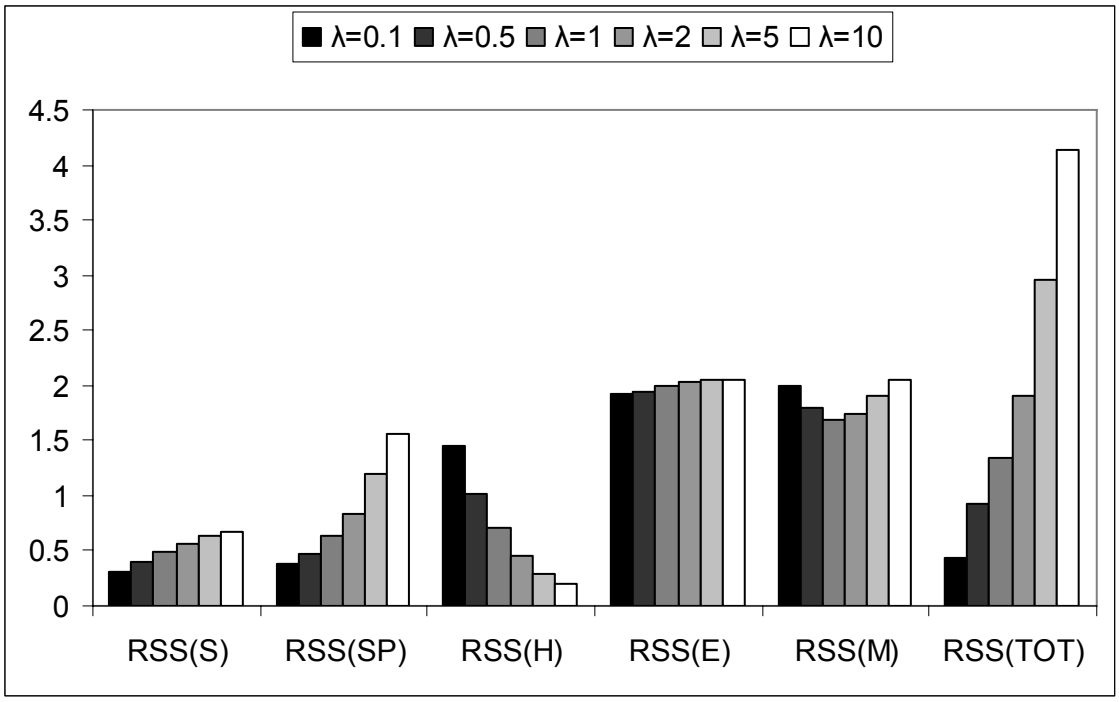

Fig. 4.5. Unweighted residual sum of squares for the estimated datasets as the weight (λ_H) on the hydroacoustic index is increased in M1 (S-Seine age composition, SP-Spawning age composition, H-Hydroacoustic, E-Egg deposition, M-Mile-days of milt, TOT-Total sum of squares). The total sum of squares has been scaled down for comparison (by 3).

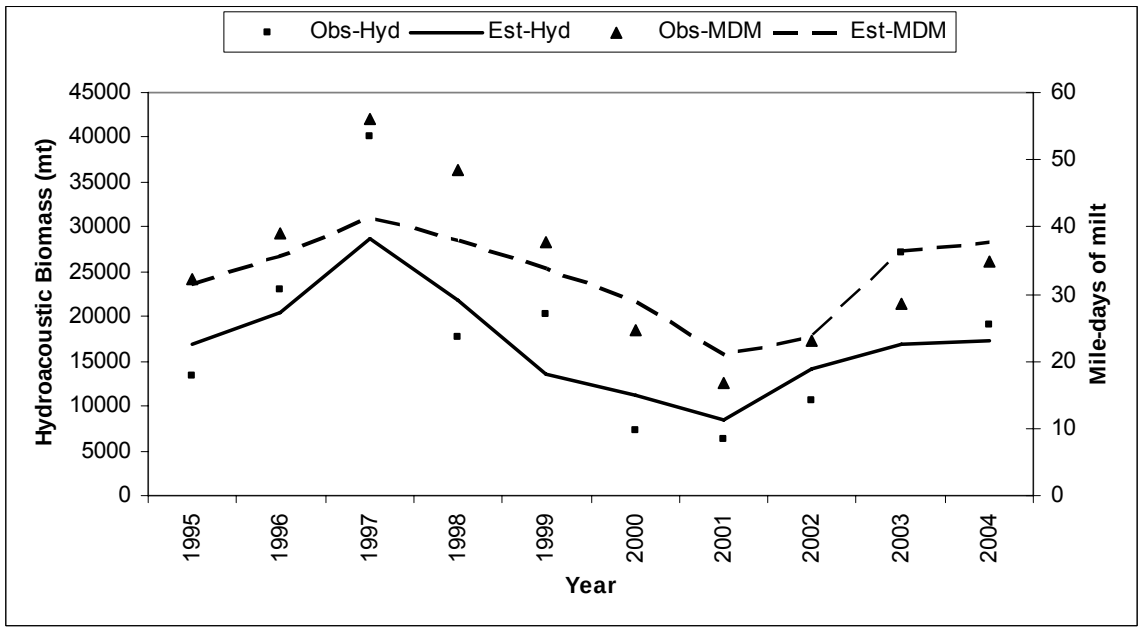

Fig. 4.6. Observed and estimated values from M1 of Mile-days of milt (MDM) and Hydroacoustic biomass (Hyd) at $\lambda_H = 0.5$.

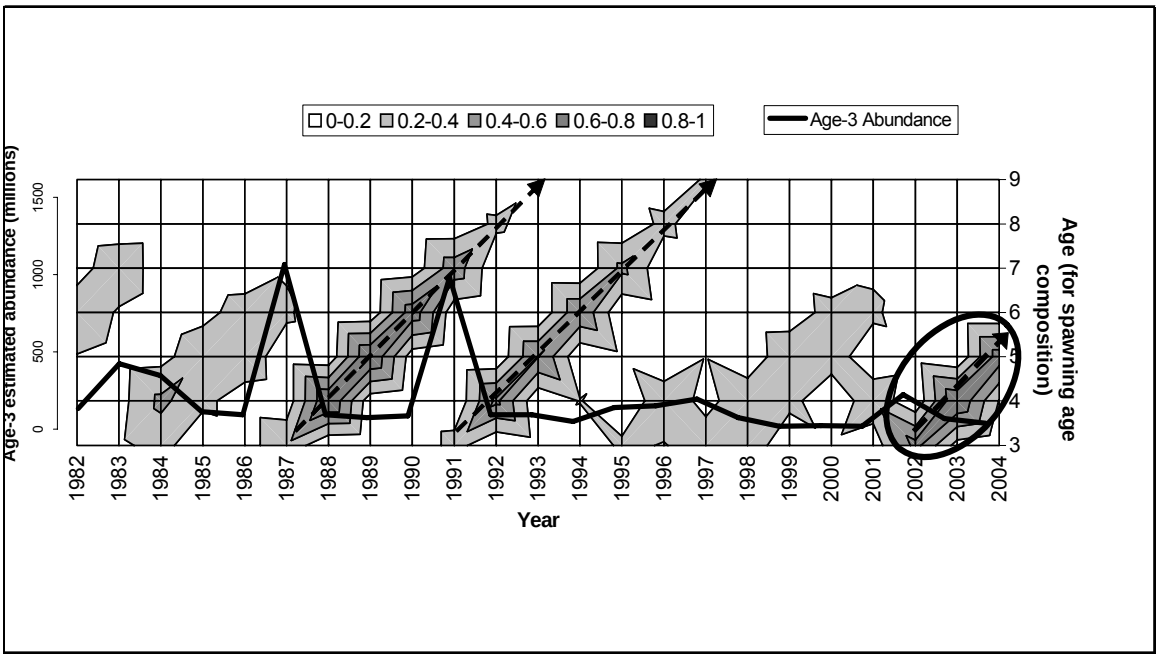

Fig. 4.7. Observed spawning age composition as it relates to initial (age-3) abundance estimates in M1. In the topographic representation of the spawning age composition each shade corresponds to a 20% increase in proportion of spawners.

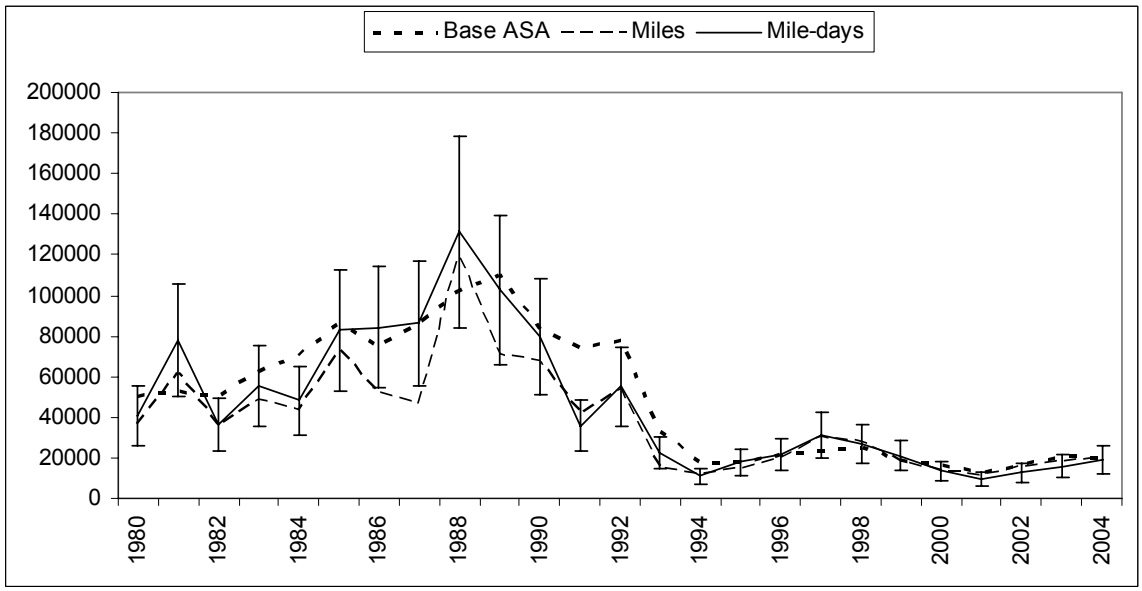

Fig. 4.8. Predicted hydroacoustic biomass obtained from regression with miles of spawning as compared to M0 (dotted line). The series Miles represents a regression with miles of milt (B = 719.64·M, dashed line), and the series Mile-days with mile-days of milt (B = 554.1·M, solid line). 95% confidence intervals have been included with the Mile-days series.

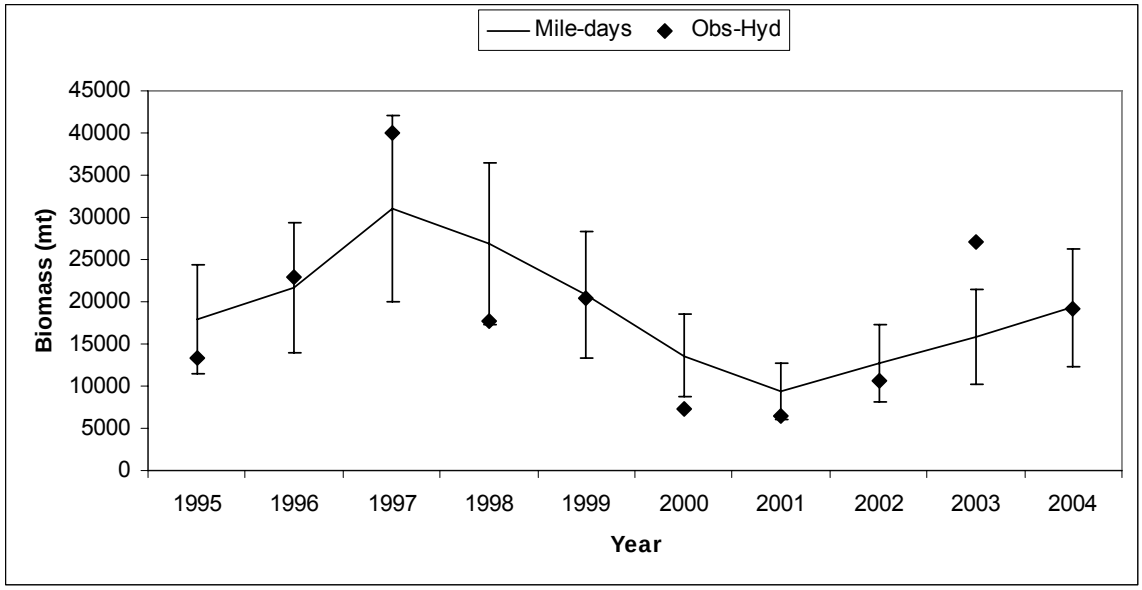

Fig. 4.9. Observed hydroacoustic survey biomass (series Obs-Hyd, diamond points) compared to the regression calculation using Mile-days of milt (steady line) to estimate biomass with 95% confidence intervals.

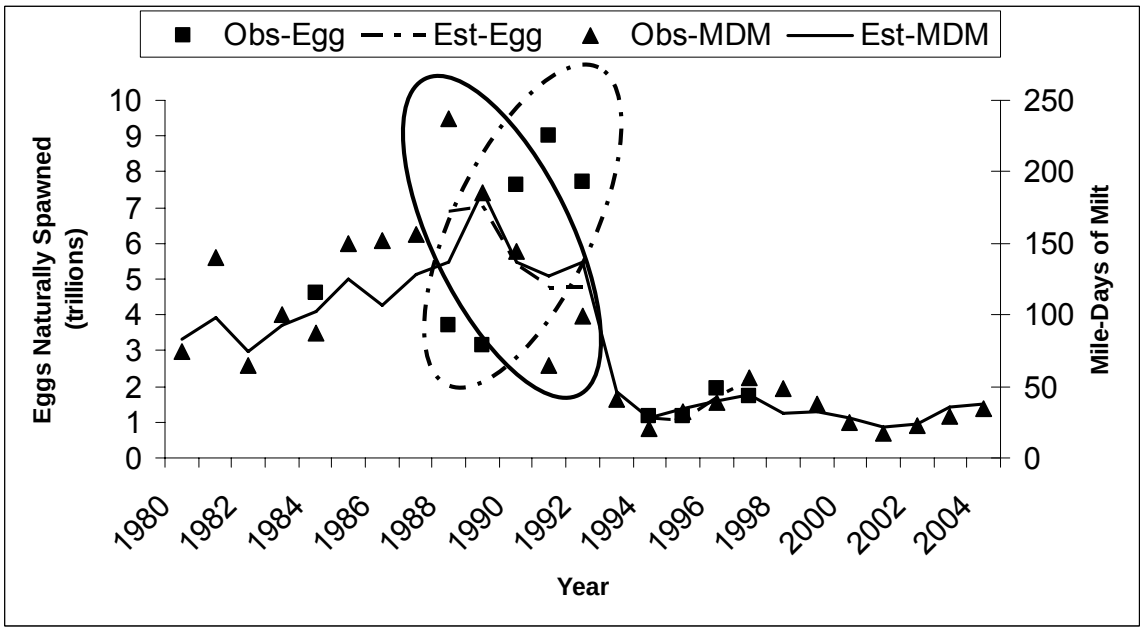

Fig. 4.10. Observed and estimated mile-days of milt compared to the egg deposition survey. Observed values are charted as points (Egg-square, Mile-days-triangle) while M1 estimated values are lines (Egg-dashed, Mile-days-solid).

Chapter 5

Role of *Ichthyophonus hoferi,* viral hemorrhagic septicemia virus, and cutaneous ulcers in preventing recovery of a Pacific herring (*Clupea pallasi*) population

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Abstract

Failure of Pacific herring population recovery is a result of age-dependent mortality from three pathogens: the mesomycetozoan *Ichthyophonus hoferi*, viral hemorrhagic septicemia virus (VHSV), and filamentous bacteria (associated with cutaneous ulcers). Beginning in 1993 with a severe outbreak of VHSV and ulcers, epidemics have cycled through the Pacific herring population of Prince William Sound, Alaska, U.S.A., about every 4 years. Epidemics of VHSV-ulcers in 1993 and 1998 were followed by epidemics of *I. hoferi* that peaked in 2001 and 2005. Comprehensive epidemiological study from 1994 – 2002 included complete necropsy examination of 230 – 300 fish each spring and 40 – 160 fish each fall (total n = 3173 fish). Continued annual spring sampling from 2003 to 2006 (n = 150 – 308 per year) analyzed fish age, cutaneous ulcers, and gross heart lesions consistent with *I. hoferi* infection. Mortality was best estimated, through modifications of an age structured assessment model, using (1) a disease index that combines the prevalence of VHSV and ulcers, and (2) the prevalence of severe cases of *I. hoferi*.

Keywords – Pacific herring, *Clupea pallasi*, Prince William Sound, *Ichthyophonus hoferi*, disease, population abundance, modeling

Introduction

Epidemics in marine organisms have increased in number and frequency during the last 30 years (Harvell et al. 1999; Sherman 2000), but we are only beginning to unravel the complex interactions of disease with predation, recruitment, and environmental change (Harvell et al. 2002; Harvell et al. 2004). Among marine fish, studies have documented long-term population cycles (e.g., Patterson et al. 2005), and population decline has been associated with disease epidemics worldwide. Most epidemics in marine fish have been associated with *Ichthyophonus*

hoferi (e.g., Daniel 1933; Sindermann 1958; Mellergaard and Spanggaard 1997), but other epidemics are more consistent with a viral outbreak (Tester 1942; Crockford et al. 2005) or bacterial pathogens (Overton et al. 2003). Less studied are the effects of endemic disease on survival and recruitment over relevant time scales at the population level. The association of a single pathogen in a marine fish population has been followed for up to four years (Patterson 1996), but longer-term effects of disease on marine fish populations had not been examined until our study of the Pacific herring (*Clupea pallasi*) population of Prince William Sound, Alaska, was initiated in 1994 (Marty et al. 2003).

Members of the Order Clupeiformes—herring, sardine, anchovy, menhaden, and shad—are among the most abundant fish species worldwide. The Pacific herring population of Prince William Sound, Alaska, provides an excellent model for studying the role of disease in population dynamics of this group of fish. Pacific herring are schooling fish that historically ranged throughout coastal regions of the North Pacific. They are an important component of the ecosystem as secondary consumers, and they also serve as high quality prey for many marine birds and mammals (Womble et al. 2005; Suryan et al. 2006). In Prince William Sound, Pacific herring first spawn when 3 – 5 years old, live up to 15 years, and weigh up to 300 g. Mature fish spawn every year, but strong recruitment into the adult population rarely occurs more often than every four years. The most accurate biomass estimates are made in early April, when fish aggregate in shallow water to spawn. After spawning, fish disperse and do not reaggregate near spawning areas until late fall. Feeding is minimal during the winter (Foy and Norcross 2001).

A severe epidemic killed about 75% of the Prince William Sound Pacific herring population over the winter of 1992 – 1993 (Fig. 5.1). Fish surviving the 1993 epidemic were lethargic, and many had external hemorrhage. The first-ever Pacific herring isolates of the North American strain of VHSV were cultured from pooled samples from these fish (Meyers et al. 1994). Subsequent laboratory study confirmed that VHSV can kill juvenile fish (Kocan et al. 1997), but the relation of VHSV to population decline of adult fish in Prince William Sound in 1994 could not be determined. Because disease involves poorly understood interactions between three major variables: the host, the pathogen, and the environment (Hedrick 1998), the mere presence of a pathogen is not equivalent to disease. Therefore, comprehensive disease study was initiated in 1994 to determine the cause of ongoing population decline. When the population failed to recover, comprehensive study was continued through 2002, with continued annual focused study through 2006. The most plausible hypothesis for the 1993 epidemic is that lack of food resources during the summer of 1992 led to poor body condition in the spring of 1993, increasing the risk for an epidemic (Pearson et al. 1999; Carls et al. 2002; Marty et al. 2003).

From 1994 – 2000, population change was best modeled using a disease index that combined the prevalence of VHSV and ulcers. Survival was less in years where the virus-ulcer index was high, and survival was greater in years when the virus-ulcer index was low (Marty et al. 2003). *Ichthyophonus hoferi* was a significant pathogen for individual fish, and the pathogenicity of *I. hoferi* for Pacific herring was confirmed through laboratory study (Kocan et al. 1999). However, for the first 7 years of field study prevalence of *I. hoferi* was positively correlated with fish age but not with population change (Marty et al. 2003). Indeed, mean sample age and *I. hoferi* prevalence were highly correlated ($r^2 = 0.80$, Fig. 5.2). This changed in the fall of 2000. Age-adjusted prevalence of *I. hoferi* increased by nearly 50% between the spring and fall of 2000, remained high through the spring of 2001, then dropped back to endemic levels by fall of 2001. The prevalence of *I. hoferi* again increased from 2003 until 2005, when it reached the highest levels recorded in 13 years of study. The objective of this paper is to

describe these recent outbreaks of *I. hoferi* and their integration into a population model that combines the virus-ulcer disease index with the prevalence of severe cases of *I. hoferi*. We introduce the concept of a "disease regime" to help explain persistent failure of population recovery.

Methods

Major diseases

This study builds on previous work with the Pacific herring population of Prince William Sound, Alaska. Sample and analysis methods during the final 2 years of comprehensive study (2001 and 2002) were maintained as constant as possible to methods established during the first 7 years of study (Marty et al. 1998; Davis et al. 1999; Marty et al. 2003). Pacific herring, were sampled at random and subjected to complete necropsy during two seasons: 1) spring (March and April) – 1994 through 2002, sample size ranged from 233 to 300 fish per year; and 2) fall (October and November) – 1995 through 2001, sample size ranged from 40 to 160 fish per year. From 1994 – 2000 fish were all sampled from bays on the north end of Montague Island (e.g., Stockdale Harbor, Rocky Bay, and Zaikof Bay). During the spring of 2001 and 2002, fish numbers were more evenly distributed between the Northeast and Montague areas of Prince William Sound; therefore, spring sampling was split between the Northeast region (2001, n = 220; 2002, n = 200) and the north end of Montague Island (2001, n = 80; 2002, n = 100). Samples collected in November 2001 were split between the Northeast region (n = 40) and the north end of Montague Island (n = 60). In all, 3173 Pacific herring were subjected to complete necropsy. Analysis for each fish included gross examination, histopathology of 10 organs, and culture of head kidney and spleen for virus isolation. From 1994 – 1996, samples were frozen at –80 °C within 72 hours of collection and later thawed for inoculation onto cell culture. Experimental evidence during that time demonstrated the potential for significant but unpredictable loss in titer resulting from freezing; therefore, all samples analyzed after 1996 were prepared for cell culture without previous freezing. Gross and microscopic lesions were scored as none (0), mild (1), moderate (2), or severe (3). Body weight, length, and age (from scales) determined from each fish linked our data with the historical database compiled by the Alaska Department of Fish and Game on this population since the early 1970s. For all fish with severe external lesions, kidney was cultured for bacteria (all were negative). To determine the sample prevalence and severity of infection with *I. hoferi*, a sum-Ichthyophonus score was calculated by adding the severity scores for all organs examined; summed scores from 10 organs yielded a maximum possible sum-Ichthyophonus score of 30.

Comprehensive study from 1994 – 2002 provided the validation needed to continue focused study by fisheries management biologists from 2003 through 2006. Focused study after 2002 included determination of fish age (from scales), semiquantitative scoring of the external lesion focal skin reddening as none (0), mild (1), moderate (2), or severe (3), and gross examination of the heart for white foci consistent with *I. hoferi* infection; samples were analyzed only during early spring, near the time of spawning (2003, n = 308; 2004, n = 250; 2005, n = 150; 2006, n = 150). Virus culture for VHSV was not done from 2003 – 2006 because prevalence of ulcers was less than 1% and previous study provided evidence that low ulcer prevalence was highly correlated with low VHSV prevalence (Marty et al. 2003).

Disease parameters were chosen for modeling purposes based on previous results and biological relevancy. The best explanatory variable for data from 1994 – 2000 was an index calculated by multiplying the relative frequency of VHSV+ fish with the relative frequency of

fish with severe focal skin reddening or ulcers (Marty et al. 2003). Because some VHSV+ fish without ulcers had evidence of disease, the prevalence of ulcers used to calculate the disease index was limited by a lower bound of 0.5%. For example, in 1997 the sample VHSV prevalence was 14.6% but the sample ulcer prevalence was 0.0%; the VHSV-ulcer index used in the model (0.073%) was calculated by multiplying 0.146 by the lower bound of 0.5% for ulcer prevalence. For *I. hoferi*, two variables were used to describe cases that were likely to be significant on a population scale. From 1994 – 2002, we used the sample prevalence in which the sum-Ichthyophonus score was greater than 10. From 2003 – 2006 we used the gross-Ichthyophonus score as the best estimator of significant *I. hoferi* prevalence; the gross-Ichthyophonus score was also used to estimate the 10-organ histopathology prevalence for 2003 – 2006 based on a linear regression between gross-Ichthyophonus score and 10-organ histopathology score in the 6 years in which both were determined (1997 – 2002).

Population modeling of disease

An age-structured assessment model (Quinn et al. 2001; Marty et al. 2003) is updated with four more years of data to evaluate the impact of disease on population abundance. The model provides an estimation framework to integrate the various sources of information about Pacific herring in Prince William Sound from 1980 – 2004, including age compositions from the purse-seine fishery and spawning surveys, egg production estimates, mile-days of milt from aerial surveys (Quinn et al. 2001), and a biomass index from hydroacoustic surveys (Hulson et al. In review). These observations are compared to comparable model quantities in a least squares setting to obtain parameter estimates of recruitment, abundance, and biomass.

Least squares components are included for the purse seine age composition (in years when the fishery was open: 1980 – 1988, 1990 – 1992, 1997 – 1998), spawning age composition (since 1982), egg survey estimates on a logarithmic scale (from 10 years between 1984 and 1997), the milt index on a logarithmic scale (all years 1980 – 2004), hydroacoustic biomass measurements (1994 – 2004), and deviations from a Ricker spawner-recruit curve. Model selection follows established procedures (Anderson et al. 1998), including the use of an information criterion, AICc (Akaike Information Criterion, corrected) as a model comparison statistic. Details about the calculation of AICc are given in Hulson et al. (in review). The difference Δ between a given model and the model with the lowest AICc value is the primary statistic for choosing appropriate models. Following (Anderson et al. 1998, p. 128), we reject models with $\Delta > 4$ and include candidate models with $\Delta \leq 4$. We further eliminate models if they are not biologically realistic.

We assume that disease increases the natural mortality M linearly for all adult ages (ages 3 and older), or

$$M = M_0 - \sum_i \beta_i x_{it} \,, \tag{1}$$

in which M_0 is instantaneous natural mortality from sources other than disease (assumed constant), x_{it} is the ith disease prevalence variable in year t, and β_i is a disease coefficient governing the magnitude of the effect. Equivalently, disease lowers the corresponding natural survival $S = \exp(-Z)$. This is a slight modification of the former model (Quinn et al. 2001; Marty et al. 2003) in which survival was linearly related to disease made to enhance estimation robustness.

The age-structured assessment model contains information about the Pacific herring fisheries in Prince William Sound, Alaska, which include purse-seine, gillnet, and pound

fisheries in the spring (mainly for roe), and a food and bait fishery in the summer and fall. Recruitment occurs at age 3 and there are parameters for recruit abundance, $\{N_{3,t}\}$, for all years (and for all abundances in the first year, 1980). From these parameters and the survival model (1), abundance at each subsequent age a and year t is estimated from the equation

$$N_{a+1,t+1} = \left[\left(N_{a,t} - C_{\text{seine}} - C_{\text{gill}} - C_{\text{pound}}\right) \times S_t^{1/2} - C_{\text{food}}\right] \times S_t^{1/2}, \quad (2)$$

in which N = abundance, C = annual catch-at-age, and $S^{1/2}$ = half-year survival, calculated as the square root of S. In equation (2), we assume that the disease variable measured in year t+1 at the time of spawning affects the population in the last half-year of year t (before recruitment, spawning, and spring fisheries) and the first half-year of year t+1. Another assumption is that total catch for the seine fishery and catch-at-age for the other fisheries are assumed measured without error in the model. The estimated number of spawners is the mature abundance after the spring fisheries, or

$$\text{Spawners}_t = \sum_{\text{a}} \text{mat}_a \left(N_{a,t} - C_{\text{seine}} - C_{\text{gill}} - C_{\text{pound}}\right) \quad (3)$$

in which mat is the proportion mature. Spawning biomass is similar to equation (3) but also multiplies by weight-at-age.

The other parameters in the model are logistic selectivity parameters for the spawning survey for the purse seine fishery, natural mortality for the plus group (aged 9+) relative to other ages, a calibration coefficient that converts the milt index into total egg production, a calibration coefficient that converts hydroacoustic biomass to total biomass, and parameters for estimated disease prevalence in 1992-1993 (because there is only very limited information from this period; Quinn et al. 2001). Natural mortality M_0 from all other sources was assumed to be 0.25 in accord with usual Pacific herring assessments in Alaska. Further details about the model are given in Hulson et al. (in review). The maturity variable mat_a in (3) is set to 1 for ages 5+ and estimated for ages 3 and 4 separately. In preliminary analyses of the extended dataset, we noticed a positive trend in the spawning age composition residuals for ages 3 and 4 after 1997. This trend could only be removed by estimating separate maturity parameters for the time period 1980 – 1997 and 1998 – 2003.

Seven model scenarios were constructed to examine which population processes were important in explaining Pacific herring dynamics. Scenario 0 is the null model, in which disease information is not used, so that natural mortality is assumed constant and equal to 0.25. Five scenarios use combinations of the VHSV-ulcer index and the prevalence of significant *I. hoferi* (Table 5.1); the first four scenarios are stratified by age group (i.e., ages 3 and 4 in one group; ages 5+ in the other group): (1) both disease variables are included; (2) just the VHSV-ulcer index is used; (3) just the significant *I. hoferi* prevalence is used; (4) VHSV-ulcer index is used for ages 3 and 4, and significant *I. hoferi* is used for ages 5+; and (5), the VHSV-disease index pooled over all ages is used. Finally scenario 6 is the same as scenario 4, except that it uses the full prevalence of *I. hoferi* from Marty et al. (2003) that was unadjusted for threshold effects (i.e., all fish with sum-Ichthyophonus > 0 were used to calculate prevalence; for 2003 and 2004, gross prevalence of *I. hoferi* in the heart was used as the best estimate of significant *I. hoferi*).

Results

The first major epidemic caused by *I. hoferi* in the Prince William Sound population was detected in fall 2000. By spring 2001, the prevalence of *I. hoferi* (38%) was more than 50% greater than it had been in any previous year of study (Fig. 5.3), or in any other Alaskan population studied in the past decade (e.g., populations from Sitka, Craig, and Auke Bay; Carls et al. 1998; Davis et al. 1999; G.D. Marty, unpublished observations). By spring 2002, prevalence of *I. hoferi* had returned to historical levels (Fig. 5.3). Before 2001, prevalence of *I. hoferi* steadily increased within individual year classes nearly every year (Fig. 5.4). Likewise, the increase in *I. hoferi* prevalence was greatest from spring 2000 to spring 2001 for nearly all year classes. For those year classes that had enough survivors in spring 2002 to contribute to the overall sample, *I. hoferi* prevalence decreased from 2001 to 2002 in more than one year-class.

Gross analysis of the heart for white foci consistent with *I hoferi* infection provided an acceptable approximation of *I. hoferi* prevalence determined from histopathology of 10 organs. A simple first order linear regression of gross *I. hoferi* prevalence (x) vs. prevalence determined by 10-organ histopathology (y) for spring samples from 1997 through 2002 yielded the equation $y = 1.81x + 2.05$ ($r^2 = 0.89$; Fig. 5.5). A second order regression better fit the data ($r^2 = 0.99$) but was not considered reliable for extrapolating beyond the data, as needed for 2005 when gross-Ichthyophonus prevalence was 25% (Fig. 5.3).

Sample prevalence of viral hemorrhagic septicemia virus and skin ulcers in spring 2001 was slightly higher than in 2000, but still at historically low levels (Fig. 5.3). In 2002, the prevalence of VHSV (14%) was among the highest of the 9 years studied, but relatively low ulcer prevalence (0.7%) provided evidence that adult fish were not significantly impaired by the VHSV outbreak; this pattern was similar to 1997 (Fig. 5.3). In 2002, recruitment of the 1999 year-class was relatively strong, providing abundant 3-year olds (Fig. 5.6). The VHSV-ulcer outbreaks of 1994 and 1998 were associated with the greatest prevalence of depleted mesenteric fat stores. By comparison, years with high VHSV but low ulcer prevalence (1997 and 2002, Fig. 5.3) had the greatest proportion of 3-year-olds in the population, but prevalence of depleted fat stores was unremarkable (Fig. 5.6).

Over time, VHSV-ulcer associated disease in Prince William Sound Pacific herring oscillated in a roughly 4-year cycle, the amplitude of which decreased with each cycle since 1993 (Table 5.1). VHSV-ulcer outbreaks tend to be more common when the prevalence of young fish in the population is high. Prince William Sound Pacific herring had a major VHSV-ulcer outbreak in 1993, moderate disease in 1997–1998, and mild disease in 2002. As the amplitude of VHSV-ulcer outbreaks decreased over time, we have evidence that *I. hoferi* outbreaks may also be cyclic. The *I. hoferi* peak prevalence in 2005 completed a 4-year cycle from the last peak in 2001 (Fig. 5.3).

Population modeling of disease

Summary statistics of the fits of the seven models to the data are given in Table 5.2. There were 324 observations among the six datasets. The numbers of parameters among the seven models ranged from 38 to 46. There were some differences in the unweighted RSS components among models, with the highest values often occurring with the null model 0, indicating poorer fits to those datasets. The total weighted residual sum of squares, a measure of the overall lack of fit to the datasets, is much higher for model 0 than for the six models with disease information. The log likelihood values show the same relationships among models as RSS, except that higher values indicate better fitting models.

The AICc values used for model selection indicate substantial differences among models (Table 5.2). Compared to the null model 0 that used constant natural mortality after 1993, the disease models 1 – 6 fitted the available data much better. Model 4 is the most parsimonious model, in which the adjusted *I. hoferi* index is used for older ages and the VHSV index is used for younger ages. The other model with $\Delta \leq 4$ is Model 6, which instead used the original *I. hoferi* index for younger ages.

Estimates of spawning biomass are generally similar among models (Fig. 5.7), presumably due to its trend being well determined from the time series for egg surveys, milt, and hydroacoustics. Overall, estimated spawning biomass increased until 1989, decreased somewhat until 1992, dropped precipitously in 1993, and has oscillated slightly at a low level near 20,000 mt since then.

Estimated recruitment in 1991 is much lower in Model 0 than the others, because its constant mortality requires fewer fish killed to be compatible with data sources (Fig. 5.8). There are some small differences in estimated recruitment among models after 1992. The overall pattern in recruitment is a recurring strong pulse every four years (1983, 1987, 1991) and then persistently low numbers after 1992.

Estimated natural mortality for ages 3–4 stands out from background natural mortality of 0.25 in 1993 and 1997 (Fig. 5.9a). All disease models produced similar estimates in 1993. Model 2 had the highest value in 1997, with slightly lower and similar values for Models 1, 4, and 6; all used VHSV indices. Models 0, 3, and 5 had low values near 0.25 in 1997; model 3 did not use VHSV indices and model 5 did not stratify by age when using VHSV. Estimated natural mortality for ages 5–9 stands out from background natural mortality for all years after 1992 in Models 1, 3, 4, 6 (Fig. 5.9b), with a range of levels from 0.5 to 1 after 1993. These values are traced to the use of the *I. hoferi* index. There appears to be an increasing trend in natural mortality of ages 5 + after 2000. All disease models produced similar estimates in 1993 at a level nearly identical to younger ages. The disease event in 1992–93 occurred across all ages, and it seems to be due to VHSV-ulcers. The disease event in 1997 – 1998 seems to be influenced by both VHSV-ulcers and *I. hoferi*. After 2000, disease affects mainly older fish and is due to *I. hoferi*.

The estimated mature proportions of age 3 and 4 fish after 1997 are substantially larger than those before (Fig. 5.10). This signifies that there has been greater proportion of younger ages that engage in spawning in more recent times.

The fit of the best model 4 to the datasets has no unwelcome patterns in the seine or spawning age composition residuals (not shown). The fit to the egg survey information is poor in the late 1980's, for unknown reasons. The fit to the milt information is fairly good, except for some lack of fit in the early 1990s. There is a strong data conflict between the egg survey and milt information during 1988 – 1992, which has not been resolved (Hulson et al. In review).

One remaining question is whether disease affected recruitment at age 3 since the early 1990s. We correlated log recruitment estimates with the 5 disease time series in Table 5.1 to answer this question. Correlation analysis was performed with no lag and then with a lag of 1 year in the disease information (Table 5.3). Correlations between the VHSV index and log recruitment with no lag are positive, but only for age group 5+ is it significant. No correlations are significant with a lag of 1. Correlations between *I. hoferi* and log recruitment with no lag and with lag 1 are not significantly different from 0. Because there was no significant and negative correlation between log recruitment and disease, there is no evidence to suggest that recruitment was negatively affected by disease.

Discussion

Spring 2001 marked the beginning of the third significant epidemic in the Prince William Sound Pacific herring population since the early 1990s. In the 1990s, high prevalence of VHSV and ulcers was associated with significant decline in population biomass (Quinn et al. 2001; Marty et al. 2003). By comparison, the epidemic of 2001 was associated with unusually high prevalence of *I. hoferi*. The cause of the increase in prevalence of *I. hoferi* is unknown, but it might be associated with high mortality in previous years. Before the 1993 epidemic, prevalence of *I. hoferi* in samples from Prince William Sound from 1989 through 1992 was as low as 3.4% (n = 60, mean age = 7.4 years, spring 1991 samples; Marty et al. 1999). At the height of the 1993 epidemic, prevalence of *I. hoferi* was only about 5% (Marty et al. 1998), but in 1994 *I. hoferi* prevalence increased to 23% (Quinn et al. 2001), despite little change in population age structure. We also have good evidence of VHSV-related population decline in 1998 that predated the fall 2000 increase in *I. hoferi* prevalence by two years.

Disease might significantly affect recruitment (Marty et al. 2003). Two of the lowest recruitment estimates on record, in 1994 and 1999, followed increased natural mortality of adults in 1993 and 1998. However, based on updated modeling results, there is no compelling biological relationship between ln recruitment and disease from 1994 to 2004. Previously, Marty et al. (2003) did find a significant relationship between VHSV-ulcers over all ages lagged one year and recruitment. Therefore, the relationship detected by Marty et al. (2003) was either spurious, or else there has been a change to no effect of VHSV-ulcers on recruitment after 2000.

There is also ambiguity in whether or not background mortality from other sources (such as predation) differs among age groups. While our data suggest that smaller, younger Pacific herring are more susceptible to predation, there is not much information in Prince William Sound to validate this hypothesis. From the results in this study, it cannot be ruled out that background natural mortality has become higher more recently and is greater for younger ages. The fact that Pacific herring abundance decreased dramatically in the mid-1990s raises the possibility that density-dependent increases in natural mortality have occurred with the potential decline in the ratio of Pacific herring as prey to the predator populations.

Also interesting is the apparent increase in the maturity on the spawning grounds inferred from the model fits to the spawning age composition data. It is possible that the reduction in Pacific herring abundance has increased either the rate of maturation or the migration of mature fish to the spawning areas. Nevertheless, it would be valuable in the future to investigate the maturity of Pacific herring in the population as a whole through field studies outside the spawning season.

How and at what age Pacific herring are naturally infected with *I. hoferi* needs further investigation. In another Pacific herring population, *I. hoferi* was identified in age 0 fish (Kocan et al. 1999). Prevalence within a year class consistently increases with age over time (Hershberger et al. 2002; Marty et al. 2003), and minimal change in age-class distribution over time in Prince William Sound Pacific herring is consistent with infected fish living for several years with *I. hoferi*. This pattern of increased prevalence with age could be a result of small numbers of fish in a year class being infected every year. Alternatively, large numbers of fish might be infected when young, but growth of *I. hoferi* in the host increases to diagnosable levels in only a small percentage of the fish each year; the relative number of fish reaching diagnosable levels of infection each year varies, perhaps as a result of different environmental conditions.

Nine years of intensive disease study dramatically increased our understanding of the interaction of disease with changes in population biomass, and comprehensive study provided the validation needed to implement ongoing annual study by management biologists. We have clearly established that disease information improves our ability to estimate population abundance, and that prevalence of any single pathogen is not sufficient to significantly improve estimates of population abundance except in catastrophic disease episodes like 1993. The advantage of long-term study was most obvious with *I. hoferi*, which did not significantly alter population biomass estimates until the 8th year of comprehensive study (2001).

Waiting until population decline is detected to determine disease prevalence might lead to false conclusions on the cause of population decline. Twice in Prince William Sound, changes in disease prevalence were quantified a year before changes in population abundance were detected by traditional abundance estimates. High virus and ulcer prevalence was documented in April 1998, whereas population decline was not detected until 1999 (Marty et al. 2003). Likewise effects of the *I. hoferi* outbreak in 2001 were not detected at the population level until 2002. Indeed, study of disease in the population only in 2002 might have attributed population decline to the relatively high prevalence of VHSV in 2002 instead of the increased prevalence of *I. hoferi* in fall 2000 and spring 2001.

Disease resulting from the combination of VHSV and ulcers in Prince William Sound Pacific herring is oscillating in roughly 4-year cycles, the amplitude of which is decreasing with each cycle since 1993. Prince William Sound Pacific herring had a major VHSV-ulcer disease outbreak in 1993, moderate disease in 1997–1998, and mild disease in 2002. However, as the VHSV-ulcer outbreaks have decreased in severity, the significance of *I. hoferi* has increased. Our original hypothesis was that disease was a sporadic event associated with exceeding carrying capacity (Marty et al. 1998), but the 1998, 2001, 2002, and 2005 disease events occurred when the population was relatively low.

The Pacific herring population of Prince William Sound, Alaska, is being controlled by a "disease regime" preventing population recovery. Defined, a population is controlled by a disease regime when disease-related mortality exceeds recruitment over a 5-year period (i.e., greater than the normal periodicity of abundant year classes). A "healthy regime" is one in which recruitment exceeds disease-related mortality over a 5-year period, as occurred in Prince William Sound in the 1980s. Population biomass will not recover to support both predators and commercial fisheries until VHSV-ulcers, *I. hoferi*, and other yet unrecognized pathogens no longer dominate population dynamics. Validation of this disease regime hypothesis will require continued surveillance until the population recovers, a process that might take many years, if not decades.

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Tables

Table 5.1. Time series of a VHSV-ulcer index and prevalence of significant cases of *Ichthyophonus hoferi* for spring samples of Prince William Sound Pacific herring. Data are stratified by age groups 3–4 and 5+. Also given is the VHSV-ulcer index pooled over all ages, as used in Marty et al. (2003).

	ages 3–4		ages 5–9		pooled ages
Year	VHSV-ulcer index[a]	significant *I. hoferi*[b]	VHSV-ulcer index	significant *I. hoferi*	VHSV-ulcer index
1994	1.04%	15.8%	0.06%	7.5%	0.14%
1995	0.14%	3.8%	0.03%	12.0%	0.06%
1996	0.00%	5.1%	0.00%	11.3%	0.00%
1997	0.09%	4.5%	0.05%	10.2%	0.07%
1998	0.60%	2.9%	0.16%	15.2%	0.44%
1999	0.01%	3.2%	0.00%	11.2%	0.01%
2000	0.00%	0.0%	0.00%	11.2%	0.00%
2001	0.01%	8.0%	0.01%	20.0%	0.01%
2002	0.14%	2.7%	0.04%	15.8%	0.10%
2003	0.01%	8.1%	0.003%	30.3%	0.005%
2004	0.05%	4.3%	0.003%	16.4%	0.005%

[a]VHSV-ulcer index calculated by multiplying the relative frequency of VHSV+ fish with the relative frequency of fish with ulcers, with a lower ulcer bound of 0.5%; VHSV was not determined in 2003 or 2004, but was assumed the same as in 1999.

[b]Significant cases of *I. hoferi* for 1994 – 2002 are the prevalence of cases with sum-Ichthyophonus score >10; for 2003 and 2004, we used the prevalence of gross *I. hoferi* lesions in the heart.

Table 5.2. Summary statistics of fitting the seven models to Prince William Sound Pacific herring data.

	Sample Size	Data Weight
SeineAC	88	1
SpawnAC	158	1
Hydro	10	0.5
Egg	10	0.5
Milt	25	0.5
Ricker	23	0.03
Total	324	

Model	0	1	2	3	4	5	6
Parameters	38	46	42	42	42	40	42
Unweighted RSS							
Seine	0.26	0.41	0.24	0.45	0.40	0.25	0.40
Spawn	0.48	0.48	0.40	0.54	0.48	0.44	0.48
Hyd	1.49	1.02	1.32	1.08	1.02	1.47	1.03
Egg	3.49	1.94	1.95	1.99	1.94	2.03	1.94
Mile-days	2.35	1.79	2.14	1.68	1.81	2.12	1.81
Ricker	25.17	22.11	23.86	20.95	22.14	23.30	22.22
Comparison							
Weighted RSS	5.16	3.93	4.07	4.00	3.93	4.21	3.94
ln L	148.57	191.33	185.84	188.59	191.22	180.60	191.01
AICc	-210.74	-275.05	-274.84	-280.33	-285.59	-269.61	-285.18
Δ	74.85	10.53	10.75	5.26	0.00	15.98	0.41

Table 5.3. Correlations among log recruitment estimates from Model 4 and disease time series from Table 1. Correlations with no lag use recruitment estimates and disease data from 1994 – 2004; correlations with lag 1 use recruitment estimates from 1995 – 2004 and disease data from 1994 – 2003.

	Ages 3-4		Ages 5-9		Pooled ages
	VHSV	*I. hoferi*	VHSV	*I. hoferi*	VHSV
Correlation (no lag)	0.355	-0.084	0.621	-0.084	0.408
P- value	0.285	0.805	0.042	0.806	0.213
Correlation (lag 1)	0.019	0.521	0.041	-0.109	0.036
P-value	0.959	0.123	0.910	0.765	0.922

Figures

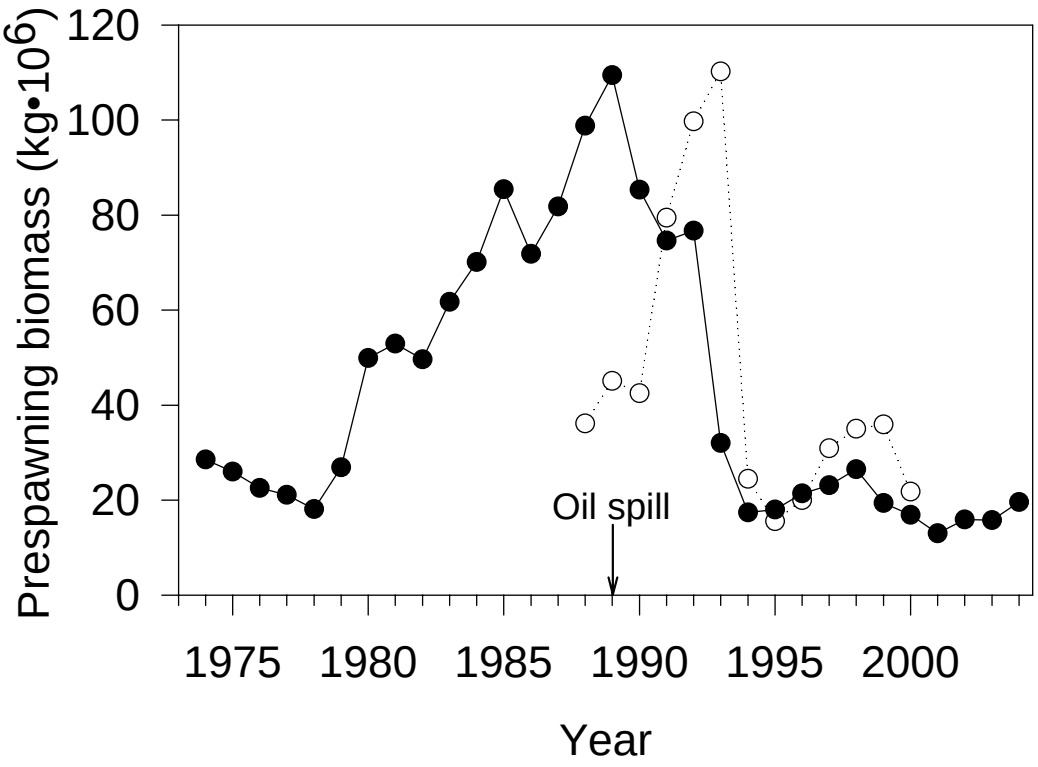

Fig. 5.1. Biomass estimates of mature Pacific herring in Prince William Sound, Alaska. Unexploited spawning biomass projected in the year before spawning (○; source, Alaska Department of Fish and Game, Cordova, Alaska) and calculated after spawning (best estimate, ●) using an age structured assessment model modified by a disease index after 1993. The *Exxon Valdez* oil spill occurred in Prince William Sound in 1989.

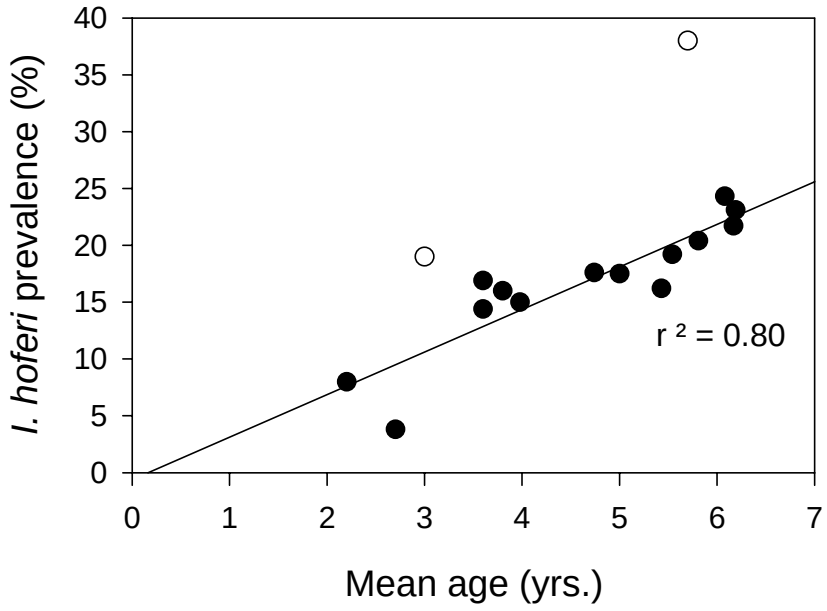

Fig. 5.2. Mean age vs. prevalence of *Ichthyophonus hoferi* determined by histopathology in spring samples (1994 – 2002) and fall samples (1995 – 2001) from Prince William Sound, Alaska. Prevalence of *I. hoferi* is greater during the epidemic in fall 2000 and spring 2001 (○) than in all other samples, classified as endemic samples (linear regression, ●).

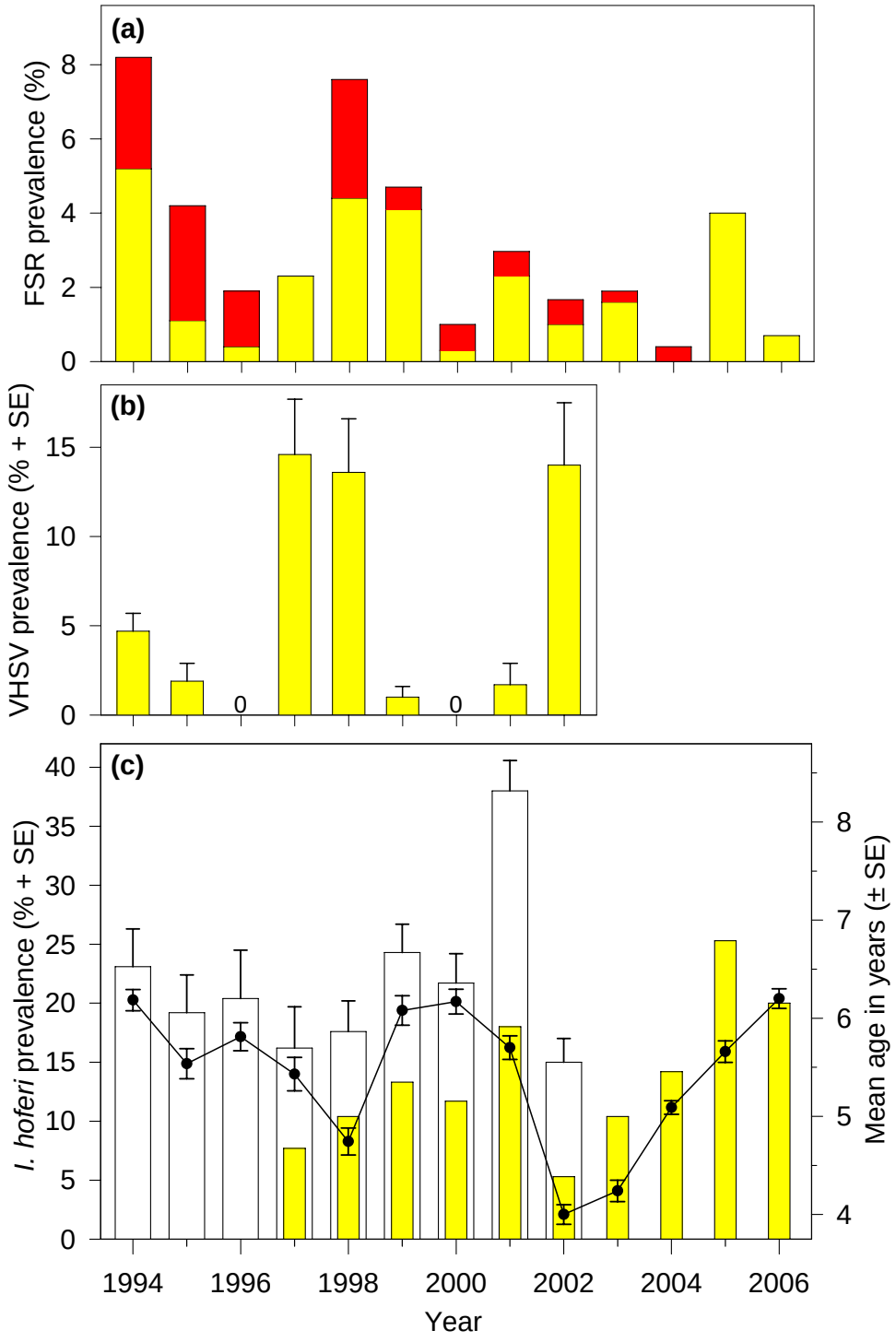

Fig. 5.3. Prevalence of lesions and pathogens in Pacific herring sampled in the spring from Prince William Sound, Alaska. (a) External lesion focal skin reddening; moderate (light bars) and severe (dark bars). (b) Viral hemorrhagic septicemia virus (VHSV; determined 1994 – 2002 only). (c) *Ichthyophonus hoferi* determined by histopathology of 10 organs (open bars, 1994 – 2002) or by gross examination of the heart (light bars, 1997 – 2006), and mean age (●) of fish in the samples (all years). SE = standard error.

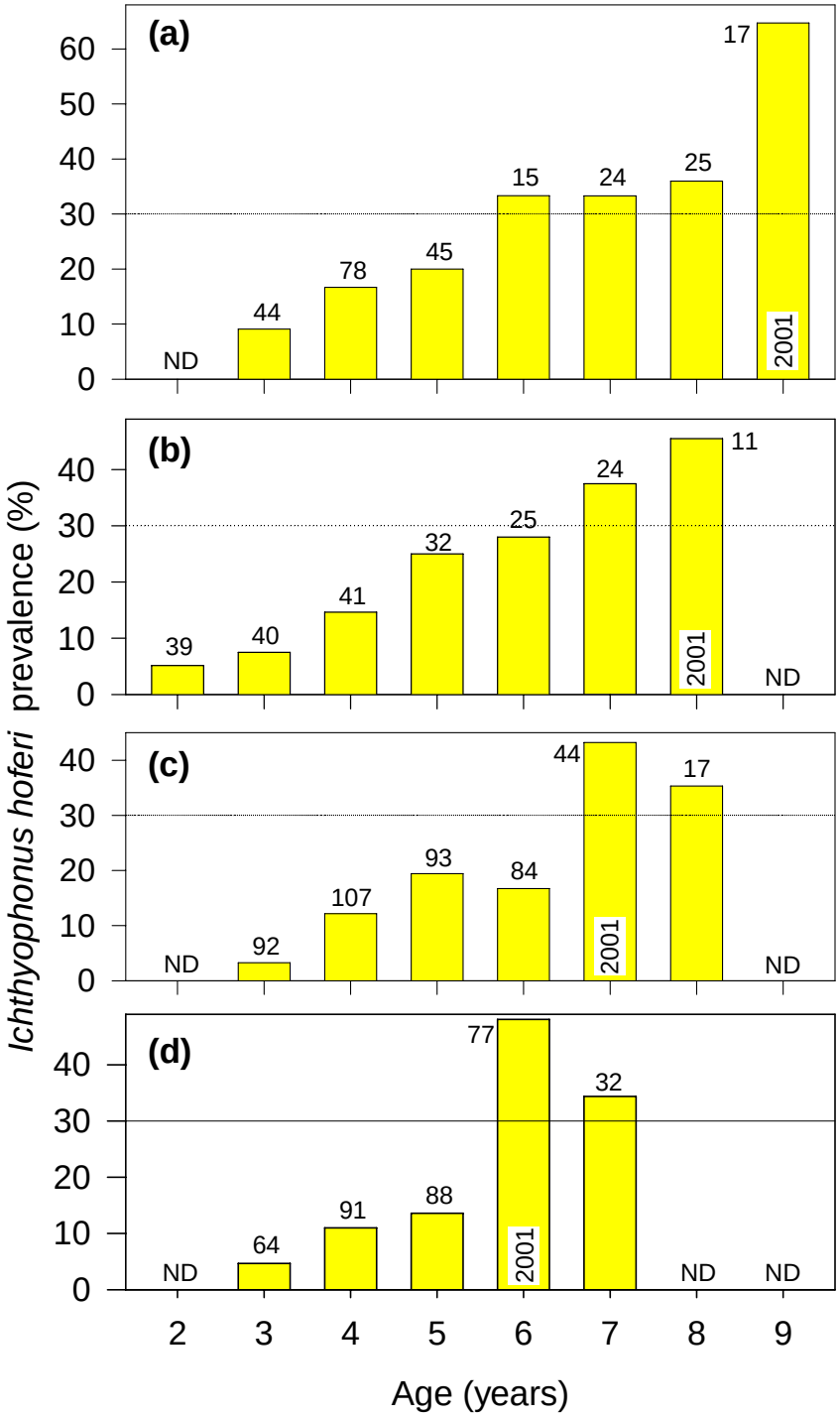

Fig. 5.4. Prevalence of *Ichthyophonus hoferi* in spring samples within large year-classes: (a) 1992; (b) 1993; (c) 1994; and (d) 1995 year classes. Numbers near top of bars designate number of fish examined. Reference lines are at 30% prevalence for each year-class graph. ND = no data.

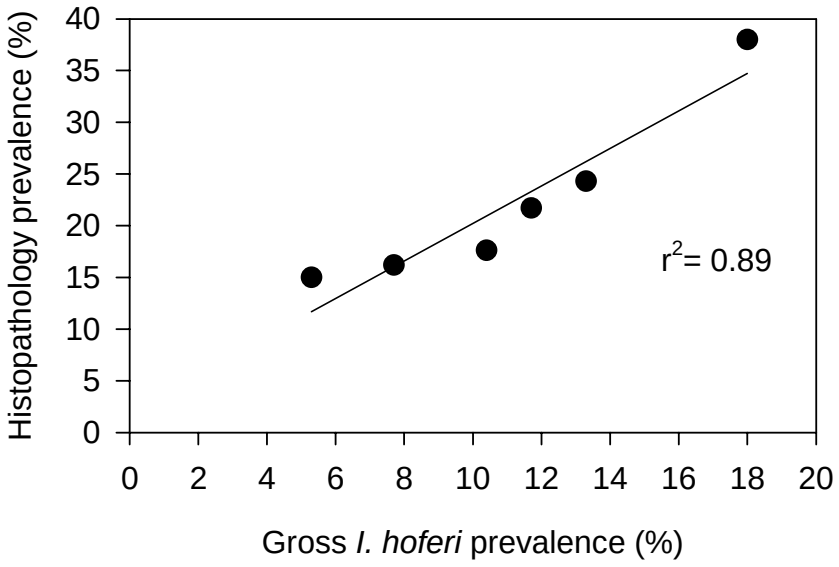

Fig. 5.5. Linear regression of gross *I. hoferi* prevalence and *I. hoferi* prevalence determined by 10-organ histopathology for spring samples from 1997 through 2002 from Prince William Sound, Alaska.

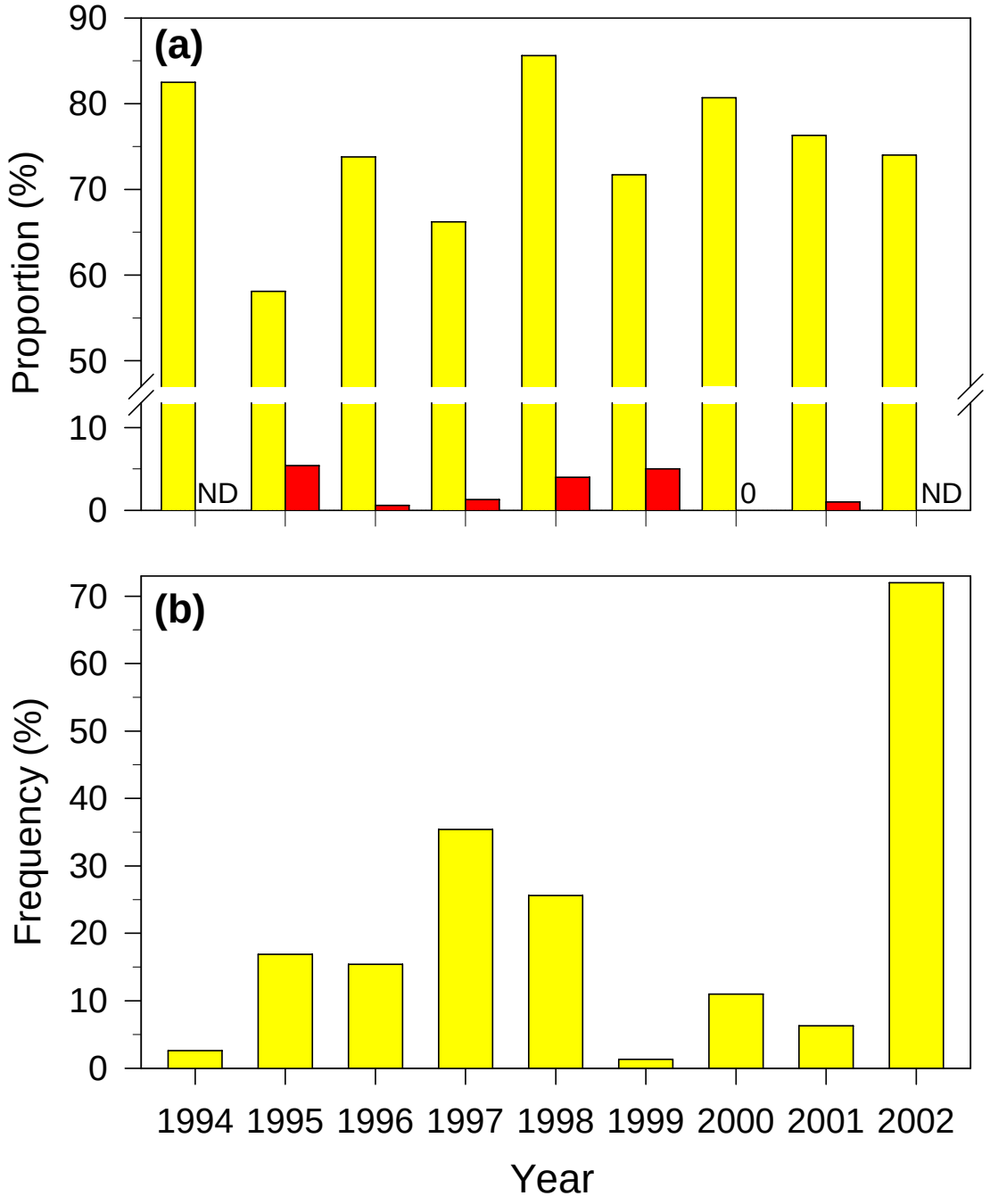

Fig. 5.6. Fat stores and recruitment over time; (a) proportion of fish with complete atrophy of abdominal mesenteric adipose tissue (score = 0); (b) frequency of 3-year-olds in spring samples. ND, no data; light bars, spring samples; darker bars, fall samples.

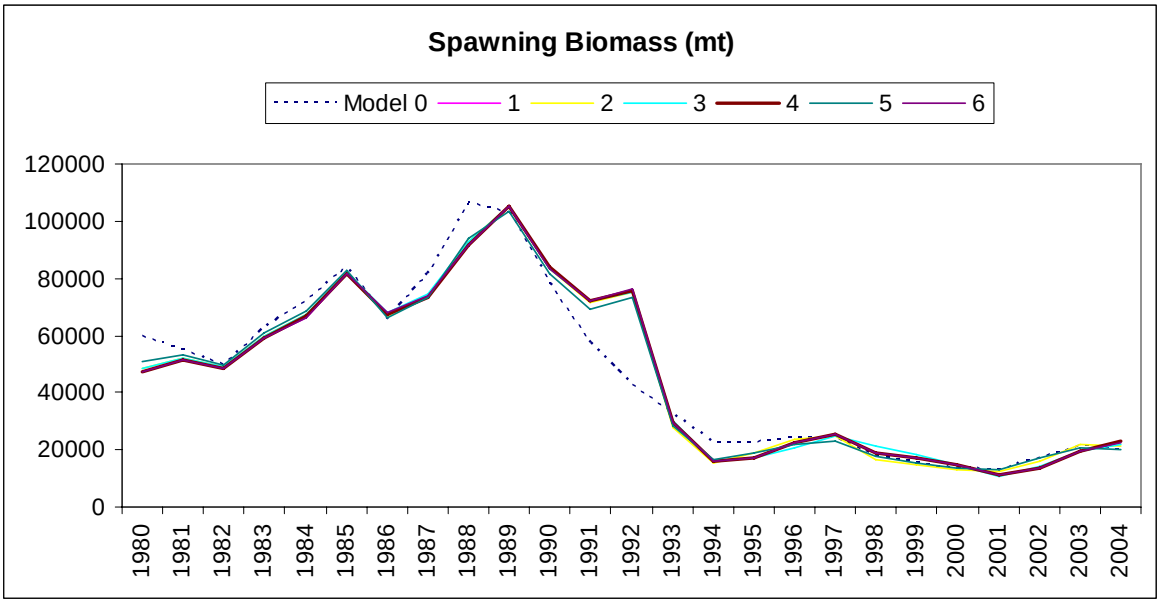

Fig. 5.7. Estimated spawning biomass from the alternative model scenarios: (0) null model with no disease; (1) age-stratified, both disease variables included; (2) age-stratified, just the VHSV-ulcer index; (3) age-stratified, just the significant-*I. hoferi* prevalence; (4) age-stratified, the VHSV-ulcer index for ages 3 and 4, the significant-*I. hoferi* prevalence for ages 5+; (5) the VHSV-disease index pooled over all ages; (6) same as (4) but with the unadjusted *I. hoferi* prevalence.

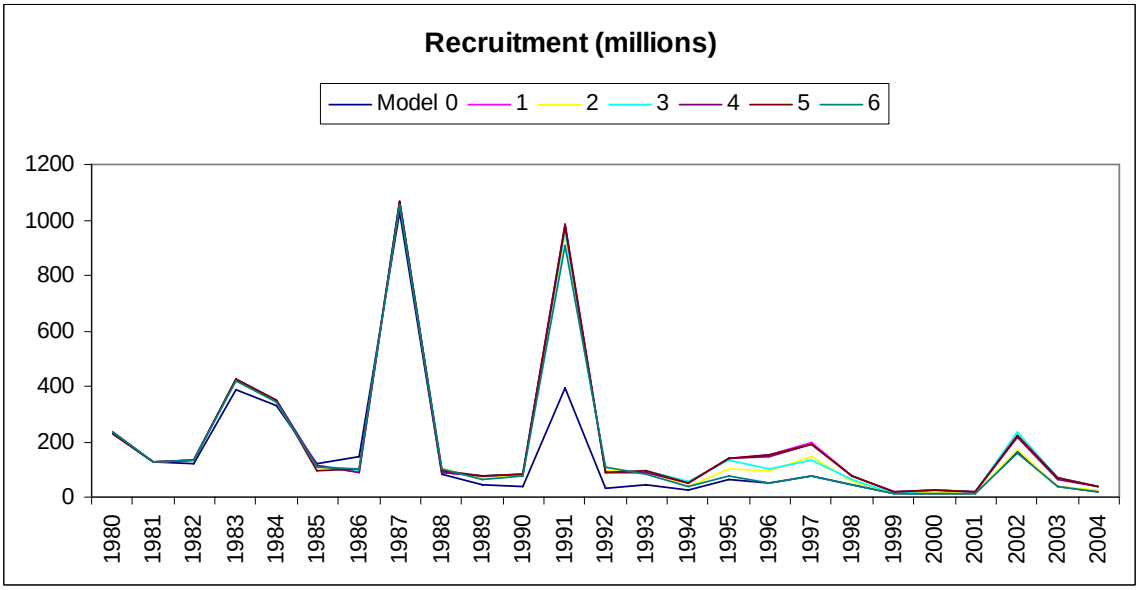

Fig. 5.8. Estimated recruitment at age 3 from the alternative model scenarios.

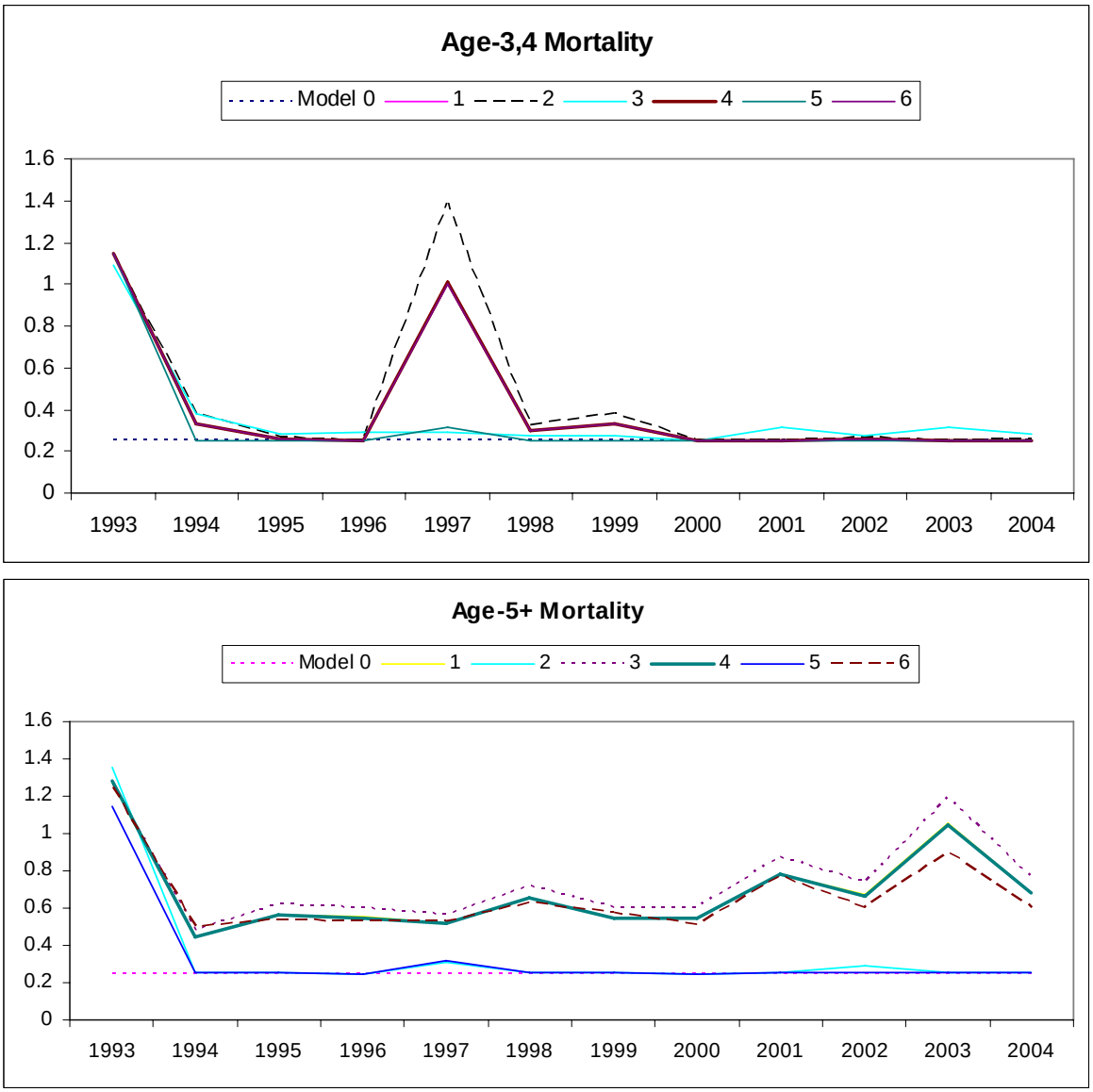

Fig. 5.9. Estimated natural mortality (including disease mortality) at ages 3-4 and at ages 5+ from the alternative model scenarios.

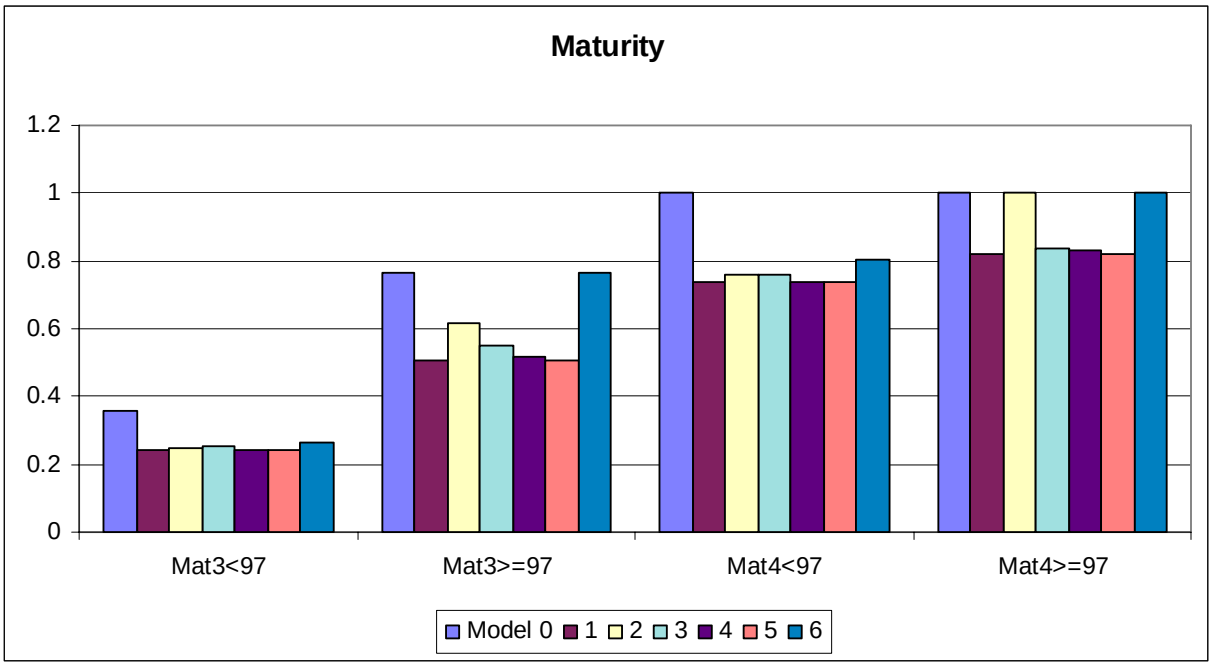

Fig. 5.10. Estimated proportion of reproductively mature fish at ages 3 (Mat3) and 4 (Mat4) before 1997 (<97) and 1997 and after (>=97) from the alternative model scenarios.

Chapter 6

Genetic Diversity in Pacific Herring (*Clupea pallasi*) from Prince William Sound, Alaska

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Abstract

The Pacific herring (*Clupea pallasi*) population in Prince William Sound (PWS) collapsed in 1993, four years after the Exxon Valdez oil spill. Recovery since the collapse has been poor at best. Given that the PWS population is "upstream" and distant from other stocks, that fishing pressure in the early part of the century was uncontrolled, and that the population crashed in 1993 with poor recovery since, there has been speculation that the genetic diversity within the survivors may be limited, producing a genetic bottleneck. Since it is thought to receive little migrant input from outside, there is concern that the resident population may not be genetically diverse enough to cope with changing environmental conditions and might be unable to expand back to its former population sizes. This study examines the data on genetic diversity of herring from PWS and compares it with diversity measurements from other populations in Alaska and the west coast, where most populations are healthy and sustained, but also with a few populations that are known to be stressed. This study concludes that genetic diversity in herring from Prince William Sound is high and does not contribute to the problems of poor recovery.

An investigation of the genetic structure of PWS herring shows that gene flow is significant between southwest PWS and the Gulf of Alaska as well as within the Sound itself, consistent with the theorized metapopulation model of herring dynamics. PWS herring can be reliably differentiated on a genetic basis from populations in Bering Sea and Bristol Bay, but subpopulations within PWS cannot be reliably differentiated. Because of large interannual genetic variation, "It seems unlikely that further work with neutral DNA markers will firmly resolve the question of whether demographically independent stocks occur within Prince William Sound or even in the northern Gulf of Alaska" (Bentzen et al. 1998).

By all available measures, genetic diversity in PWS herring collected in 1995 and 1996 was comparable to that of other healthy Northeast Pacific herring populations in Alaska, British Columbia and Puget Sound. Both gene diversity (heterozygosity) and allelic diversity (the number of alleles per locus) are high in PWS herring. The genetic diversity of PWS herring is similar to that of herring from Cherry Point, Washington but significantly higher than that of herring from San Francisco Bay, California. All measurements examined fail to demonstrate evidence of a genetic bottleneck among PWS herring capable of reducing recruitment success. According to observed genetic diversity, the 22,000 metric ton minimum spawning biomass threshold needed to conduct a commercial fishery is expected to protect the long-term genetic diversity of PWS herring. Even currently low population levels appear to be at least one thousand times higher than the upper bound on the evolutionarily effective population size of PWS herring.

Keywords – Pacific herring, *Clupea pallasi*, Prince William Sound, genetic diversity,

Introduction

Over the last century, the Prince William Sound herring (*Clupea pallasi)* population has sustained numerous stresses including over 80 years of heavy fishing pressure, the 1989 *Exxon Valdez* oil spill (EVOS), and a population collapse in 1993 from which it apparently has not recovered (Carls et al. 2002). There were large, uncontrolled commercial fisheries on Prince William Sound (PWS) herring from 1904 until 1967 (Brown and Carls 1998) until the population size became reduced. During the 1960's, there were parallel herring population declines throughout the North Pacific (Schweigert 2002, Brown 2002). Monitoring of herring biomass in PWS began in the early 1970's, at which time the estimated biomass was about 30 metric tons (Marty et al. 2003). Commercial fishing resumed as the estimated biomass of PWS herring increased throughout the 1980's to a high of 113,200 metric tons in 1989 (Brown and Carls 1998, Marty et al. 2003). The fishery was closed following the March, 1989 oil spill. Approximately half of the egg biomass was deposited within the trajectory of the spilled oil, and the contribution of the 1989 year class to the 1993 spawning population was only 25% of that forecast (Carls et al. 2002, Marty et al. 2003). Commercial herring fisheries in PWS were reduced in 1993 and subsequently closed until 1996 due to the extremely low spawning biomass in 1994 (13,900 metric tons). A minimum spawning biomass of 22,000 metric tons was established as the threshold for opening the commercial fishery. In 1997, there was a limited commercial harvest of the 29,000 metric ton population; however, the fishery has remained closed since then with spawning biomass estimates dropping to around 9,550 metric tons in 2001 (Steve Moffitt, Alaska Dept. of Fish and Game, personal communication).

Clupeids, including Pacific herring, typically have "boom or bust" population cycles, even in unstressed populations (Schweigert 2002). Before the oil spill, strong year classes historically dominated the PWS spawning populations about every four years and the spawning population was dominated by the 1984 year class at the time of the spill (Brown and Carls 1998; Norcross et al. 2001). Juvenile herring remain in nearshore habitats up to two years and subsequently recruit into the spawning population (Norcross et al. 2001). Large aggregations of adult herring migrate to overwintering areas in central and eastern PWS, then return with fairly high fidelity to their approximate natal areas in preparation to spawn (Hay and McKinnell 2002). In PWS, eggs are laid on seaweed and rocks in the intertidal and shallow subtidal zones in April and hatch about 24 days later.

The timing of the EVOS on March 24, 1989, coincided with the aggregation of the spawning population in nearshore areas. An estimated 25% to 32% of the egg biomass was exposed to *Exxon Valdez* oil (EVO) during early development at concentrations in the low parts per billion range which are known to cause biologically significant sublethal effects following laboratory exposures, including premature hatching of smaller larvae, reduced growth, morphological malformations and genetic (chromosomal) damage (Brown et al. 1996; Hose et al. 1996, Carls et al. 2002). While the egg biomass deposited in oiled and unoiled areas was approximately equal, the abundance of viable pelagic larvae was reduced by almost a thousand-fold within the oil trajectory (Norcross et al. 1996). Larvae that remained in bays within the oil trajectory throughout the summer were smaller and had higher rates of malformations and genetic damage than those from outside oiled area (Hose et al. 1996) but the impact of oil could not be assessed because so little was known regarding the life history and ecology of larval and juvenile herring. Subsequent studies of larval (age-0) herring (Norcross et al. 2001) suggest that significant proportions of larvae from unoiled spawning sites would have migrated into nursery areas within the oil trajectory and that the reduced nutritional status of the smaller larvae from

oiled nursery areas would reduce over-winter survival, contributing to the 52% population reduction estimated for the 1989 year class (Brown et al. 1996). Effects on juvenile (the strong 1988 year class age-1) herring were not assessed following EVOS, but it is now known that age-1 and age-2 herring form mixed schools in bays in PWS and along the Kenai Peninsula (Norcross et al. 2001). Thus it is possible that some portion of juvenile herring were exposed to oil dispersed in the water column or adherent onto zooplanktonic prey items like *Neocalanus* copepods (Carls et al. 2005). Adult herring from oiled areas had liver lesions and immunosuppression consistent with laboratory exposure to oil (Kocan et al. 1997). Laboratory-exposed fish subsequently developed viral hemorrhagic septicemia virus (VHSV), and similar liver lesions were detected in VHSV-positive PWS herring when the population collapsed (1993-1994) (Meyers et al. 1994).

Estimation of long-term survival and the formulation of conservation plans for PWS herring require both knowledge of its population structure and an assessment of its genetic diversity. At the time of the spill, PWS herring were managed by the Alaska Department of Fish and Game as a discrete population but recent research on microsatellite and mitochondrial DNA has failed to show consistent differences that would distinguish PWS herring from those at other Gulf of Alaska regions (Seeb et al. 1999). There was significant genetic variation between herring from PWS and Kodiak Island in 1995 and again in 1996, but the magnitude of the variation was less than the interannual differences at the same site. Seeb et al. (1999) noted that the lack of stable differences in microsatellite and mitochondrial DNA may hamper the use of these genetic markers for management of the PWS population.

The historical high fishing pressure and the lack of recovery in the PWS herring population after the 1993 collapse suggest the possible loss of genetic diversity as an explanation for reduced recruitment success. Genetic diversity is frequently measured by the number of alleles per locus, heterozygosity, and the proportion of polymorphic loci (Kuo and Jantzen 2003; Habicht et al 2004). A second approach is to compare the genetic diversity of PWS herring with that of less stressed populations in the Bering Sea, Gulf of Alaska, Southeast Alaska, British Columbia and Puget Sound (Seeb et al. 1999, Beacham et al. 2002) and against populations known to be stressed such as Cherry Point, Washington and San Francisco Bay, California (Small et al. 2004, Gustafson et al. 2005). Such comparisons are important because they can provide a framework for interpreting the overall genetic diversity of the PWS population at a time of low recruitment (1995-1996).

There is concern that the resident PWS herring population may have experienced a genetic bottleneck, leaving the population with insufficient genetic diversity to cope with changing environmental conditions and unable to expand back to its former population sizes. Genetic (or population) bottlenecks reduce adaptive potential by decreasing genetic variation, increasing the rate of inbreeding, and the fixation of mildly deleterious genes, thereby increasing the potential for extinction (Cornuet and Luikart 1996). Several factors give some credence to the theory that at least one genetic bottleneck may have occurred in PWS herring since 1900. The PWS population is “upstream” and distant from other stocks, fishing pressure in the early part of the century was uncontrolled (Brown and Carls 1998), embryos and larvae from oiled areas of PWS experienced genetic damage and high mortality (Hose et al. 1996; Brown et al. 1996), and the population crashed in 1993 with poor recovery since. There has been speculation that the genetic diversity within the survivors may be sufficiently limited to form a genetic bottleneck that increases the possibility of population extinction. Two analyses of the population genetics of post-collapse PWS herring (fish from 1995 and 1996, Seeb et al. 1999; fish from

1996, Small et al. 2004) yielded data that can be used to examine their genetic diversity. This study analyzes those data and compares them with genetic diversity measurements from other herring populations in Alaska and the Northeast Pacific, where most populations are healthy and sustained (Beacham et al. 2002), but also with a few populations that are known to be stressed (Gustafson et al. 2005).

Methods

Allozymes.

Early investigations of genetic variation involved the use of polymorphic proteins or allozymes that demonstrate a range in molecular weights. Protein variants were separated using starch gel electrophoresis and visualized using histochemical stains. Thus, allozyme variation is a measurement of phenotypic diversity, rather than genotypic diversity. The single study of population structuring in Pacific herring (Grant and Utter 1984) using allozymes showed genetic differences among Bering Sea, Bristol Bay, Gulf of Alaska (GOA) and Southeast Alaska populations. A more recent statistical analysis of the allozyme data (Bentzen et al. 1998) showed that the proportion of the genetic variance within basins was not significant; that is, stocks within the GOA could not be separated.

DNA Testing.

With the advent of DNA technology, direct markers of DNA variation could be assessed in both coding and noncoding (or silent) sequences. Using restriction endonucleases, restriction fragment length polymorphisms (RFLPs) can detect single base substitutions in the base recognition sequences of specific restriction enzymes. However, RFLP analysis of mitochondrial DNA (mtDNA) and ribosomal DNA (rDNA) has not uncovered population-specific patterns in Pacific herring from PWS (mtDNA, Bentzen et al. 1998) or British Columbia (mtDNA, Schweigert and Withler 1990; rDNA, Domanico et al. 1996).

The invention of polymerase chain reaction (PCR) technology allowed any genomic region from single individuals and to be amplified and analyzed without the necessity of cloning. The development of PCR-based technologies for population genetics and genome mapping focused on variability in microsatellite DNA, relatively short (1 - 6 base pairs [bp]), frequently occurring, highly polymorphic tandemly repeated sequences (O'Connell and Wright 1997). Repeat sequences can consist of dinucleotides such as $(GT)_n$ and $(AC)_n$ or tetranucleotides $(GATA)_n$ (O'Connell and Wright 1997; DeWoody and Avise 2000). These repeats can be isolated by radioactively labeling one of two primers that are complementary to the flanking sequence on either side of the repeat sequence. Length differences of the repeat sequence are then determined by resolving the amplification products on a sequencing gel. DNA microsatellites, particularly in marine fishes, are so polymorphic that variants are usually "binned" into groups of different length. Microsatellite analysis is limited only by the necessity to develop species-specific primers, although primers designed for one species (for example, salmon) can often be modified for use in a related species (herring). However, mutations within the primer region can create "null alleles" that are weak or not visible after PCR amplification. Also, the mutational mechanisms involving the gain or loss of repeat sequences of microsatellite DNA are not fully known, so phylogenetic relationships cannot be established with certainty (O'Connell and Wright 1997). However, comparisons between the evolution of nuclear and mitochondrial loci can be used to infer differing responses to evolutionary pressures (Seeb et al. 1999). One significant technical problem of DNA microsatellite analysis is slippage of repeat

units during PCR amplification, causing PCR artifacts or stutter bands (O'Connell and Wright 1997). Most current analyses rely on tri- or tetranucleotide repeats since they are less prone to slippage than are dinucleotide repeats and can be visualized using nonradioisopic techniques.

Because of problems inherent in analyzing microsatellite DNA repeat variations, recent research has focused on the detection of DNA sequence variations at a single nucleotide position within the genome, most notably using high-throughput analysis of single nucleotide polymorphisms (SNPs) (Schlotterer 2004). At present, polymorphic SNPs have not been identified for Pacific herring, although SNP technology is being developed for salmonids (Smith et al. 2005).

The development of mitochondrial DNA (mtDNA) markers follows a similar method except that a DNA segment is analyzed using a single restriction enzyme or any combination of enzymes prior to PCR amplification (Bentzen 1997). Because mtDNA is single-stranded, numbers of haplotypes are compared instead of the number of alleles.

Existing Studies on PWS Herring Genetics.

Most of the DNA microsatellite papers reviewed in this report are based upon muscle tissue from herring collected in 1995 and 1996 from Prince William Sound. Single collections in 1995 were made of PWS spawning stocks from Rocky Bay and Port Chalmers on Montague Island, Matthews Bay (a southeast isolate), and Fish Bay (a northeast isolate) as well as from the west side of Kodiak Island (thought to be an ancestral stock in the Gulf of Alaska) (Seeb et al. 1999 and reports therein). These samples were compared with those from spawning populations from the Bering Sea (Norton Sound) and Bristol Bay (Togiak Bay) collected in 1991. In 1996, herring were again collected at Kodiak Island, Togiak, Norton Sound and all the PWS sites. Small et al. (2004) based their studies on herring collected in 1996 from an unreported location in PWS and other northwest Pacific collections of varying years.

The studies reviewed in this report encompass the available genetic information on Pacific herring in PWS:

1. Seeb et al. (1999) contains reports by:

a. O'Connell et al. (1998a) on development of 6 DNA dinucleotide microsatellites in 1995 PWS herring

b. O'Connell et al. (1998b) on the use of 5 of the 6 DNA microsatellites developed in (a) to differentiate between PWS and other Alaskan herring populations in 1995

c. Wright and Dillon (1997) on the same 5 DNA microsatellites used in (b) to differentiate between PWS herring collected in 1995 and 1996 and other Alaskan herring populations. Genetic variability of herring from four PWS sites, Kodiak Island, Togiak Bay and Norton Sound (n=100 each) was compared between 1995 and 1996. Because these contain the 1995 genetic analyses presented in (b) above (O'Connell et al.1998b) as well as comparable data for 1996, Wright and Dillon's data are used in this report where interannual comparisons are warranted.

d. Bentzen (1997) developed RFLP methods for analyzing a two kilobase (kb) segment of the ND1 gene of mitochondrial DNA in 1995 PWS herring and other Alaska herring.

e. Bentzen et al. (1998) used methods developed in (d) as well as new methods to compare mtDNA variability in 1995 and 1996 PWS herring and other Alaskan herring (n=100 at each of the 7 sites). Differences in tissue preservation (490 in 1995 versus 390 in 1996) and the type of restriction enzyme used (range: 445-563) were thought to alter the number of samples that could be successfully analyzed.

2. Small et al. (2004) contains results on genetic diversity of 11 different microsatellite DNA loci among 1996 PWS herring and other populations from Alaska, Washington and California. They primarily analyzed microsatellites with tetranucleotide repeats (developed by Olsen et al. 2002 from Bering Sea herring) because of the technical problems associated with dinucleotide repeats in microsatellite DNA.

Diversity Measurements.

Herring collected from PWS in 1995 and 1996 would represent the generation surviving the 1993 population collapse. Hence, these fish would be expected to have reduced genetic variability if the population decrease was sufficiently severe. Two measures can be directly obtained from the DNA analyses that define genetic diversity, DNA fragment lengths (alleles for diploid DNA, haplotypes for mtDNA) and fragment diversity (expected heterozygosity, H_e, for diploid DNA; haplotype diversity for mtDNA). Because microsatellites are highly polymorphic, there are numerous possible alleles, up to 70 in marine fish which demonstrate extreme polymorphism (O'Connell and Wright 1997). H_e is the estimated fraction of all individuals who would be heterozygous for any randomly chosen locus and represents potential genetic variability. Parallel measures for mtDNA are the number of haplotypes (H) and haplotype diversity (h).

Published data (O'Connell et al. 1998a,b; Wright and Dillon 1997, Bentzen 1997 and Bentzen et al. 1998 in Seeb et al.1999; Small et al. 2004) and accompanying raw data for Wright and Dillon (1997) were examined for differences in genetic diversity. Site-specific values for N and H_E are given in Appendix 7a (data from Wright and Dillon [1997]) and Appendix 8a (data from Small et al. 2004) and the parallel values for mtDNA in Appendix 9a (data from Bentzen (1997). Missing H_E values (Wright and Dillon 1997) and haplotype diversities (h) were calculated from raw data supplied by James Seeb (jimseeb@adfg.ak) using the BOTTLENECK program (version 1.2.02, Cornuet and Luikart 1996). Analysis of variance (ANOVA) was used to test for differences in N, H, H_e, and h among sites, followed by t-tests if the results of the ANOVA were significant. If variances among sites to be tested were not similar, parallel nonparametric tests were performed. Because of the significant interannual differences in genetic diversity measurements (Seeb et al. 1999), data from 1995 and 1996 were analyzed separately when sequential data are available. If the sample size was less than 100, collections within that site were grouped and calculations performed on the grouped data. Significance (p) values for one-tailed t-tests were used since a decrease in these measured indicated a reduction in genetic diversity. Significance levels of multiple tests were adjusted using a Bonferroni adjustment.

Bottleneck Measurements.

Three measures were used to test for the presence of a recent genetic bottleneck or a less severe reduction in effective population size among PWS herring, the number of alleles (N) or haplotypes (H), M values, and heterozygosity excess (Garza and Williamson 2001). The M value, a mean ratio of the number of alleles (N) to the range in allele size (r), is smaller in recently reduced populations (Garza and Williamson 2001). Following a population reduction, rare alleles are usually lost first, however, their loss will only reduce the allele range (r) if they are the largest or smallest allele. Therefore, N should be reduced more quickly than r, yielding a reduced M value. These values were calculated for each site and are shown in Appendices 1b-3b. Since microsatellite alleles were binned in the herring studies analyzed here (O'Connell et

al. 1998b; Small et al. 2004), the allele range was corrected for binning before calculating the M values. N, H and M values were analyzed using ANOVA followed by pairwise t-tests as described above for all data sets since the M statistic is capable of detecting a genetic bottleneck within one generation of the population decrease. Depending on population size, N and/or M may be reduced for up to 500 generations although M recovers more rapidly than N (Garza and Williamson 2001).

Heterozygosity excess is found following a population bottleneck since allelic diversity is reduced faster than heterozygosity. That is, "there will be a deficit of rarer alleles relative to the number expected in a population at equilibrium. Because rarer alleles contribute little to expected heterozygosity, there will be an excess of observed heterozygosity when compared with a population at equilibrium with an equivalent number of alleles" (Garza and Williamson 2001 citing Cornuet and Luikart 1996). This transient excess of heterozygosity can be detected for 25-250 generations, depending upon the magnitude of the population decrease, population heterozygosity, the variability of the loci, and the numbers of loci and individuals sampled (Cornuet and Luikart 1996). Because of power limitations, only two data sets were appropriate for this analysis, Bentzen et al. (1997) and Small et al. (2004). Heterozygosity excess was tested in these two sets with the BOTTLENECK program (version 1.2.02, Cornuet and Luikart 1996) using 1000 iterations assuming the infinite-allele model (IAM). Analyses were also performed assuming the stepwise mutation model (SMM), but did not always yield a result because of the extraordinarily high number of microsatellite alleles (up to 70). Significance of the heterozygosity excess and deficit was determined using the Wilcoxon signed-rank test.

Review of studies of genetic discreteness of PWS herring

Discreteness of PWS Herring.

Alaskan herring population dynamics may be best described using a metapopulation model where neighboring stocks (populations) are linked through migration (Hay et al. 2001; Beacham et al. 2002; Gustafson et al. 2005). Alaskan herring can be divided into three populations, Eastern Bering Sea, Central Alaska (or Gulf of Alaska, GOA), and Southeast Alaska (Hay et al. 2001) with many smaller "local populations" such as PWS (Brown and Norcross 2005). Herring from PWS, as part of the large GOA stock, can be reliably differentiated on a genetic basis from other Alaskan stocks such as the more northern Bering Sea stocks that includes Bristol Bay as well as from the more southern Southeast Alaska stock (Grant and Utter 1984; Seeb et al. 1999). Regardless of the type of genetic distance method Seeb et al. (1999) used, Bering Sea collections (Togiak Bay and Norton Sound) always clustered together in 1995 and again in 1996. Samples from the Gulf of Alaska clustered with each other both years, but GOA populations from the two years did not cluster together. Mitochondrial DNA analyses showed differences in haplotype frequencies between the GOA and the Bering Sea with little overlap between the two (Bentzen et al. 1998). Although they thought that mtDNA might be used to distinguish between PWS and Kodiak Island herring, as with the microsatellite analysis, the magnitude of site-specific genetic variation among sampling years was equal to or greater than the variation within locations. Unlike all of the other genetic studies comparing these two populations, Small et al. (2004)'s genetic markers failed to discriminate between PWS and Bering Sea (Norton Sound) herring.

Population Structure of PWS Herring.

Within each year, herring from eastern PWS were genetically distinct from southwestern PWS herring which were more similar to Kodiak Island herring, 300 km southwest and downstream via the Alaskan Coastal Current (Wright and Dillon 1997, Brown and Norcross 2005). However, differences were not temporally consistent since allele frequencies differed significantly at one or more loci in successive years. The information in Seeb et al. (1999) suggests that the use of genetic markers to consistently differentiate among subpopulations of PWS herring may not be possible. Because of this large interannual variation, "It seems unlikely that further work with neutral DNA markers will firmly resolve the question of whether demographically independent stocks occur within Prince William Sound or even in the northern Gulf of Alaska" (Bentzen et al. 1998).

Brown and Norcross (2005) attribute this lack of genetic differentiation to extensive mixing of various life history stages from the eastern, northern and southwestern (Montague Island) spawning locations and larval transport out of PWS through Montague Strait into the GOA. The highest rate of larval retention occurs in the eastern spawning region, intermediate in the northern region and low retention for the southwestern spawning sites. Dispersal of PWS larvae is expected to follow ocean circulation patterns, but once juveniles acquire the capability to swim against currents, they form mixed aggregations with nearby adults to exploit favorable feeding, overwintering and spawning areas (McQuinn 1997, Brown and Norcross 2005). Thus in Pacific herring, homing sites may be decided by mature members of the "aggregation" and not established in juveniles until maturation or the first spawning (Hourston 1982). However, it is estimated that general spawn site fidelity is between 75% and 95% for Pacific herring but there is variability in exact site selection (Haegele and Schweigert 1985, Hay and McKinnell 2002, Brown and Norcross 2005). Since spawn site fidelity is key to maintaining the integrity of a population (Sinclair 1988), it follows that the potential for learned spawning site plasticity would argue against the perpetuation of unique local populations within PWS. Brown and Norcross (2005) state that, exclusive of unusual predation, recruitment should be correlated with abundance at age-1, itself dependent on sufficient energy stores in age-0 fish for successful overwintering (Norcross et al. 2001).

Comparison of genetic diversity between PWS herring and other northeast Pacific herring populations

Measurements of Genetic Diversity.

Two measurements of genetic variation can be used to compare the genetic health of Pacific herring populations using existing studies on allozymes and microsatellite DNA. Allelic variation is necessary for organisms to adapt to environmental change. Thus, the proportion of loci that are heterozygous is a general indicator of genetic diversity. (Since loci for microsatellite analysis are chosen for their polymorphism, determining the number of polymorphic loci is not possible in microsatellite studies). The allelic diversity or the number of alleles at a particular locus (N) is applicable to existing data on Pacific herring. The loss of alleles, particularly rarer ones, is one of the initial and most sensitive measurements of a reduction in the genetically effective population size or a genetic bottleneck. The corresponding measure for mitochondrial DNA is the number of haplotypes (H). Gene diversity within a population is typically measured as expected heterozygosity (H_e), defined as the proportion of individuals that would be heterozygous at a particular locus if the population mated randomly. The analogous measure is

mtDNA haplotype diversity (h) within a population; Bentzen et al (1998) calculated mtDNA haplotype diversities in PWS herring.

As the genetically effective size of a population is reduced, genetic drift promotes the loss of rarer alleles (Garza and Williamson 2001). Two statistics have been developed to detect this change. The M value, a ratio between the number of alleles and their overall range in allele size, can detect single population reductions for more than 100 generations (Garza and Williamson 2001). Because rare alleles contribute relatively little to expected heterozygosity (H_e), the deficit in rarer alleles in a bottlenecked population relative to a population at equilibrium will be evident as an excess of observed heterozygosity (H_o) (Cornuet and Luikart 1996). Testing for significant increases in this "excess heterozygosity" forms the basis of Cornuet and Luikart's (1996) BOTTLENECK program. Similar to the M value, excess heterozygosity can detect reductions in the genetically effective population size as well as genetic bottlenecks (Habicht et al. 2004).

Comparison of Genetic Diversity Between Prince William Sound and Other Northeast Pacific Herring Populations.

There are only two years for which the genetic diversity of PWS herring has been evaluated, 1995 and 1996, years that followed the 1993 population collapse. Thus, the comparisons presented in this report would be expected to utilize data from PWS fish with the most severely restricted genetic diversity.

Despite their reduced population size, the genetic diversity of PWS herring in 1995 and 1996 was high (Tables 6.1, 6.2). Heterozygosity (H_e) in microsatellite DNA in the PWS population averaged 0.90 between all available studies with a range of 0.860 to 0.922. These values bracket the mean heterozygosities of herring from southeast Alaska, 0.88, and British Columbia, 0.86, using a different set of microsatellites (Beacham et al. 2002). DeWoody and Avise (2000) calculated the overall heterozygosity in 12 species of marine fishes to average 0.79. Parallel values for the average number of alleles per locus (N) in marine fish is 20.6 (DeWoody and Avise 2000) compared with an overall mean of 21.9 for PWS herring in the four studies summarized in this report. Up to 42 alleles per loci were present in PWS herring (Appendix 8a, Small et al. 2004). (Site-specific comparisons for southeast Alaskan and British Columbia herring were not reported [Beacham et al. 2002]). Such high genetic diversity is characteristic of marine fishes with large geographic distributions (DeWoody and Avise 2000).

In the Alaska Department of Fish and Game data series (Wright and Dillon 1997; O'Connell et al. 1998a,b) genetic diversity in PWS herring was similar to that of the other Alaskan sites, Kodiak Island, Norton Sound and Togiak Bay (Table 6.1). The number of alleles (N) and heterozygosity (H_e) were not significantly different at any of these sites. When broken down by station, PWS did not exhibit consistent differences in allelic diversity (N) for either year or for gene diversity (H_e) in 1995 or 1996. Although more alleles were usually observed in 1996 than in 1995, the contribution from technical improvements in 1996 was not defined (Wright and Dillon 1997).

In a spatially comprehensive survey using primarily tetranucleotide microsatellites, Small et al. (2004) compared genetic diversity among Pacific herring from Alaska, British Columbia, Washington and San Francisco Bay, California (Table 6.2). Heterozygosities were almost identical throughout this entire range. Regardless of whether the population is stressed (PWS, Cherry Point and San Francisco Bay) or not stressed (Norton Sound, Strait of Georgia and Puget Sound). The latitudinal pattern among the numbers of alleles decreased from north to south,

although only herring from San Francisco Bay had significantly fewer alleles than did PWS herring. For 5 of the 11 loci studied, herring from San Francisco Bay (SFB) had the lowest number of alleles (Appendix 8a) and the predominant alleles were different between SFB and PWS at five loci (Cha 107, Cha 134, Cpa-K, Cpa-H and Cpa-130, data not shown). Although PWS herring were not compared in multiple years in that study, many other sites were. Like Wright and Dillon's (1997) results, large temporal differences were found within populations for both the number of alleles (Appendix 8a) as well as for allele distributions and often for predominant allele sizes (Small et al. 2004). Similar differences existed for spawning and nonspawning herring collected at Cherry Point in 1999. Therefore, the large interannual variability noted by Wright and Dillon (1997) was probably not simply the result of improved technical methods in 1996. And, as noted by Seeb et al. (1999), the temporal instability of genetic data within the PWS population will limit the use of genetic markers for discrete stock management.

Results from mitochondrial DNA samples were consistent with those of DNA microsatellites, demonstrating that the genetic diversity among PWS herring is relatively high (Table 6.3). Haplotype diversities (h) were not significantly different between PWS and other Alaskan sites. The number of haplotypes (H) at PWS was significantly higher than those from Togiak Bay (p value = 0.02) and Norton Sound (p value = 0.001). Available information on mtDNA genetics is less consistent than microsatellite DNA (all mitochondrial polymorphism is linked on the mtDNA loop, low sample sizes usually reduce the number of haplotypes detected) and detailed but also supports the conclusion that genetic diversity of PWS herring is comparable to that of other Alaskan populations (Bentzen 1997).

Estimation of Effective Population Size (N_E) of PWS Herring.

Because not all individuals directly contribute to the gene pool, the evolutionarily effective population size (N_e**)** is an important measurement for conservation of genetic diversity, particularly when related to the population census size. It has been assumed that the N_E for PWS Pacific herring is far lower than its census size (J. Seeb, pers. comm..), but N_E has not been estimated for this population.

N_E can be calculated from microsatellite data using a classic stepwise mutation model: Heterozygosity at equilibrium (H_{eq}) = $1 - (1 + 8\ N_e\ \mu)^{-0.5}$ where μ is the mutation rate of neutral alleles (Ohta and Kimura 1973). A study using microsatellite DNA variation yielded a range of 250 to 25,000 for the effective sizes of marine fish populations estimated from a mean expected heterozygosity of 0.63 and a range of microsatellite mutation rates as 10^{-2} to 10^{-4} per locus (microsatellites mutate more frequently than does coding DNA) (DeWoody and Avise 2000).

Mean heterozygosities at equilibrium (Table 6.4) were calculated using the BOTTLENECK program (Cornuet and Luikart 1996) with allele counts from Wright and Dillon (1997) and Small et al. (2004). As in DeWoody and Avise (2000), published expected heterozygosites were used for sites referenced by Beacham et al. (2002) since H_{eq} values were not given. N_e was then calculated using the equation above. The harmonic mean of the upper and lower range (Hauser et al. 2002) was calculated for point estimates. The two estimates of the effective population size of PWS herring are 1275 from Wright and Dillon's (1997) data yielding an equilibrium heterozygosity of 0.862 and 822 using Small et al.'s (2004) data yielding 0.829, within the range given in DeWoody and Avise (2000).

The calculated range of PWS herring's effective population (415-64,400) is well below the census population estimated for 1995 (137 million fish from biomass of 14,900 metric tons) and 1996 (220 million fish from biomass of 24,000 metric tons) (Steve Moffitt, ADF&G, personal communication). The 1995 census population is used for calculations since it is the more conservative estimate. This N_e /N ratio of approximately 4.7×10^{-4} to 3×10^{-6} brackets the estimate for New Zealand snapper (1×10^{-5}, Hauser et al. 2002). Those researchers stated that a census population necessary to maintain long-term genetic adaptability would be 50 million individuals. Thus, the 22,000 metric ton minimum (approximately 202 million fish) for opening the commercial fishery in PWS should be protective of the estimated effective population for genetic adaptation of the PWS herring population.

Effective population sizes at the other two stressed locations were with the same order of magnitude as that of Prince William Sound (Table 6.4). At Cherry Point, Washington, herring census populations reached a record low of 733 metric tons in 2000 (Hershberger et al. 2005). Small et al.'s (2004) data yielded a heterozygosity value of 0.811 and the N_e for the Cherry Point herring population ranges from 337-33,700 fish. Beacham et al.'s (2002) higher heterozygosity value yields a N_e range of 1238-123,800 fish. However, the year 2000 census population was well above this estimated effective population range (census population of approximately 8.4 million fish; Table 6.4 using Gustafson et al.'s [2005] weight for year 3 fish of 87 g). Thus, at its lowest abundance, the range of the N_e /N ratio for the Cherry Point herring population is therefore 1.5×10^{-2} to 4×10^{-5}, one to two orders of magnitude smaller than that of PWS. In 2004, the number of Cherry Point herring has increased to an estimated 15 million (Gustafson et al. 2005). The two estimates of N_E for San Francisco Bay herring, 731 and 1440, are similar to those of Prince William Sound. Hay et al. (2001) report the 2000 spawning biomass to be about 25,000 metric tons. Given Schweigert's (2002) weight of 100 g for age 4 herring from San Francisco Bay, this biomass corresponds to 250 million fish and a N_e /N ratio of 3×10^{-4} to 1.5×10^{-6}. The N_e /N ratio for San Francisco Bay is similar to that of PWS.

Because they are based on heterozygosity values similar to those of PWS herring, N_e estimates for unstressed populations are comparable to that of PWS (Table 6.4). The overall mean N_e for unstressed populations was 1262, very close to the average of the three stressed populations, 1231. To calculate census population abundance, spawning biomass estimates were divided by the average 4 year old fish (213 g for Bering Sea, 130 for other sites, Hay et al. 2001). Since the estimated abundances of these unstressed populations are much larger than that of PWS (230 to 923 million fish), N_e /N ratios are accordingly smaller. Because the estimates of N_e presented here are primarily dependent upon estimates of microsatellite mutation rates and, to a lesser degree, on site-specific heterozygosity measurements, confirmation of effective populations by another method would be helpful, possibly using temporal changes in allele frequency (Waples 1989). This method has been used for some marine fishes (Hauser et al. 2002, Turner et al. 2002) but has large confidence intervals (the upper bound frequently is infinity) and requires a second set of parallel microsatellite measurements at least one generation removed.

Examination of PWS Herring DNA Data for Evidence of Genetic Bottleneck or Low N_E .

Recently, characteristics for determining genetic bottlenecks and less drastic reductions in N_E have been applied to fish populations (Habicht et al. 2004). The number of alleles per locus (N) is an effective signal of reduced N_e that may not be as severe as drastic, low N_E bottlenecks. Two measurements, M values (number of alleles/range of alleles, Garza and

Williamson 2001) and heterozygosity excess (Cornuet and Luikart 1996) are useful for detecting recent, low N_e bottlenecks (Habicht et al. 2004). Because mutation restores lost alleles over time, M values and heterozygosity excess are suitable for detecting genetic bottlenecks for maxima of approximately 100 and 25-250 generations, respectively. The number of alleles can remain depressed for longer periods of time than the other two measures and the two measures could be used to differentiate between populations that have experienced recent or lengthy reductions (Garza and Williamson 2001). These preceding measurements can also analogously be applied to mtDNA haplotypes.

The number of microsatellite alleles per locus (N) has been examined in this report for two data sets and failed to show any reduction in post-collapse PWS herring (Tables 6.1, 6.2). None of the four Alaskan herring populations studied by O'Connell et al. (1998b) and Wright and Dillon (1997) demonstrated statistically reductions in allelic diversity. Similarly, none of the Alaskan, British Columbia or Washington herring populations, Cherry Point included, had significantly reduced allelic diversity (Small et al. 2004). Only San Francisco Bay herring had significantly lower allelic diversity than PWS herring with an approximate 18% reduction ($p=0.044$ after Bonferroni correction, Table 6.1).

The M value, calculated by dividing N by the range of alleles (in base pairs) is a measure of the loss of rare alleles (Garza and Williamson 2001). Differences in M values are reported to be statistically less sensitive than the number of alleles (N) (Habicht et al. 2004); however, low average M values are indicative of recent N_E reductions. For a data set of at least seven loci, an average M value of <0.68 denotes a recent population decrease (Garza and Williamson 2001). Although Wright and Dillon's (1997) data assessed only five loci, all of the PWS sites had average M values above 0.68 in both 1995 and 1996 (Appendix 7b). Similarly, all sites studied by Small et al. (2004) using 11 microsatellites had average M values exceeding 0.68. Thus, absolute M values do not support the existence of a recent genetic bottleneck.

There were no site-specific differences among M values for the 5 microsatellites in either 1995 or 1996 (Wright and Dillon 1997) or for the set of 11 microsatellites (Small et al. 2004) (Table 6.1). One finding of interest is the relative preponderance of M values = 1.00 among San Francisco Bay herring. Although they had smaller ranges compared to other herring populations, allele distributions were continuous for three of the 11 loci (only one other population, Port Gamble, had continuous distributions for four loci; most had M values = 1.00 at only one or two loci). San Francisco Bay is the southern limit for a viable herring population (Hay et al. 2001) and these genetic markers suggest that this population may have been small for a relatively long time. Garza and Williamson (2001) note that "in the case of sustained small population size, the number of alleles, and therefore the overall genetic diversity, continues to decline…., even when the value of M has partially recovered….Mutation and drift together can actually cause the value of M to increase following a reduction." Depending upon the mutation processes, the authors state that newly polymorphic loci will have M=1. The San Francisco Bay herring population became reduced after El Nino events in 1984, 1993 and 1997 (Hay et al. 2001).

Excess heterozygosity is also indicative of a recent, low N_e bottleneck (Habicht et al. 2004). After a bottleneck but before a population again reaches equilibrium, fewer but higher frequency alleles remain after rarer alleles are eliminated. This loss in allelic diversity results in higher (excess) heterozygosity and can be evaluated using the BOTTLENECK program (Cornuet and Luikart 1996). None of the stressed herring populations surveyed by Small et al. (2004) demonstrated an excess in heterozygosity (Table 6.2), even at San Francisco Bay where the number of alleles was decreased. As in Habicht et al. (2005), a reduction in the number of

alleles was the most sensitive test and is detects a reduction in e_E not as severe as a genetic bottleneck. The lack of a heterozygosity excess among San Francisco Bay herring is consistent with unaffected M values, indicating the absence of a recent genetic bottleneck. Of the nonstressed herring populations, only Puget Sound, showed a significant heterozygosity excess (p value = 0.042 without Bonferroni correction). Heterozygosity excess tests performed assuming the stepwise-mutation model (SMM) all showed significant heterozygosity deficiency (data not shown), similar to the results of Habicht et al. for tributary-spawning salmon (2004). Neither of the other bottleneck measurements (N and M), particularly the more sensitive measure (N), showed a significant difference, so the validity of this single positive test is doubtful that a recent bottleneck has occurred in the Puget Sound herring population.

Mitochondrial DNA is also amenable to testing for recent bottlenecks, and none of these tests demonstrated the presence of a bottleneck among PWS herring (Table 6.3). The number of haplotypes and M values were similar between PWS herring and other populations in Alaska. Although M values can be used comparatively for mtDNA, absolute mtDNA M values (average $M < 0.68$ using microsatellite DNA signals a bottleneck, Garza and Williamson [2001]) are not valid for the determination of a bottleneck. The test for heterozygosity excess is suitable for use with mtDNA (Cornuet and Luikart 1996) and was also not significant for PWS herring. Although the two western PWS sites (Rocky Bay and Port Chalmers) appeared to have higher numbers of haplotypes than the eastern sites, differences were not statistically significant. MtDNA for the overall PWS population as well as three sites within PWS (St. Matthew's Bay and both western sites, Rocky Bay and Port Chalmers) demonstrated a significant heterozygosity deficiency. All tests assuming the SMM also showed a heterozygosity deficiency at these sites. Such results are indicative of a population expansion (Cornuet and Luikart 1996), but since mtDNA generally evolves on a slower time scale than does nuclear DNA, this population increase would have occurred many generations ago, possibly reflecting the population expansion during the 1980's or earlier.

Despite the low numbers of mtDNA haplotypes found among Togiak Bay and Norton Sound herring, there was not a heterozygosity excess at these sites. Data on spawning biomass for Bering Sea herring only goes back to 1980, but this population is much larger than GOA populations and appears to have only sustained a drop from almost 500,000 metric tons in 1985 to about 140,000 metric tons in 2000 (Hay et al. 2001), not a change sufficient to reduce its effective population size (Table 6.4).

Maintenance of Genetic Diversity Among Alaskan Herring Populations.

The genetic resiliency of PWS herring, and possibly herring in general, is related to a large census population:effective population ratio. This high ratio should be protective of genetic adaptability during large population swings inherent in herring population dynamics. Other fish species that have shown significant reductions in N_e have very different life histories from herring. Hauser et al. (2002) demonstrated that reductions in effective population size and loss of microsatellite diversity in the southern population of New Zealand snapper (*Pagrus auratus)* was temporally related to overfishing. New Zealand snapper, a long-lived species has discrete northern and southern genetic populations with little migration between the two. Lower effective population sizes have also been documented in tributary-spawning populations of sockeye salmon from the Kvichak River, Alaska drainage (Habicht et al. 2004). Populations above migration obstacles had reduced allelic diversity (N) and excess heterozygosity, differences which signaled a reduction in N_E.

In addition to a relatively low number of individuals capable of sustaining genetic diversity in the PWS herring population (low N_e), this stock presumably receives gene flow from nearby populations from GOA (Wright and Dillon 1997). Genetic flow between PWS and Kodiak Island is equivalent to a range of 33 to 256 genetic migrants per generation, with the highest value between Kodiak Island and Rocky Bay. Within PWS, the highest genetic exchange rate is between Rocky Bay and the eastern districts, Fish Bay in the northeast (347 migrants per generation) and St. Matthew's Bay in the southeast (95). Far less gene flow occurs between the two eastern sites (43 fish) and between Port Chalmers and any of the other PWS sites (41 to 47 migrants). This pattern is similar to the population structure described by Brown and Norcross (2005) consisting of a migratory southwestern population with two other non-migratory populations in the east and north. Dispersal of larvae from spawning grounds follows clockwise local currents where some are retained in PWS bays while others are transported out of PWS through Montague Strait into the GOA (Norcross et al. 2001). As these herring age, they migrate to nursery areas along eastern PWS, the far side of Montague Island and along the Kenai Peninsula, probably forming mixed aggregations with GOA herring. Straying is thought to result from the association of individuals with aggregations composed of fish from other stocks, which then migrate to the group's natal region (Hay and McKinnell 2002). Genetic structuring over smaller spatial areas appears to be through differentiation by spawning sites or spawn timing (Hay et al. 1999).

Until the population collapse, spawning in PWS appeared to be dominated by strong year classes appearing about every four years (Brown and Carls 1998). Genetic variability between year classes might explain the interannual differences observed in previous genetic studies of PWS herring (Wright and Dillon 1997, Bentzen et al. 1998). In 1995, the 1988 year class was most abundant (45%), followed by the 1992 year class (24%). However in 1996, the 1992 year class was dominant (33%) with the 1988 year class comprising 26% (Steve Moffitt, ADF&G, personal communication). Additional genetic variability between year classes might occur due to low differential survival following unfavorable environmental conditions during the larval period or in juvenile-adult aggregations. Factors known to substantially reduce the survival of a larval cohort are advection from nursery areas, low food (Brown and Norcross 2005), and poor egg quality producing small larvae with yolk deficiency (Hershberger et al. 2005). Following the EVOS, there was a large reduction in the 1989 year class in PWS (Brown et al. 1996; McGurk and Brown 1996). In 1993 and 1998, juvenile-adult aggregations experienced high mortality from disease, best correlated with the prevalence of viral hemorrhagic septicemia virus (Marty et al. 2003). A recent study has shown that skewed year class composition toward younger, age-2 herring producing poor quality larvae (small with yolk deficiencies) appears at least partially responsible for recent declines in recruitment at Cherry Point, Washington (Hershberger et al. 2005). Another factor capable of producing genetic heterogeneity between year classes is sweepstakes spawning (Hedgecock 1994). However, the only genetic study using a larval cohort of a marine fish, Atlantic cod (*Gadus morhua*), concluded that there was no evidence of any temporal or spatial family structure among the larvae (Herbinger et al. 1997).

Improving Measurement of Genetic Diversity of PWS Herring.

Data on the genetic diversity of PWS herring have only come from the years 1995 and 1996, long after the initiation of fishing pressure in the early 1900's (Brown and Carls 1998). A more robust estimation of genetic diversity and temporal trends could be obtained if a time-series of herring has been archived. Hauser et al. (2002) used DNA from archived scales to examine

changes in microsatellite variation, providing estimates of the effective population size using temporal fluctuations in allele frequencies and changes in heterozygosity rather than the method used here which relies on estimates of microsatellite mutation rates in organisms other than fish (DeWoody and Avise 2000). However, the large genetic variation observed in PWS herring between 1995 and 1996 is expected to complicate temporal comparisons.

Genetic studies on PWS herring have not been stratified by year classes, an essential refinement expected to provide meaningful data given the large interannual genetic variation observed and the cyclic domination of herring year classes in spawning (Bentzen et al. 1998). This omission gains importance from Small et al.'s (2004) finding that the genetic structures of spawning and nonspawning herring differ at Cherry Point, Washington. Examination of microsatellite DNA genetics of a larval cohort in PWS using the likelihood methods of Herbinger et al. (1997) for cod would allow the estimation of a minimum N_e for the spawning aggregation that produced the larval cohort (O'Connell and Wright 1997) free of assumptions of microsatellite mutation rates.

Although numerous studies of DNA microsatellite variability have provided useful information on genetic variability and effective population size histories in fish (Hauser et al. 2002; Habicht et al. 2004), much effort is currently being focused on the development and use of single nucleotide polymorphisms (SNPs) in fish genetics (Smith et al. 2005). It is expected that future genetic studies of Pacific herring will investigate variation in SNPs as well as microsatellite DNA.

Conclusions with respect to PWS herring recruitment success

The genetic structure of PWS herring has been examined here with respect to its possible contribution to the lack of recovery the PWS since the 1993 collapse. Several findings argue that the genetic diversity of PWS herring is not constricted and does not contribute to the problems of poor recruitment observed since 1993.

1. The PWS herring population is not a genetically discrete population within the Gulf of Alaska since it shares much gene flow with the Kodiak Island population.
2. Genetic diversity in PWS herring is high and similar to that of healthy herring populations from elsewhere in Alaska, British Columbia and Washington.
3. There is no evidence for a genetic bottleneck in post-collapse PWS herring.
4. Estimates of the genetically effective population size of PWS herring are several orders of magnitude below census populations, even at post-collapse levels.

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Tables

Table 6.1. Comparison of DNA microsatellite heterozygosities (H_e), number (N) of alleles and M values of Prince William Sound herring with other sites in Alaska, British Columbia, Washington and California. H_e and N are from cited references, M values calculated from data in those references. H_e, N and M values shown as mean (standard deviation). Alaskan populations: PWS = Prince William Sound; KI = Kodiak Island; TB = Togiak Bay, Bristol Bay; NS = Norton Sound, Bering Sea.

Location	Collection	H_e	N of alleles	M value	Sample size	Reference
PWS	1995?	0.904 (0.023)	27.5 (7.7)	0.90 (0.08)	200	O'Connell et al. 1998a
PWS	1995	0.906 (0.032)	20.2 (4.1)	0.81 (0.12)	200	O'Connell et al. 1998b
KI	1995	0.899 (0.033)	20.0 (6.0)	0.64 (0.13)	50	O'Connell et al. 1998b
TB	1991	0.865 (0.083)	18.6 (4.8)	0.67 (0.10)	50	O'Connell et al. 1998b
NS	1991	0.836 (0.092)	19.2 (6.8)	0.82 (0.10)	50	O'Connell et al. 1998b
PWS	1995	0.922 (0.022)	19.8 (3.8)	0.81 (0.12)	200	Wright and Dillon 1997
PWS	1996	0.927 (0.023)	22.6 (6.5)	0.80 (0.11)	200	Wright and Dillon 1997
KI	1995	0.906 (0.038)	19.8 (5.7)	0.73 (0.10)	50	Wright and Dillon 1997
KI	1996	0.922 (0.017)	22.0 (5.7)	0.76 (0.13)	50	Wright and Dillon 1997
TB	1991	0.871 (0.082)	18.0 (4.1)	0.74 (0.08)	50	Wright and Dillon 1997
TB	1996	0.848 (0.042)	21.2 (5.8)	0.69 (0.09)	50	Wright and Dillon 1997
NS	1991	0.877 (0.070)	19.2 (7.2)	0.83 (0.10)	50	Wright and Dillon 1997
NS	1996	0.876 (0.058)	19.6 (6.1)	0.75 (0.09)	50	Wright and Dillon 1997

Table 6.2. Comparison of DNA microsatellite heterozygosities (H_e), number (N) of alleles, M values and heterozygosity excess of Prince William Sound herring with other sites in Alaska, British Columbia, Washington and California. H_e and N are from Small et al. (2004), M values and heterozygosity excess calculated from data in Small et al. H_e, N and M values shown as mean (standard deviation). Alaskan populations: PWS = Prince William Sound; Norton Sound, Bering Sea. British Columbia: SG = Nurthumberland, Strait of Georgia. Washington (WA): Puget Sound (PS); Cherry Point (CP). California: San Francisco (SF). NA = not available.

Location	Collection	H_e	N of alleles	M value	Heterozygosity Excess (p value)	Sample size
PWS	1996	0.860 (0.086)	19.5 (8.5)	0.81 (0.13)	0.120	96
NS	1996	0.844 (0.102)	20.0 (8.1)	0.76 (0.12)	0.183	100
SG	1999	0.838 (0.099)	18.2 (7.1)	0.85 (0.13)	0.206	96
WA-PS	1999-2002	0.835 (0.112)	17.9 (6.9)	0.79 (0.09)	**0.042**	672
WA-CP	1999-2003	0.844 (0.109)	17.9 (6.8)	0.79 (0.12)	0.260	480
CA-SF	1998	0.818 (0.121)	**16.0 (5.8)***	0.83 (0.19)	0.350	67

***** 0.01<p value<0.05 after Bonferroni correction

Table 6.3. Comparison of mitochondrial DNA haplotype diversities (h), number (N) of haplotypes, M values and heterozygosity excess from Prince William Sound herring with other sites in Alaska. N and h are from Bentzen (1997, Table 4), M values and Heterozygosity Excess calculated from data in Bentzen. N, h and M values shown as mean (standard deviation). Alaskan populations: PWS = Prince William Sound; Sites within PWS: SMB = St. Matthew's Bay, FB = Fish Bay, PCh = Port Chalmers, RB = Rocky Bay; KI = Kodiak Island; TB = Togiak Bay, Bristol Bay; Norton Sound, Bering Sea.

Location	Collection	h	N of haplotypes	M value	Heterozygosity Excess (p value)	Sample size
PWS	1995	0.536 (0.254)	7.5 (4.7)	0.67(0.23)	0.996**	400
SMB	1995	0.474 (0.270)	5.3 (2.2)	0.61 (0.33)	0.988**	100
FB	1995	0.524 (0.276)	7.6 (4.8)	0.66 (0.22)	0.980	100
PCh	1995	0.544 (0.256)	9.1 (5.8)	0.72 (0.21)	0.996**	100
RB	1995	0.571 (0.264)	9.4 (6.2)	0.67 (0.17)	0.988**	100
KI	1995	0.576 (0.296)	6.7 (4.4)	0.61 (0.28)	0.766	100
TB	1995	0.496 (0.344)	**4.9 (3.4)***	0.68 (0.36)	0.055	100
NS	1995	0.417 (0.295)	**4.0 (1.2)***	0.58 (0.35)	0.711	100

* 0.01<p value<0.05 after Bonferroni correction
** 0.01<p value<0.05 for heterozygosity deficit, no Bonferroni correction

Table 6.4. Heterozygosities at equilibrium (H_{eq}), effective population sizes (N_e) and census sizes (N) for stressed and healthy herring populations in the northeast Pacific. Census sizes are for the year closest to the year in which genetic analyses were completed.

		N_e		N_E/N Ratio		
	H_{eq}	Harmonic Mean (Range)	N	Based on Harmonic Mean	Reference for H_{eq}	Year and Reference for N
Stressed						
Prince William Sound	0.862	1275 (644-64,400)	137×10^6	9.3×10^{-6}	Wright and Dillon 1997	1995 Marty et al. 2003
	0.829	822 (415-41,5000)	137×10^6	6.0×10^{-6}	Small et al. 2004	
Cherry Point	0.748	667 (337-33,700)	8.4×10^6	7.9×10^{-5}	Small et al. 2004	2000 Gustafson et al. 2005
	0.90*	2451 (1238-123,800)	8.4×10^6	2.9×10^{-4}	Beacham et al. 2002	
San Francisco Bay	0.819	731 (369-36,900)	250×10^6	2.9×10^{-6}	Small et al. 2004	2000 Hay et al. 2001
	0.87*	1440 (727-72,700)	250×10^6	5.8×10^{-6}	Beacham et al. 2002	
Healthy						
Northern Bering Sea	0.874	1535 (775-77,500)	657×10^6 includes	2.3×10^{-6}	Wright and Dillon 1997	2000 Hay et al. 2001
	0.832	851 (430-43,000)	Bristol Bay	1.3×10^{-6}	Small et al. 2004	
Bristol Bay	0.873	1522 (762-76,200)		2.3×10^{-6}	Wright and Dillon 1997	Hay et al. 2001
Gulf of Alaska	0.883	1784 (901-90,100)	230×10^6	7.8×10^{-6}	Wright and Dillon 1997	2000 Hay et al. 2001
Southeast Alaska	0.878*	1638 (827-82,700)	230×10^6	7.1×10^{-6}	Beacham et al. 2002	2000 Hay et al. 2001
Strait of Georgia	0.815	699 (353-35,300)	770×10^6	9.1×10^{-7}	Small et al. 2004	2000 Beamish et al. 2001
	0.872*	1485 (750-75,000)	770×10^6	1.9×10^{-6}	Beacham et al. 2002	
Puget Sound	0.798	582 (294-29,400)	923×10^6	6.3×10^{-7}	Small et al. 2004	2004 Gustafson et al. 2005

* Values are expected heterozygosities

Chapter 7

An Early Life History Model for Pacific herring in Prince William Sound, Alaska

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Abstract

Using published data, we integrated information about survival and its uncertainty in egg, larval and juvenile life stages into a mathematical model to characterize the early life history of Pacific herring. The early life history (ELH) model predicted survival after the first year to be 118 herring out of one million eggs, with a 95% confidence interval of (5, 2,822). Our modeling efforts support Hjort's concept of mortality in the larval stages as the most "critical period" in determining year-class strength of herring fisheries. Estimates of survival of the egg stages, fall juvenile stage and winter juvenile stage were two orders of magnitude greater than the survival of the larvae stage. A single-stage sensitivity analysis demonstrated that the largest influence on total survival was daily mortality in the larval stage. An interaction sensitivity analysis of all possible paired life stages showed that the combination of the egg stage with the larval life stage contributed the most to total survival. Environmental processes, including food availability, water temperature, and transport processes, are key factors in the larval stage.

Keywords – Pacific herring, *Clupea pallasi*, Prince William Sound, early life history model

Introduction

Fluctuations in fish abundance are often due to variations in the strength of individual year classes (Walford, 1938). In turn, year-class strength is a function of the egg production in a given year and the subsequent high mortality found in the early life stages, from eggs through larvae to juveniles. High larval mortality is coupled with high fecundity in many marine fishes (May, 1973), leading to the "critical period" concept (Hjort, 1914). Mortality in the earliest larval stages is hypothesized to be the most critical period in determining year-class strength of herring fisheries (Hjort, 1914).

A foundation for part of this concept was the "larval drift hypothesis", i.e., that spawning takes place in a relatively restricted area (Damas, 1909; Schmidt, 1909). Eggs and larvae then drift passively with the currents and disperse. Later the larval drift hypothesis was expanded into a generalized pattern of fish migration (Harden Jones, 1968). Eggs and larvae are distributed passively by surface-layer currents into a nursery area, juveniles grow to sub-adult stage in a nursery area that offers survival benefits in some form, and then actively migrate to join adults, and the adult stock migrates to localized spawning area during spawning season and returns to feeding grounds afterwards.

Hjort's critical period hypothesis was further modified in the famous "match/mismatch" hypothesis that added the concepts of critical depth and stratification in the zooplankton production cycle (Cushing, 1975). Variations in temporal overlap of zooplankton with larval fish could affect larval herring feeding success and their ultimate survival (Blaxter, 1985). Additional work on clupeoids revealed that there was insufficient evidence to determine if food or transport was the cause of recruitment variation (Lasker, 1985).

The hydrodynamic and meteorological events that influence water movement affect year-class recruitment by affecting the vertical stability of the water column, thereby providing concentrations of suitable food (Lasker, 1978), and by affecting transport of larvae to areas of good or bad food supply and predator fields (Fortier and Leggett, 1982; Frank and Leggett, 1982; Crecco et al., 1983; Crecco and Savoy, 1984; Lambert and Ware, 1984; Leggett et al., 1984; Sherman et al,, 1984), estuarine nursery areas (Nelson et al., 1977; Shaw et al., 1985), and areas of recruitment to adult stocks (Bailey, 1981; Parrish et al., 1981; Bolz and Lough, 1984; Power, 1986).

While the concepts of the critical period and its causes were based on Atlantic herring (*Clupea harengus*), they may well be applicable to Pacific herring (*Clupea pallasii*), a congener that was not recognized as a separate species until recently (Mecklenburg et al. 2002). In March – May (Steve Moffitt, Alaska Dept. of Fish and Game, Cordova, AK, personal communication), age-4 and older herring spawn on 23 – 168 km of coastline in Prince William Sound (PWS) (Norcross et al., 2001). Pacific herring are demersal spawners that deposit and fertilize their eggs on brown and red filamentous algae and red foliose algae in shallow sub-tidal waters of PWS (Gerke, 2002). However, rather than type of vegetation, location of egg deposition may also be a function of the depth in a given area (Haegele and Schweigert, 1985). Some eggs become detached from substrate and accumulate in bottom depressions, where invertebrate predation is significant (Haegele, 1993). Herring are especially vulnerable to egg mortality because they incubate for up to 21 days in shallow locations (Rooper et al., 1999). Many of the herring eggs are lost to predation (Bishop and Green, 2001), wave-action, and exposure (Rooper et al., 1999). The surviving herring eggs hatch as larvae in May (Brown et al., 1996). After herring larvae are advected from natal areas, the planktonic larvae drift counter-clockwise through the open water of PWS pushed by surface currents, buoyant forces, and meteorological forces (Brown et al., 1996). For up to three months larvae are transported around PWS and subject to starvation and predation (Norcross and Brown, 2001).

Metamorphosis of the larval herring begins to occur in June or July (Stokesbury et al., 2002). Herring become nektonic and swim to favorable habitats, and thus are no longer at the mercy of the currents. In August, the young herring begin to form schools and aggregate at the heads of bays far from coastal waters (Brown et al, 2002; Stokesbury et al., 2000). These populations stay isolated in their respective nursery areas through the first two winters (Stokesbury et al., 2000). From August to October, age-0 juvenile herring survival depends on food availability, competition, predation, and disease (Stokesbury et al., 2002). During over-wintering, food resources are limited and herring depend on energy stores. Water temperature, photoperiod, and prey availability affect the mortality of over-wintering herring (Paul and Paul, 1998). Herring survival to age-1 is not equal in all nursery bays in PWS (Patrick, 2000; Norcross et al., 2001).

Inadequate understanding of factors regulating recruitment is the major obstacle to building population dynamics models that accurately predict the ranges of future stock sizes and yields (Sissenwine, 1984). Improved understanding of this variability in abundance of early life

stages of fishes before they enter the exploitable stock could lead to better sustainable yields from fishery resources.

A previous study estimated stage-specific survival of Pacific herring during the first year (Norcross and Brown, 2001). That study was based on life-stage specific ranges of survival and resulted in cumulative upper and lower survival limits. Here we expand that study using knowledge derived from additional publications. As a further improvement, we use statistical distributions instead of ranges to determine overall survival and its uncertainty.

Methods

Model

The model for the mortality of herring during early life history is the standard year-class model (Quinn and Deriso, 1999, chapter 1). Let N_0 be the initial egg production at age 0; $N(t)$, the number of individuals on day t; and z_t, the daily instantaneous mortality. The standard abundance equation is then

$$N_t = N_0 \exp\left(-\sum_t z_t\right), \quad (1)$$

in which the time unit is one day. In terms of daily survival fraction,

$$s_t = \exp(-z_t). \quad (2)$$

Mortality changes as a function of life stage (Brown, 2003), so we let z_i be the constant instantaneous daily mortality in life stage i. To obtain the cumulative mortality for each stage the mortality per day was multiplied by the number of days d_i for the life stage, or

$$Z_i = d_i \cdot z_i. \quad (3)$$

Survival is then

$$S_i = \exp(-Z_i). \quad (4)$$

The total mortality, Z_{tot}, combines the cumulative mortalities of sequential life stages, including the egg stage, larval stage, and late summer and winter juvenile stages. Therefore, total mortality during early life history is

$$Z_{tot} = \sum_i Z_i, \quad (5)$$

with corresponding survival

$$S_{tot} = \exp(-Z_{tot}). \quad (6)$$

Estimates of survival and their standard errors (SE) come from the literature (see Data section below) and can be converted into instantaneous daily mortality rates by using the inverse relationship of (4) combined with (3), or

(7) $$z_i = -\ln(S_i)/d_i .$$

The estimates are assumed to be independent, as they come from separate studies. The delta method (Seber, 1982, p. 7-9) is used to convert the standard error of survival to that of mortality:

(8) $$\mathrm{SE}(Z_i) = \frac{\mathrm{SE}(S_i)}{S_i} = \mathrm{CV}(S_i).$$

The SE for cumulative mortality from (5) is

(9) $$\mathrm{SE}(Z_{tot}) = \sqrt{\sum_i \mathrm{SE}^2(Z_i)},$$

with corresponding SE of survival from the delta method of

(10) $$\mathrm{SE}(S_{tot}) = S_{tot}\mathrm{CV}(Z_{tot}) = S_{tot}\frac{\mathrm{SE}(Z_{tot})}{Z_{tot}}.$$

The underlying statistical distribution of mortality is assumed to be the normal distribution according to common practice. Consequently, the distribution of survival is lognormal. This approach allows determination of 95% confidence intervals, unlike the approach of Norcross and Brown (2001) that multiplied lower (or upper) limits of individual confidence intervals.

Data

We considered four life stages in the first year of life as particularly influential on herring survival: egg (first 21 days), larval (next 92 days), fall juvenile (next 92 days), and winter juvenile (next 135 days) (Norcross and Brown, 2001). Published estimates for herring survival at each life stage were evaluated for incorporation into the survival model. We developed a ranking system in which the highest, Rank 1, was for a study of Pacific herring in PWS that contained a standard error estimate (SE). Rank 2 was for a study of Pacific herring that had a standard error estimate but was conducted on herring outside of PWS. Rank 3 was for a study that contained information on Pacific herring in PWS, but had no standard error estimates available. Data were not included in the model from non-PWS herring with no standard error estimates or any data for Atlantic herring, *Clupea harengus*.

Egg Stage

Herring egg stage survival is affected by two measurable sources of mortality, one above the water and one below the water. Our survival estimate was derived from both subsurface invertebrate predation (Haegele, 1993) and duration of air exposure (Rooper et al., 1999). Crabs, sea anemones, sea cucumbers, and snails consume significant amount of herring eggs (Haegele, 1993). The observed rate of oophagy on herring spawn among the epibenthic invertebrates of British Columbia (Rank 2) is $0.0018*d^{-1}$ (SE = 0.011) (Haegele, 1993). Amount of time of air exposure can be used as a proxy for the multiple factors determining egg loss during incubation. These factors include depth, tide, storm, and avian predation affect (Rooper et al., 1999). The

average mortality from air exposure (Rank 1) that we included in this model is $0.0654d^{-1}$ (SE = 0.0062). Herring eggs spawned at intermediate depths would have higher survival rates due to decreased predation and wave action (Rooper et al., 1999). For both sources of per-day mortality, the life stage was assumed to be 21 days in April (Haegele, 1993; Rooper et al., 1999).

Larval Stage

Larval mortality is caused by advection, predation, inability to feed, and other factors (McGurk, 1993; McGurk et al., 1993; Norcross et al., 1996). Larval mortality was estimated from 22 May to 19 July in Auke Bay, Southeast Alaska by converting catch densities of larvae into larval numbers by adjusting area for the effects of dispersion (McGurk et al, 1993). Average mortality (Rank 2) was $0.0652d^{-1}$ (SE = 0.0162), the value that we used for larval herring mortality in the model. These survival estimates included the first feeding mortality estimates of McGurk (1989); therefore, any post-hatch mortality due to cranial-facial deformities is incorporated. Mortality estimates from Southeast Alaska were comparable with the geometric mean mortality of Pacific herring larvae ($0.083d^{-1}$) along the British Columbia coast (McGurk, 1993). We assumed that the larval period in Prince William Sound was 92 days from larval hatch on 1 May to metamorphosis on 31 July (Stokesbury et al., 2000).

Fall Juvenile Stage

Predation is the greatest mortality to age-0 herring from the time of metamorphosis and movement into bays through fall and into October (Stokesbury et al., 2000; 2002). Estimates of juvenile mortality came from herring densities in four nursery areas within PWS in fall 1996 and 1997 (Stokesbury et al., 2002). Instantaneous mortality rates were estimated from acoustic observations of cohorts of fish followed from late summer through spring. The instantaneous mortality rates varied among bays, cohorts, and ages, with young-of-the-year herring always sustaining higher mortality. The instantaneous mortality (Rank 1) for age-0 herring averaged over four bays was $0.0090d^{-1}$(SE = 0.002) in 1996 and $0.0161d^{-1}$ (SE = 0.0121) in 1997. For input to the model we used an average of the mortality estimates and standard error rates (0.0126, SE = 0.0062). The fall juvenile period was assumed to be 92 days from 1 August to 31 October.

Winter Juvenile Stage

Mortality of age-0 winter herring is due to starvation (Paul and Paul, 1998; Paul et al., 1998; Foy and Paul, 1999) and predation (Stokesbury et al., 2002). There are no data available with which to estimate predation, but the effects of starvation can be estimated. The dependence of herring on stored energy reserves to survive the first winter takes into account the empirical relationship of a species with temperature, individual mass, and respiration (Arrhenius and Hansson, 1996; Foy and Paul 1999). A variation of a bioenergetics model was used to describe winter fasting of age-0 herring in PWS (Patrick, 2000). Input energy flux to the fish is denoted by η. Output energy flux from the fish is split into two sub-groups: basal respiration and swimming speed, $R(\nu,T,m)$, where ν is the swimming speed, T is temperature, and m is individual mass, and $L(\eta,T,m)$ is the assimilation costs due to egestion and excretion,

$$\frac{dm}{dt} = \frac{1}{\hat{E}}\left(\eta - L(\eta,T,m) - R(\nu,T,m)\right), t \in \left[t_0, t_1\right] \quad (11)$$

This model (Patrick, 2000) is further modified and simplified. The model assumes that no feeding occurs during the first winter; therefore η is zero. The model also assumes that there is no predation through the winter months and that no migration occurs into or out of nursery bays. Mortality of age-0 herring in the model depends on energy stores (whole body energy content) and ambient winter water temperature through the first winter. Mortality for all bays over three years ranged from 1-95 % (Patrick, 2000; Norcross et al., 2001). The instantaneous mortality for age-0 herring averaged over 12 bays was $0.00354d^{-1}$. The winter juvenile period was assumed to be 135 days from 1 November through to 15 March. No estimate for standard error (Rank 3) was provided. We assumed that the coefficient of variation for winter juvenile herring was the same as for autumn juvenile herring (CV = 49%; Stokesbury et al., 2002), and hence, multiplied the CV by the estimated daily mortality to get an approximation of the standard error (SE = 0.0017).

Sensitivity Analysis:

Once the early life history (ELH) model for Prince William Sound Pacific herring had been constructed, the necessary subsequent step was to determine the sensitivity of the overall survival in the first year to perturbations of survival in each life stage. A single-stage sensitivity analysis was performed by altering the daily mortality in each life stage (z_i) by 10%. The resulting sensitivity of the total survival was computed (S_{tot}, following eq. 6) after both increasing and decreasing the daily mortality for each life stage. Not only did we want to determine the sensitivity of S_{tot} to changes in individual life stage survival, we were interested in ascertaining how interactions between life stages affected S_{tot}. The interaction sensitivity analysis was performed by first determining all the possible paired combinations of life stages. Then within each pair the daily mortality was altered by 10%, as in the single stage sensitivity analysis. There were four possible combinations of altering the daily mortality within each pair that was investigated, either both increased, both decreased, or a combination of increase and decrease. This sensitivity analysis resulted in 40 possible combinations of life stage interactions, each with a resulting total survival.

Results

Survival estimates differed for all life stages in the first year (Table 1). Survival for Pacific herring in PWS was the lowest in the larval stage ($S_l = 0.0025$). Estimates of survival of the two egg stages (both invertebrate predation and air exposure), fall juveniles and winter juveniles were two orders of magnitude greater than the survival of larvae. Total survival is the product of individual survivals; total mortality is the sum of individual mortalities (Table 1). Total survival was estimated as 118 herring out of one million eggs ($S_{tot} = 1.181 \times 10^{-4}$). The survival estimate came from a total mortality (Z_{tot}) of 9.0437 (SE(Z_{tot}) = 1.6191). The distribution of mortality (Figure 1) was normal and the distribution of survival was log-normal (Figure 2) as a consequence of the assumptions in the model.

These estimates of first-year survival are consistent with results from an age-structured assessment (ASA) model (Hulson et al. 2006) The average of ten annual estimates of total mortality from age-0 to age-3 from the ASA is $Z_{ASA} = 10.73$ (.SE(Z_{ASA}) = 1.14). The distribution of ASA mortality was normal (Figure 1) and shifted to the right to indicate higher mortality. The corresponding distribution of survival was log-normal and shifted to the left (Figure 2). This comparison shows that the bulk of mortality during the first three years is accounted for by the mortality during the first year of life.

For further insight, we calculated abundance estimates after each life stage from an initial production of 1 million eggs. The largest percentage decrease in abundance was after the larval stage (Table 2). The probability distributions of survival became increasingly skewed to the right as life stage advanced (Figure 2). The ELH model estimated the survival to age-1 to be much closer to the lower confidence limit than to the upper limit.

The single-stage sensitivity analysis demonstrated that life stages did not contribute equally to mortality and survival (Figure 3). The largest influence on total survival was the larval stage. A 10% decrease in daily larval mortality resulted in almost a doubling of survival. A 10% increase in daily larval mortality resulted in a total survival that was an order of magnitude lower. Altering the winter juvenile daily mortality resulted in virtually no change in total survival (Figure 3). The effects of the egg stage and autumn juvenile stage were intermediate but not remarkable.

The results of the interaction sensitivity analysis of all possible paired life stages affirmed the results of the single life stage sensitivity. The larval life stage, in combination with any other life stage, contributed the most to total survival (Table 3). Total survival was maximized by decreasing the daily mortality for both the larval and egg stages (outlined in bold in Table 3). The estimated total survival of this combination was greater than any resulting total survival from the single-stage sensitivity analysis, and more than double the total survival estimated by the base ELH model. The next largest increase in survival, and comparable to the previous pair, was for a decrease in daily mortality for both the larval stage and autumn juvenile stage. This resulting difference in total survival from each of these competing pairs (larval-egg vs. larval-autumn juvenile) corresponded to an increase of only 6 herring out of 1 million eggs that survive to age-1.

In the interaction sensitivity analysis, total survival was minimized similarly by increasing the daily mortality in the larval stage. The lowest survival occurred when mortality was increased in both the egg and larval stages (outlined in dashed bold in Table 3). The resulting survival was an order of magnitude lower than the base ELH value. Similarly, an increase in daily mortality in the larval and autumn juvenile stage resulted in the second lowest total survival. Other paired combinations had minimal affect on the total survival, including the two periods of the juvenile stage, winter and autumn, and combinations of these juvenile stages with the egg stage (Table 3). However, it is important to note that when egg stage was combined with either of the two juvenile stages when the daily mortality was decreased for both life stages, the resulting total mortality was an order of magnitude smaller than the base ELH survival. Upon obtaining these results we concluded that any interaction analysis for more than two stages would produce similar results, i.e., the larval stage would dominate the resulting total survival.

Discussion

The much-discussed concept of "critical period" (Hjort, 1914) has fallen out of scientific favor in the last few decades. Therefore, it is surprising that our modeling efforts support Hjort's (1914) concept of mortality in the larval stage as the most critical period in determining year-class strength of herring fisheries. Our ELH model results agree with the earlier conclusion that the larval stage of Pacific herring is the most vulnerable (Norcross and Brown, 2001). However, the new ELH model uses statistical distributions instead of ranges to combine life stages and hence is superior. The estimated survival by life stage was similar for three of the four life stages examined (current results vs. Norcross and Brown, 2001): eggs 24% vs. 24-45%; fall juveniles 31% vs. 2-21 %, and winter juveniles 62% vs. 5-99%. However, larval survival used in the ELH

model was an order of magnitude lower (0.25% vs. 1-7%), resulting in a much lower total survival. These results are less surprising given the outcome of similar life-stage investigations of Atlantic herring on the east (Blaxter and Ehrlich, 1974; Cushing, 1990) and west (Anthony and Forgarty, 1985; Campbell and Graham, 1991) sides of the Atlantic Ocean.

Our results appear to disagree with a recent analysis that employed different types of models than we used. Use of Paulik diagrams revealed that for Atlantic herring in the North Sea, only poor year classes are determined in larval stage (< 1 month old) while strong year classes are formed at older (1-5 months old) stages (Nash and Dickey-Collas, 2005). The North Sea herring spawn September through January, as opposed to the March through April spawning in PWS. In this analysis, we considered larvae to be 0-3 months old. Therefore the difference in spawning seasons combined with the difference in age-at-life-stage interpretation could mean, while year-class strength of Pacific herring in PWS is determined during the larval stage, that there may actually be different mechanisms working early and late within that stage as determined for North Sea herring. For herring in PWS, growth rates are not constant over the duration of the larval period (Thornton, 2003), indicating differential survival over time.

Larval dynamics control year-class strength through critical periods caused by lack of food for larvae or unfavorable environmental conditions (Hjort, 1914). Lack of food available for larvae at the time active feeding begins (time of yolk-sac absorption) would be critical. In the 'match-mismatch' hypothesis, survival is dependent upon the degree of temporal 'match' between the hatching of the larvae and the spring bloom of their prey (Cushing, 1975). Based on a coupled biophysical model for PWS 1993 through 1997, larval herring survival is determined by zooplankton-dependent growth rates and water temperature (Thornton, 2003). When prey are not available, because of reduced amount of vertical stratification, survival of herring larvae is limited. Survival is inversely related to length of larval stage duration. Water temperature controls the yolk-sac phase length, with greater survival in warmer years. Herring larvae with faster growth rates experience earlier metamorphosis and higher survival to the juvenile stage (Thornton, 2003).

Low growth rates result in a longer exposure time for mortality through predation or transport out of favorable oceanographic regions (Cushing, 1990; Leggett and Deblois, 1994). A longer larval period could result in poor condition for juvenile herring that must prepare for winter (Paul et al., 1998; Foy and Paul, 1999; Norcross, et al. 2001). Transport offshore can lead to increased mortality from lack of food, salinity intolerance or increased predation pressure (Stevenson, 1962; Alderdice and Hourston, 1985; Stocker et al, 1985; McGurk, 1989; Wespestad and Moksness, 1990).

Another factor causing high mortality in larval herring is physical transportation away from areas favorable to development. Successful larval survival is dependent on both the advection pathways in PWS that result in larvae arriving at suitable nursery areas and the distribution of spawning areas. Larval advection may contribute more to larval mortality in spawning areas adjacent to open areas than inside fjords and inlets where local circulation enhances retention (Brown, 2003). Atlantic herring recruitment has been linked with spatially distinct larval retention areas, suggesting that herring population abundance depends on the area available to the post-larval stages (Iles and Sinclair, 1982). Transport of larvae to juvenile nursery areas promotes individual growth and recruitment to the adult population (Hare and Cowen, 1996). Herring use of estuaries as early juveniles suggests that the juvenile stage may also be a critical period with increased food requirements or increased per capita predation risk

(Maes et al., 2005). Our results show that getting herring larvae to survive to the juvenile stage, which can benefit from good nursery areas, is even more critical.

Advected herring larvae are not distributed evenly in PWS and distribution is largely based on the location of natal area (Brown, 2003). Spawning concentrates eggs, thus dispersal is essential for survival. The ability of larvae to enter nurseries and the quality and quantity of those nursery areas affects year-class strength and population size (Urho, 1999). Contraction of nursery areas could decrease the resilience of herring at very high stock sizes, as recruitment is heavily influenced by density dependency (Goodwin et al., 2006). Therefore the spatial diversity of spawning grounds, as the origins of larval advection, could severely influence the survival of larval and juvenile Pacific herring.

In addition to limiting the dispersal of larval herring, concentration of spawning herring could have other deleterious effects. The area of herring spawning was more than 10 times larger in 1988 (Figure 4) than that of the smallest extent, 1994 (Norcross and Brown, 2001). Not only was there a decrease in the aerial extent of spawning, there was also a reduction of the locations in PWS in which spawning occurred. Since 1990, spawning in the northern and northeast Alaska Department of Fish and Game (ADF&G) herring survey sectors was minimal or nonexistent (Figure 4). Simultaneously, the estimated spawning biomass was about five times larger in 1988 than in 1994 (Hulson et al., in review). However, while the miles of herring spawning decreased after 1988, the number of eggs deposited remained high through 1992 (Hulson et al., in review). Concentration of spawning amidst high egg production implies increased density of egg deposition. Pacific herring egg deposition can vary in density from thinly scattered layers to more than 20 layers (Haegele and Schweigert 1985), with increased density contributing to egg mortality (Hay 1985).

High stock density produces chronic stress and lessens ability of fish to meet increased energy demands (Montero et al., 1999). None of the 1989-1998 year classes of herring in PWS were strong like the 1980, 1984, and 1988 year classes (Hulson et al., in review). Increasing biomass and decreasing food availability may have led to poor nutritional status, and that alone or in combination with disease explains the 1993 decline (Pearson et al. 1999). That might mean that the disease that caused the collapse of herring in PWS in 1993 (Marty et al. 1998; 2003; Carls et al. 2002), began affecting the herring population during the years of crowding of spawning concentrations. Diseases emerge by rapidly increasing incidence or geography. Factors affecting emergence include ecological, environmental, demographic, i.e., increased contact (Morse, 1995). Factors that characterize fish diseases are population density, contagiousness, duration of infection, and immunity development (Reno, 1998). A propagative epidemic develops when a pathogen is passed between infectious and susceptible individuals (Smith et al., 2000). Fish density and pathogen concentration interact to affect outcome of disease outbreak (Bebak-Williams et al., 2002).When fish density is high, even though the number of infected fish is low, the death rate increases, time to death decreases, and probability of survival decreases (Bebak-Williams et al., 2002). This, combined with concentration of spawning areas, seems to characterize the situation is PWS 1989-1992. With the high infection rates found in herring in PWS in 1993 and 1999, the density of fish does not matter.

The ELH model provides a framework for organizing the relative importance of the vulnerable life stages that determine herring recruitment. Our model increases our understanding of the critical life history stages and offers insights into the mechanisms causing variable recruitment success. Further investigations are needed into the early life history of herring to

provide more accurate estimates of the effects of the physical and biological factors on each life stage.

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Table 7.1. Survival (*S*), mortality (*Z*), and standard error of mortality (SE(*Z*)) for each life stage estimated by the early life history (ELH) model.

Life Stage	Source/Period of Mortality	Survival (S)	Mortality (Z)	SE(Z)
Egg	Invertebrate Predation	0.963	0.038	0.023
	Air Exposure	0.253	1.374	0.131
Larval	Drift	0.0025	5.996	1.493
Juvenile	Autumn	0.314	1.157	0.567
	Winter	0.620	0.478	0.234
	Total:	1.18E-04	9.04	1.619

Table 7.2. Estimated abundance after each life stage from an initial population of 1 million eggs, with 95% confidence values included.

Life Stage	95% LCI	N	95% UCI
Egg	187801	243639	316078
Larval	32	606	11430
Juvenile (Autumn)	8	191	4402
Juvenile (Winter)	5	118	2822

Table 7.3. Early life history model estimated total survival (S_{tot}) resulting from paired stage interaction sensitivity analysis. Portion of table is removed due to redundant results.

Life Stage		Egg		Larval		Juvenile Autumn	
	SA Adjustment*	I	D	I	D	I	D
Larval	I	5.63E-05	7.47E-05	Not Applicable			
	D	1.87E-04	2.48E-04				
Juvenile	I	9.14E-05	1.21E-04	5.78E-05	1.92E-04		
Autumn	D	1.15E-04	1.53E-04	7.28E-05	2.42E-04		
Juvenile	I	9.78E-05	1.30E-04	6.18E-05	2.05E-04	1.00E-04	1.26E-04
Winter	D	1.08E-04	1.43E-04	6.80E-05	2.26E-04	1.10E-04	1.39E-04

*For Sensitivity Analysis (SA)-I:10% increase in mortality, D:10% decrease in mortality

Cell with Solid Bold Outline: highest survival when perturbing two stages
Cell with Dashed Bold Outline: lowest survival when perturbing two stages

Figure 7.1. Distribution of total mortality (Z_{tot}) from the early life history (ELH) model up to age-1 and the age-structured assessment (ASA) model up to age-3. The frequency of occurrence, or density corresponding to a probability density function, is plotted on the *y*-axis.

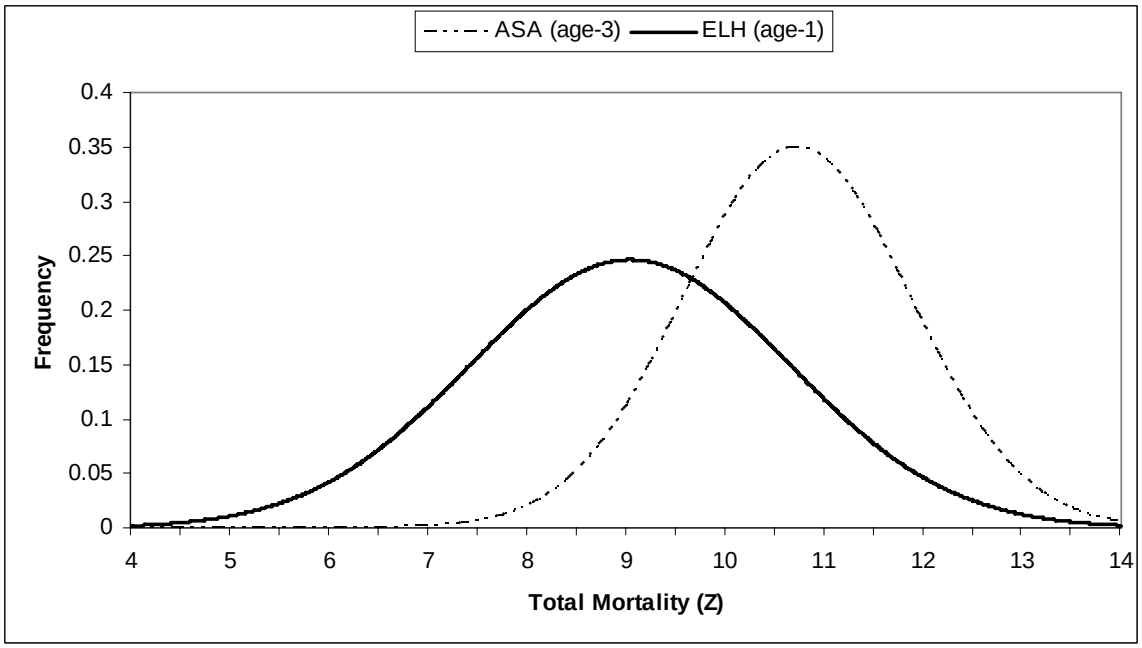
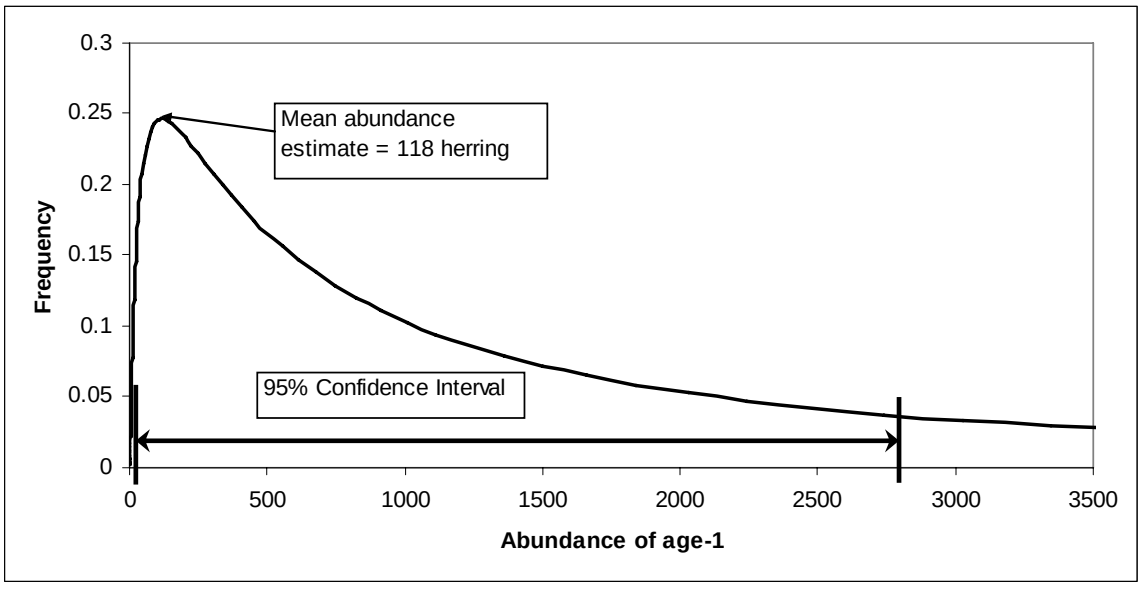


Figure 7.2. Distribution of abundance of age-1 herring after the first year of life, as computed by the early life history (ELH) model. Included are the 95% confidence range and mean abundance estimate. The frequency is plotted on the *y*-axis.



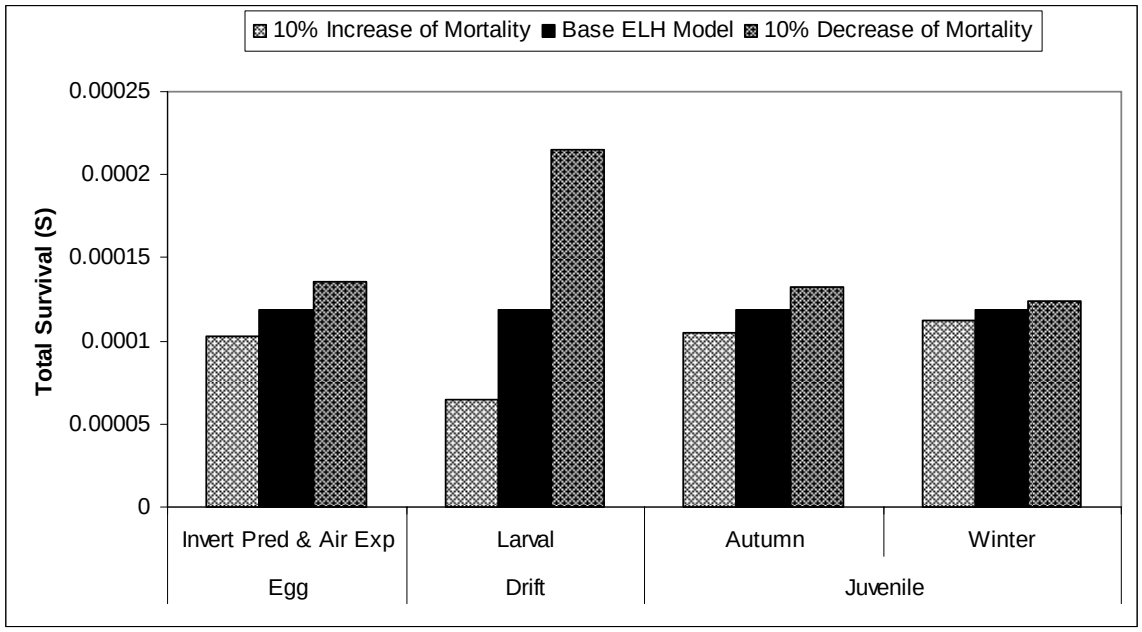


Figure 7.3. Results of single-stage sensitivity analysis. Both of the textured series are the results from increasing (left) or decreasing (right) daily mortality (z_i) while the black series is the total survival estimated from the base early life history (ELH) model ($S_{tot} = 1.181 \times 10^{-4}$). Total survival ($S_{tot}$) is plotted on the *y*-axis.

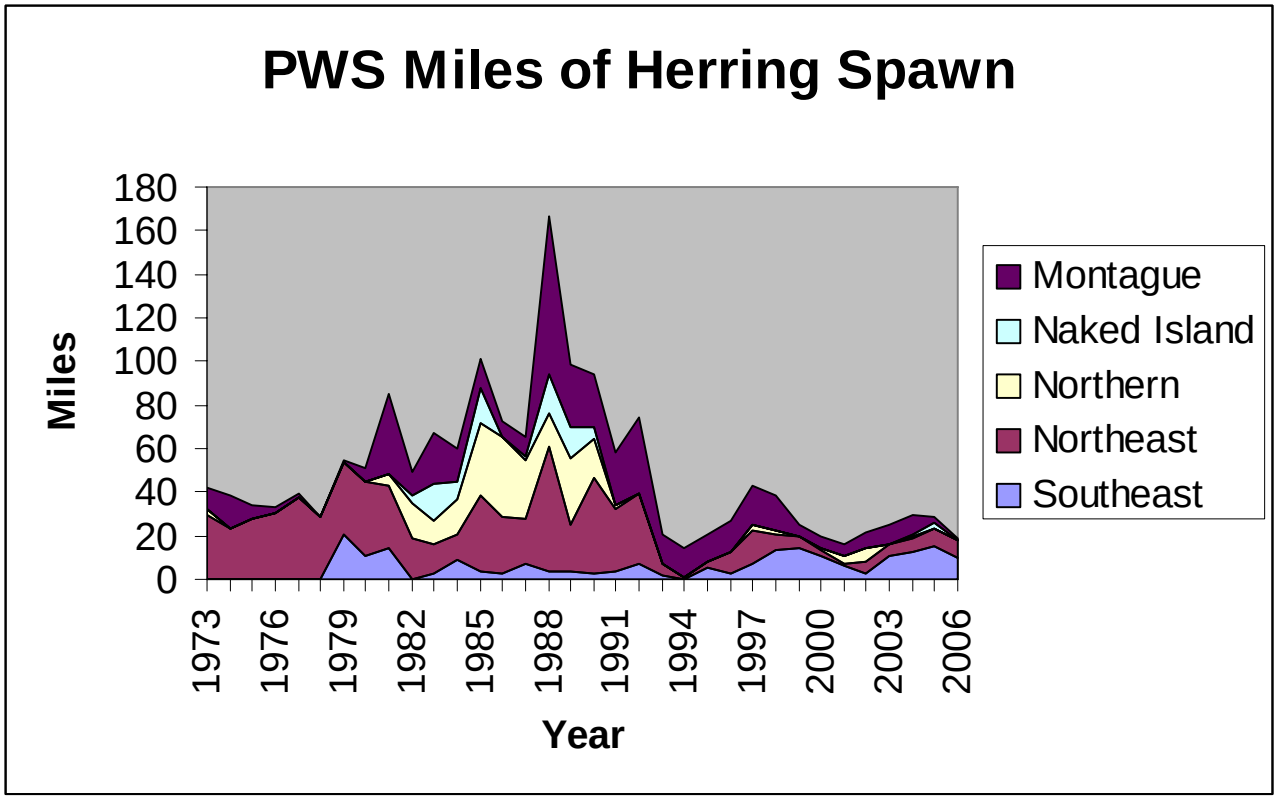
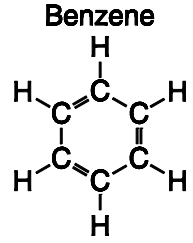


Figure 7.4. Linear miles of herring spawning in Prince William Sound, 1973 – 2006, shown by Alaska Department of Fish and Game survey area.

Appendix 1

Aromatic hydrocarbons

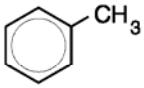
Crude oil contains a wide variety of compounds; those of greatest concern are the toxic aromatic hydrocarbons and hetrocyclic compounds. Aromatic hydrocarbons are ring compounds; benzene is the fundamental structure, with six carbon atoms (C) joined together and six hydrogen atoms (H) bonded at each corner of the resultant hexagon (see illustration at left). Double bonds are represented with double lines, though these change position so rapidly that they are considered "shared" and are typically illustrated with a circle. Various molecules often replace hydrogen, giving rise to other compounds with names such as toluene and ethyl-benzene. Aromatics with a single ring are called monoaromatic; compounds with two or more rings are typically called polynuclear (or sometimes "polycyclic") aromatic hydrocarbons (PAH; see Fig. 2). Because monoaromatic compounds typically evaporate rapidly (within hours or days), and because PAH typically remain dissolved in water for longer periods of time, toxicity research has focused on the latter in recent decades. Aromatic hydrocarbons have an electronic structure that makes them more soluble in water than non-aromatic hydrocarbons of comparable size. Atoms of elements other than carbon can be present, such as sulfur, nitrogen, and oxygen. These are heterocyclic aromatic hydrocarbons and are often included with PAH (e.g., Bence and Burns 1995; Short et al. 1996).



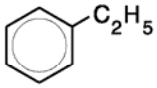
Monoaromatic hydrocarbons

Benzene

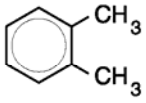
Toluene



Ethylbenzene

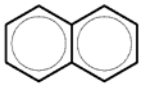


o-Xylene

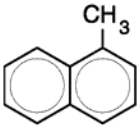


Naphthalenes

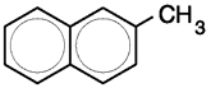
Naphthalene



1-methylnaphthalene

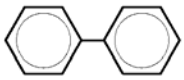


2-methylnaphthalene

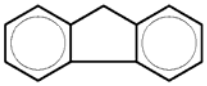


Polynuclear aromatic hydrocarbons

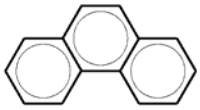
Biphenyl



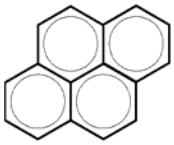
Fluorene



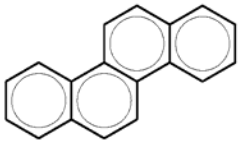
Phenanthrene



Pyrene

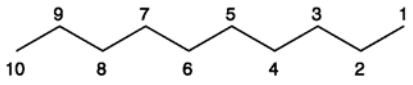


Chrysene

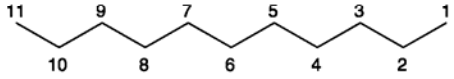


Alkanes

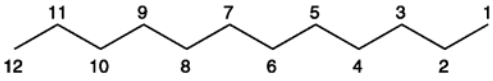
n-decane



n-undecane

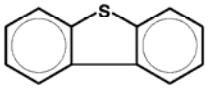


n-dodecane



Heterocyclic hydrocarbons

Dibenzothiophene



Appendix 2

Oiled rock column (ORC) assays

Oiled rock column (ORC) assays were developed as a standard procedure to test polynuclear aromatic hydrocarbon (PAH) toxicity after the *Exxon Valdez* oil spill (Marty et al. 1997). The primary purpose of this appendix is to address misunderstandings about the physical characteristics of the effluent produced by ORC. Also discussed is effluent chemistry, hydrocarbon exposure in embryos and larvae exposed to effluent, and causes of toxicity in these assays. Demonstrated are 1) ORC effluent contains primarily dissolved oil constituents; particulate oil (oil droplets) are rare, 2) oil constituents associated with fish eggs in ORC experiments are dissolved, 3) bioaccumulated polynuclear aromatic hydrocarbons (PAH) enter embryonic tissue, and 4) solutions containing particulate oil are no more toxic than those containing dissolved PAH only when compared on a tissue uptake basis.

Methods

In ORC experiments, water is passed through oiled gravel to produce water contaminated with oil constituents for use in assays (e.g., Marty et al. 1997). Effluent concentrations are controlled primarily by the quantity of oil applied to the rock, rock characteristics, and water flow. Rock diameter in these experiments is typically about 5 mm; the smaller the rock the greater the surface area of oil-water contact and the thinner the oil film will be. Oil has been applied by spraying it onto tumbling rock or by shaking oil and rock together. The rock is spread out and allowed to air dry for 24 h before use. Oiled rock can be frozen pending later use. Rock mass has ranged from 1.6 to 90 kg (about 1 to 57 L), and water flow has ranged from 0.01 to 7.3 L/min. Oil film thickness and total grams of oil are the best two predictors of initial total PAH (TPAH) concentrations in effluent; other factors include water flow rate, oil-water contact time, surface area, and weathering. Columns and flows are sized to meet experimental requirements, ranging from benchtop, e.g. zebrafish embryos (Incardona et al. 2005) to large volume, e.g., adult Pacific herring (Carls et al. 1998).

Concentrations of PAH and alkanes on oiled rock, in effluent water, and on rock were determined by GC/MS per methods of Short et al. (1996). Samples were extracted with dichloromethane after addition of perdeuterated surrogate hydrocarbon standards (naphthalene-d_8, acenaphthene-d_{10}, phenanthrene-d_{10}, chrysene-d_{12}, perylene-d_{12}, benzo[a]pyrene-d_{12}, n-dodecane-d_{26}, n-hexadecane-d_{34}, n-eicosane-d_{42}, n-tetracosine-d_{50}, and n-triacontane-d_{64}). Isolation and purification of calibrated and uncalibrated hydrocarbons was completed by silica gel/alumina column chromatography followed by size-exclusion high-performance liquid chromatography and fractionation; water samples were only processed through aluminum/silica columns. Extracts of PAH were separated and measured by gas chromatography/mass spectroscopy in the selected ion mode. Calibrated PAH were identified by retention time and two characteristic mass fragment ions, and quantified using a 5-point calibration curve. Uncalibrated PAH homologs (which included alkyl-substituted homologs of naphthalene, fluorene, dibenzothiophene, phenanthrene, and chrysene) were identified by retention times and a single characteristic

mass fragment ion. Uncalibrated PAH were quantified by using calibration curves of the most similar calibrated homologs: 2,6-dimethylnaphthalene for dimethylnaphthalenes; 2,3,5-trimethylnaphthalene for tri- and tetramethylnaphthalenes, and 1-methylphenanthrene for all alkylphenanthrene homologs. Quality control samples (including National Institute of Standards and Technology standard reference material samples, spiked samples, and blanks) were analyzed with each batch of 12 samples to assess the accuracy and precision of the analyses, and to verify the absence of laboratory contaminants introduced during processing (Short et al. 1996). Experimentally determined method detection limits (MDL) depended on sample weights, and generally were 1 ppb in sediment or tissue, and 1 to 8 pptr (ng/L) in water. Sediment and tissue concentrations are reported on a dry-weight basis; wet to dry weight ratios were measured by dehydrating 1 g wet samples for 24 h at 60°C and weighing the remaining mass. The accuracy of the hydrocarbon analyses was about ±15% based on comparison with National Institute of Standards and Technology values, and precision expressed as coefficient of variation was usually less than about 20%, depending on the PAH. Concentrations below method detection limits (MDL) were treated as zero (Short et al. 1996). Data were assembled from several studies involving Pacific herring (Carls et al. 1998, 1999; Barron et al. 2003) and pink salmon (Marty et al. 1997; Heintz et al. 1999, 2000; Carls et al. 2005).

Phytane is used an indicator of the presence (or absence) of particulate oil. Phytane is ubiquitous in crude oils and remains in refined oils if not removed during distillation (Dean and Whitehead 1961), it is considerably more resistant to microbial oxidation than are the normal alkanes (Pirnik 1977), and it has very low (<0.5 μg/L) solubility in water, based on comparisons with *n*-alkanes of comparable molecular weight (Sutton and Calder 1974).

The composition of effluent PAH changes with time, described as weathering. One way to characterize this change is with an index of weathering (w) estimated with a first-order loss-rate model developed by Short and Heintz (1997). Definitions previously used are: unweathered ($w = 0$), slightly weathered ($0 < w \leq 2$), moderately weathered ($2 < w \leq 8$), and highly weathered ($w > 8$) (Carls et al. 2001). An alternative method to characterize weathering is to estimate the total percentage of phenanthrenes (with respect to TPAH).

Results & Discussion

The PAH in ORC effluent are typically naphthalene dominated initially but as time increases the lighter PAH become relatively depleted and heavier PAH, particularly phenanthrenes, become dominant (e.g., Carls et al. 2005; Fig. 1). Industry researchers Brannon et al. (2006) report the same phenomenon.

Total PAH concentrations in ORC effluent are related to oil loading on rock and decline rapidly. Concentration declines can be described with a two-compartment exponential model (e.g., Carls et al. 2004; Fig. 2). Industry researchers Brannon et al. (2006) report similar concentration declines.

Visual evidence suggests that ORC effluent contains only or primarily dissolved oil constituents. Solutions are clear, not turbid (e.g., Carls et al. 2005). These observations are consistent with industry researcher Pearson (2002) who rarely detected oil droplets. Brannon et al. (2006) observed oil droplets in a similar experiment but did

not quantify the incidence of droplets or provide the chemical evidence necessary to quantify whole oil movement. Oil droplets are most likely to occur when columns are started, hence the protocol for 1 d or more flow before assays began and inclusion of a trap designed to exclude buoyant particles or slicks in the herring experiments.

Chemical evidence demonstrates that particulate oil was rare in oiled rock column effluent. The mean phytane concentration in oiled-rock column effluent was 0.04 ± 0.008 ug/L (n = 168) and 0.01 ± 0.005 µg/L in controls (n = 41; $P_{ANOVA} = 0.067$; Marty et al. 1997; Heintz et al. 1999, 2000; Carls et al. 1998, 1999, 2005). Correlation between dose (measured as TPAH) and phytane was poor ($r^2 = 0.111$, slope = 0.002), yet the regression was significant ($P < 0.001$) indicating the probability of encountering particulate oil increased slightly with dose. About half the phytane concentrations (90/168) in oiled treatments were within the 95% confidence range of controls. In contrast, phytane concentrations were dose dependent ($r^2 > 0.999$) in high-energy water accommodated assays where particulate oil was present (range 0.19 to 10.59, n = 8; Barron et al. 2003), proof of concept that phytane traces particulate oil.

Pacific herring and pink salmon eggs accumulated dissolved oil constituents with no evidence of particulate oil (Fig. 3). No phytane was present in herring eggs (n = 25) or pink salmon eggs (n = 34) exposed to oiled effluent (Marty et al. 1997; Heintz et al. 1999, 2000; Carls et al. 1999, 2005). This chemical evidence is consistent with the absence of visual evidence of egg coating by oil. Thus, particulate oil cannot explain the dose-dependent responses observed in these experiments.

The dominant or exclusive accumulation mechanism by embryos and larvae is uptake of dissolved oil constituents even when oil droplets are present in surrounding treatment water. This situation was tested by an alternative high-energy mixing method that produced a water-accommodated fraction of oil (WAF; Barron et al. 2003). Phytane was present in 1 of 10 WAF-exposed egg samples and possibly is an artifact due to contact with an oil slick during collection. No phytane was observed in herring larvae exposed to WAF (n = 2). Even when treated with chemical dispersant (Corexit 9527), which resulted in a turbid, brown, oil-in-water dispersion (Barron et al. 2003), particulate oil did not adhere to herring larvae (n = 2).

Particulate oil does not explain the dose-dependent responses of herring eggs and larvae assayed in water with containing particulate oil. This was clearly demonstrated by Barron et al. (2003), who compared ORC toxicity to WAF toxicity, using TPAH concentrations in tissue as the common measure (Fig. 3). Yolk-sac edema, one of the most obvious and sensitive embryo reactions to PAH increased in a dose-dependent manner, regardless of the physical nature of the assay (particulate or dissolved) and at about the same TPAH concentration in tissue. The common currency in these assays is the amount and composition of biologically available PAH. Droplets do not affect embryos and larvae directly and as previously indicated, droplets were rarely associated with eggs in any experiment.

Other alternatives put forward by industry researchers to explain the toxicity of ORC effluent fail. These include possible ammonia, sulphides, and low oxygen (Pearson 2002) and microbial metabolites (Neff et al. 2000; Page et al. 2002a; Brannon et al. 2006). Pearson (2002) clearly demonstrated that none of the potential abiotic factors are meaningful under flow-through assay conditions. Any metabolite contribution to toxicity is at most very small (<3%; see Appendix 3). Furthermore, there was no chemical

evidence of microbial activity in the oiled substrate (Appendix 3). This leaves PAH toxicity as the most parsimonious explanation for ORC effluent toxicity.

Biochemical evidence (cytochrome P4501A (CYP1A) induction) demonstrates that PAH enter embryonic tissues. This was unambiguous with both pure PAH tests and with crude oil tests where zebrafish embryos were exposed either to WAF or ORC effluent (Incardona et al. in 2005). Induction varied among PAH; chrysene was the most potent inducer tested, yet was less toxic than dibenzothiophene and phenanthrene. Although Incardona et al. (2005) demonstrate that CYP1A has generally has a protective, not causal roll in PAH toxicity, compound-specific induction patterns suggest that modes of toxic action may differ among PAH, thus allowing differences in toxicity. In particular, tricyclic PAH are highly toxic (Barron et al. 2004; Sundberg et al. 2005; Incardona et al. 2005) and have entered the environment through oil spills and as urban pollution for decades (Lima et al. 2003). Induction of CYP1A plays a protective role for tricyclic PAH (Incardona et al. 2005).

The oil film on rock or natural sediment serves as a reservoir for toxins (PAH). The oil film is relatively stationary and continues to provide toxins until depleted. Intertidal rock coated with oil can continue to serve as a PAH reservoir for long periods of time (e.g., Carls et al. 2001; Carls et al. 2004a; Short et al. 2004). Intertidal substrate is reworked by wave energy, thus oil-coated rock can be buried or re-exposed, potentially affecting the rate of PAH release. Tidal action ensures that oiled substrate in intertidal areas will be periodically flushed with water; hydraulic groundwater drainage can deliver PAH-laden water into stream channels thus potentially exposing developing fish embryos (e.g., Carls et al. 2003, Carls et al. 2004b).

Oil droplets can serve as a toxic reservoir in open water. Obviously the potential for dilution and dispersal of oil droplets is much greater than for oil-coated intertidal gravel, thus the time and affected body of water remains toxic is more limited than possible along shorelines. However, a body of water containing oil droplets is likely to remain toxic longer because of the reservoir effect than if the same body of water had originally contained only dissolved oil constituents. This important distinction may lead to differences in laboratory-measured toxicity between WAF and ORC effluent, particularly under static conditions. The distinction may also have implications for application of chemical dispersants designed to mix oil with water in an effort to limit spill impacts.

In summary, transfer of toxic PAH from oil, whether a film on water-washed rock or suspended particulates, is mediated by dissolution in water. The quantity and composition of dissolved oil constituents that are bioaccumulated are what negatively affect developing fish embryos. Oiled rock column assays closely emulate transfer of PAH from oiled intertidal substrate to aquatic organisms; the vast majority of PAH are transferred as dissolved substances. Developing fish eggs bioaccumulated almost entirely dissolved PAH. Oil droplets were rarely associated with eggs even from water containing a high abundance of oil particles. The incidence of droplets associated with developing embryos was too infrequent to explain observed embryonic responses and is consistent with lack of visible evidence of oil coating. In contrast, bioaccumulation of dissolved PAH explains embryonic response very well.

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Fig. 1. Typical shift in PAH composition in ORC effluent. Data from Carls et al. (2005).

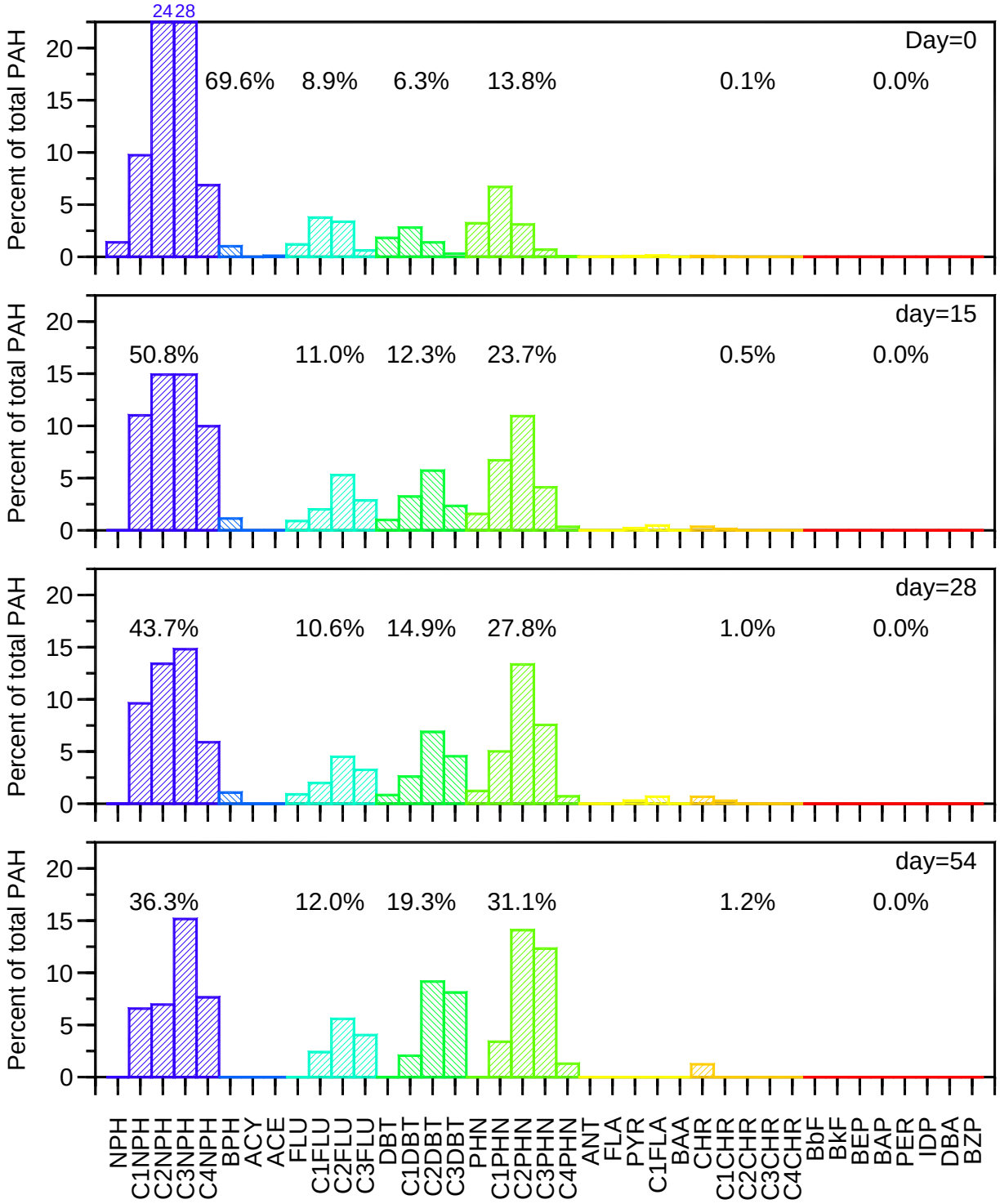


Fig. 2. Typical decline in TPAH concentration in ORC effluent. Concentrations are expressed as relative concentration (%) in this example from Carls et al. (2004).

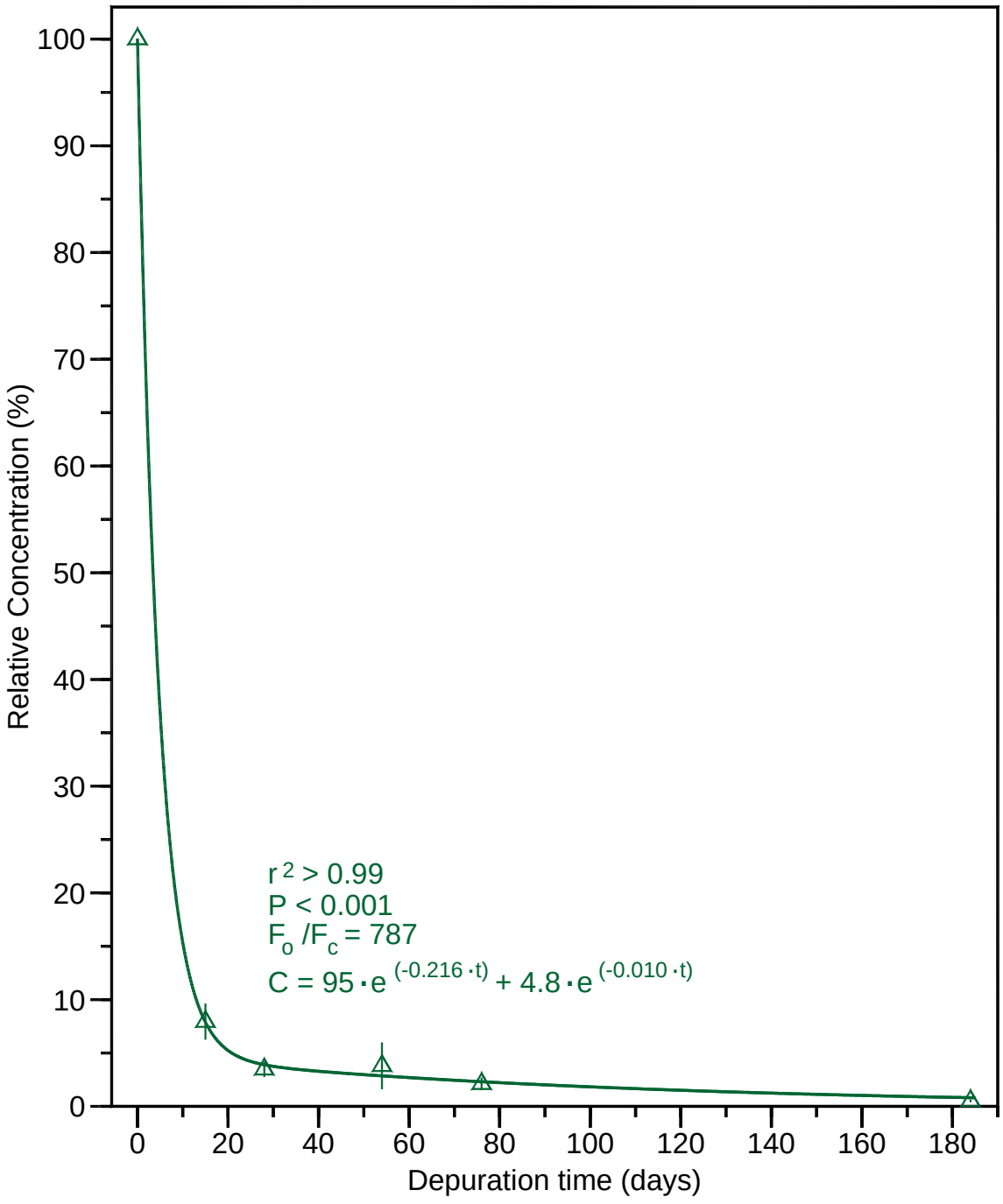


Fig. 3. Example PAH uptake and depuration by fish embryos. Herring data are from Carls et al. (1999); embryos in this test remained in contaminated water 16 d before transfer to clean water. Pink salmon data are from Carls et al. (2005); embryos remained in oil-contaminated water for the duration of the time illustrated. Blue lines represent controls; all other curves represent oiled treatments.

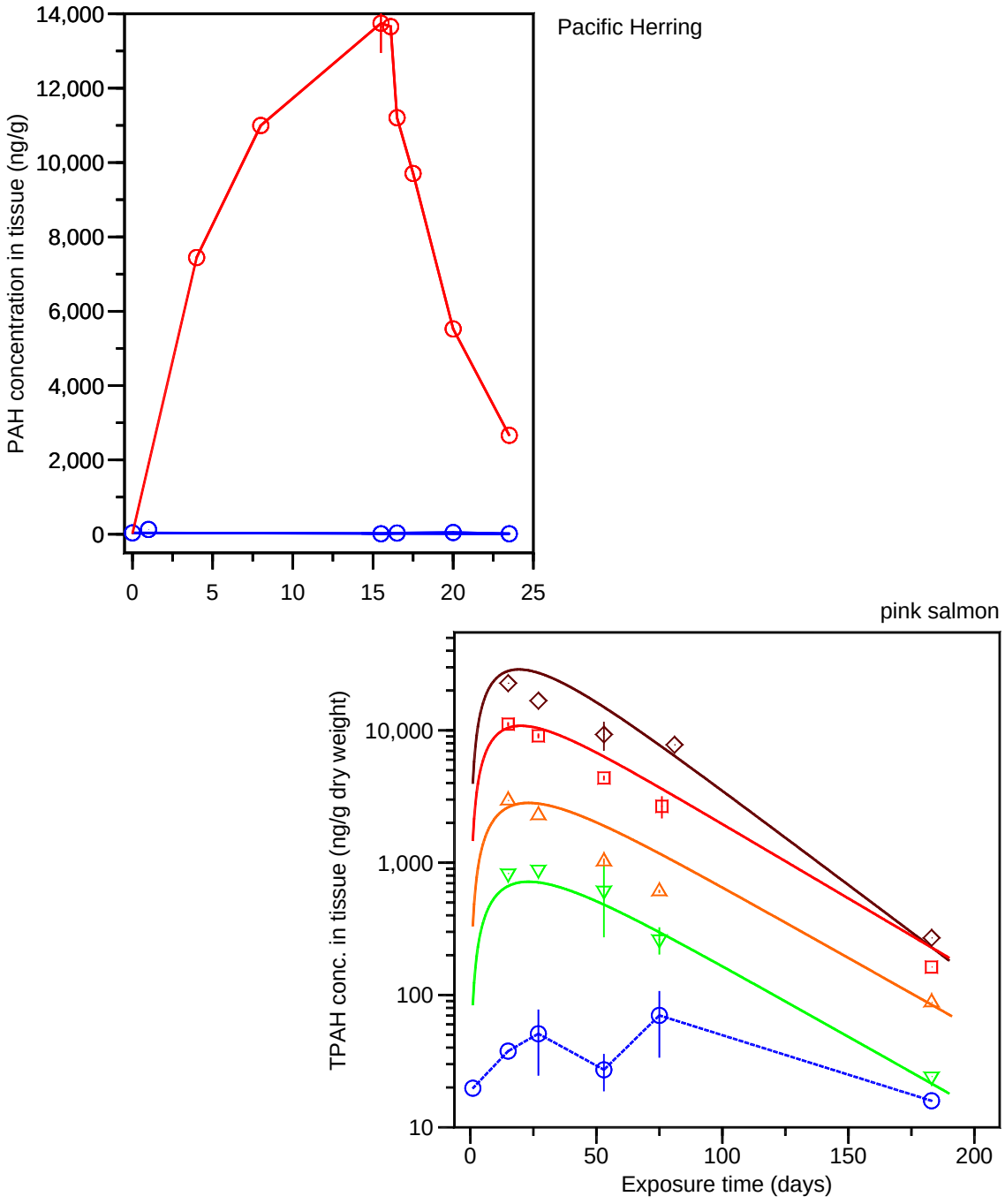
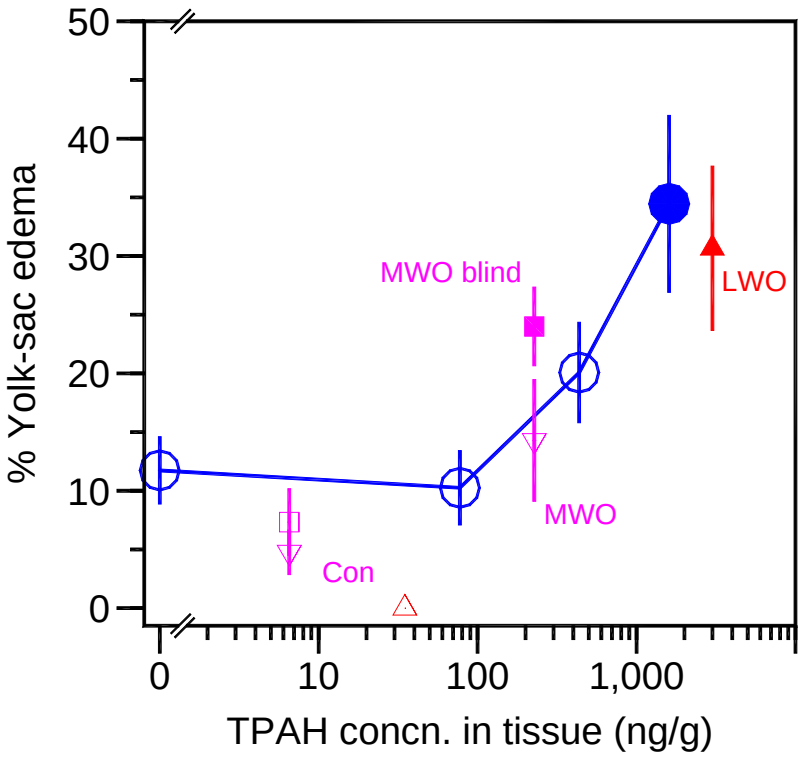


Fig. 4. Comparison of embryo response in the presence of particulate oil and in oiled rock column (ORC) effluent (redrawn from Barron et al. 2003). Circles represent mean percentage (± standard error) of yolk sac edema in herring embryos exposed for 4 d to water-accommodated fractions of oil (particulate rich) versus TPAH concentration in eggs (ng/g wet weight). Triangles represent 4 d exposures to less weathered oil (LWO) in ORC effluent and squares are 4 d exposures to more weathered oil (MWO), also ORC effluent. ORC effluent has little phytane and phytane, hence particulate oil, was never observed associated with embryos in ORC assays. Solid symbols indicate significant differences from corresponding controls.



Appendix 3

Microbial contribution to toxicity in oiled rock column (ORC) assays

Metabolites resultant from microbial degradation of oil in ORC assays have been suggested by some as an explanation for the high toxicity of effluent water (Neff et al. 2000; Page et al. 2002a). The plausibility of this assertion is examined and rejected in this appendix.

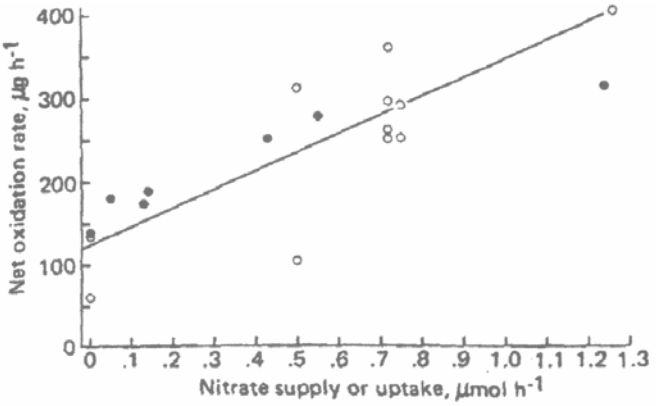
Methods

In ORC experiments, water passed through oiled gravel accumulates oil constituents, notably polynuclear aromatic hydrocarbons (PAH; Marty et al. 1997). This effluent is used for bioassays. Effluent concentrations are controlled by the quantity of oil applied to the rock, generally measured in g oil per kg rock. Concentrations of polynuclear aromatic hydrocarbons (PAH) and alkanes were determined by GC/MS; concentrations below method detection limits (MDL) were treated as zero (Short et al. 1996). There were no systematic attempts to quantify the presence (or absence) of metabolites.

Results & Discussion

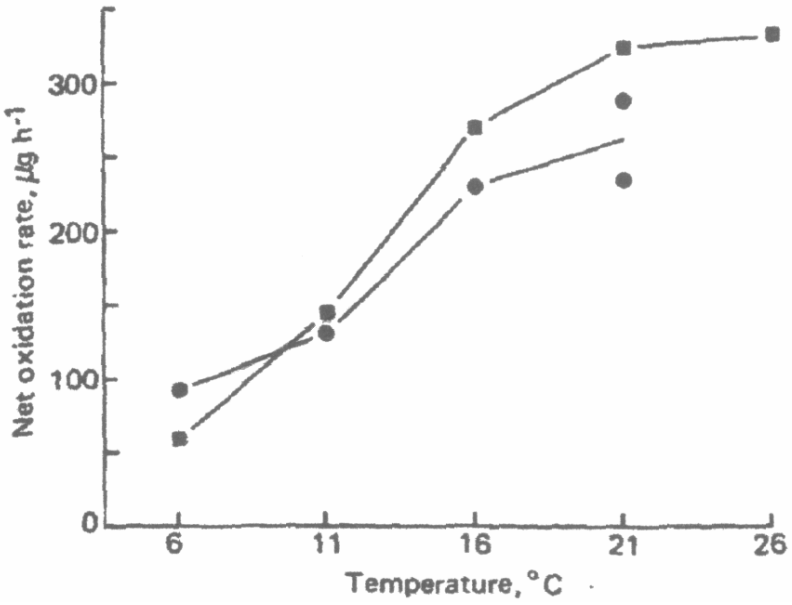
Microorganisms in the marine environment have evolved the ability to metabolize hydrocarbons (Atlas 1981; Floodgate 1984) and the resultant metabolic byproducts can be toxic (Middaugh et al. 1998; Shelton et al. 1999). Certain conditions must be met in order for microbes to utilize petroleum, including additional nutrients and sufficiently warm temperatures.

Microbial metabolism of petroleum hydrocarbons is limited by sources of nitrogen and phosphate. In studies designed to observe microbial metabolism and in industrial-scale utilization of microbial degradation of oil, the addition of fertilizer is required to increase natural degradation rates (Gibbs and Davis 1976; Oudot and Dutrieux 1989; Bragg et al. 1994; Atlas 1981; Atlas 1995; Middaugh et al. 1996, 1998; Shelton et al. 1999; Payne et al. 2005). In laboratory tests, microbial growth without the addition of fertilizer is typically negligible (Gibbs and Davis 1976; Shelton et al. 1999; Venosa, personal communication).



Example relationship between microbial growth and nutrients (Gibbs & Davis 1976).

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 25°C than at 5°C. In experimentally oiled sand-gravel columns, net oxidation rates were 3.7 times faster at 21°C than at 6°C (estimated from Gibbs and Davis 1976).



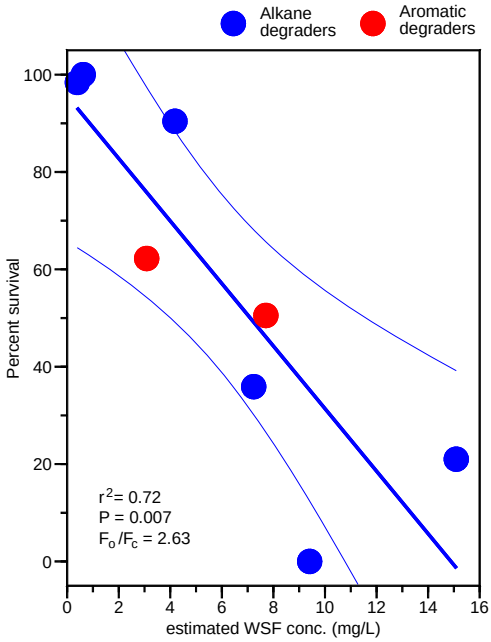
Example relationship between microbial growth and temperature (Gibbs & Davis 1976)

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). Shelton et al. (1999) reported that metabolites accumulated faster in the second week of microbial incubation at 20°C in water under static conditions, suggesting that changes in bacterial abundance occurred within this time scale.

Metabolite assays.

Several papers by one group suggest that microbial degradation of crude oil contributes to toxicity (Middaugh et al. 1996; 1998; Shelton et al. 1999). As a result of microbial activity, concentration of the water-soluble fraction (WSF) of oil increased, probably as a result of surfactant secretion, thus increasing the surface area of the weathered oil and allowing more compounds to dissolve. Aqueous hydrocarbon concentration explains the majority of the toxicity reported by Shelton et al. (1999) (our analysis): survival of grass shrimp was correlated with WSF concentration ($r^2 = 0.72$, $P = 0.007$). The type of hydrocarbons degraded, alkane or

aromatic, did not appear to influence the resultant toxicity. The response of inland silversides (*Menidia beryllina*; Middaugh et al. 2002) was almost entirely explained by changes in WSF concentration ($0.96 \leq r^2 \leq 0.98$, our analysis).



Relationship between the water-soluble fraction (WSF) concentration of oil in water and shrimp survival in microbial assays (our analysis of Shelton et al. 1999).

Oiled rock column assays.

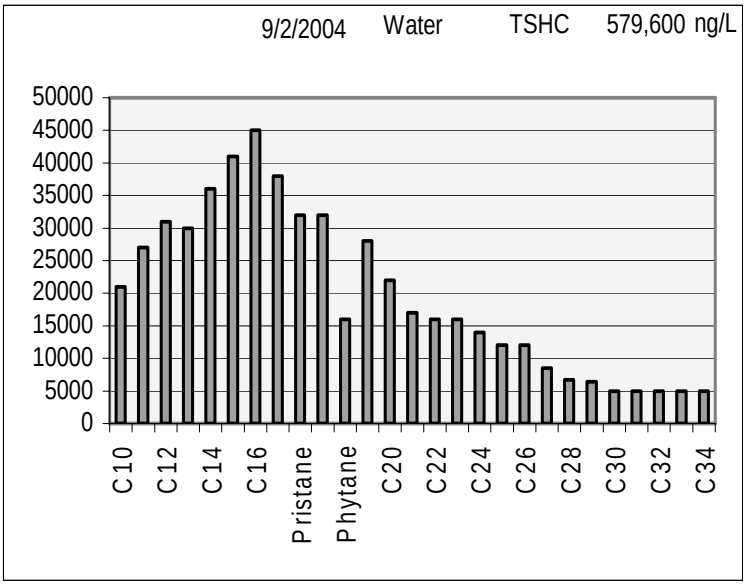
Conditions in oiled rock column assays are not optimal for microbial degradation of oil (Marty et al. 1997; Carls et al. 1999, 2005; Heintz et al. 1999, 2000). The rock was terrestrial, bare, washed, and dried before use, thus the substrate was of little or no nutritional value. No nutrients were added to the columns. The seawater passing through columns was cold (4 to 7 °C; Carls et al. 1999) and flow rates were rapid (5 to 6 L/min; Carls et al. 1999). These factors limit the potential for microbial growth.

The lack of nutrient supplements, colder temperatures (4-7°C), rapid flow-through conditions (5-6 L/minute) in ORC assays predicts slower microbial growth than the 20 °C static tests of Middaugh et al. (1996; 1998) and Shelton et al. (1999). Dividing the potential contribution of metabolic byproducts (4 to 28%; estimated from Shelton et al. 1999 and Middaugh et al. 2002) by 3–5 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), metabolites account for <2–3% of the toxicity in ORC.

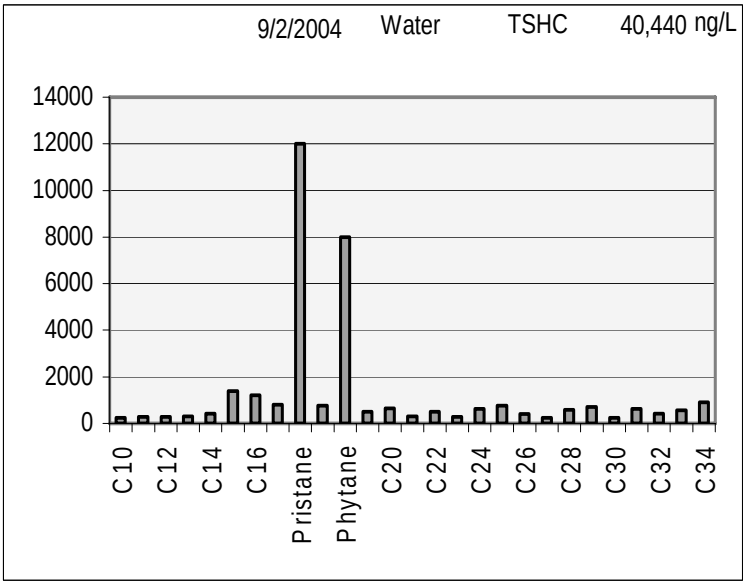
This literature-based estimate is confirmed by direct chemical evidence. Alkanes are more easily degraded than PAH, and straight chain alkanes are more easily degraded than

branched alkanes such as pristane and phytane (Alimi et al 2003). Increases in the concentrations of pristane and phytane relative to similar-sized straight-chain alkanes is evidence of microbial degradation. Such conditions occur in the biological treatment tanks at the ballast-water treatment facility in Port Valdez, Alaska (Payne et al. 2005; see figure).

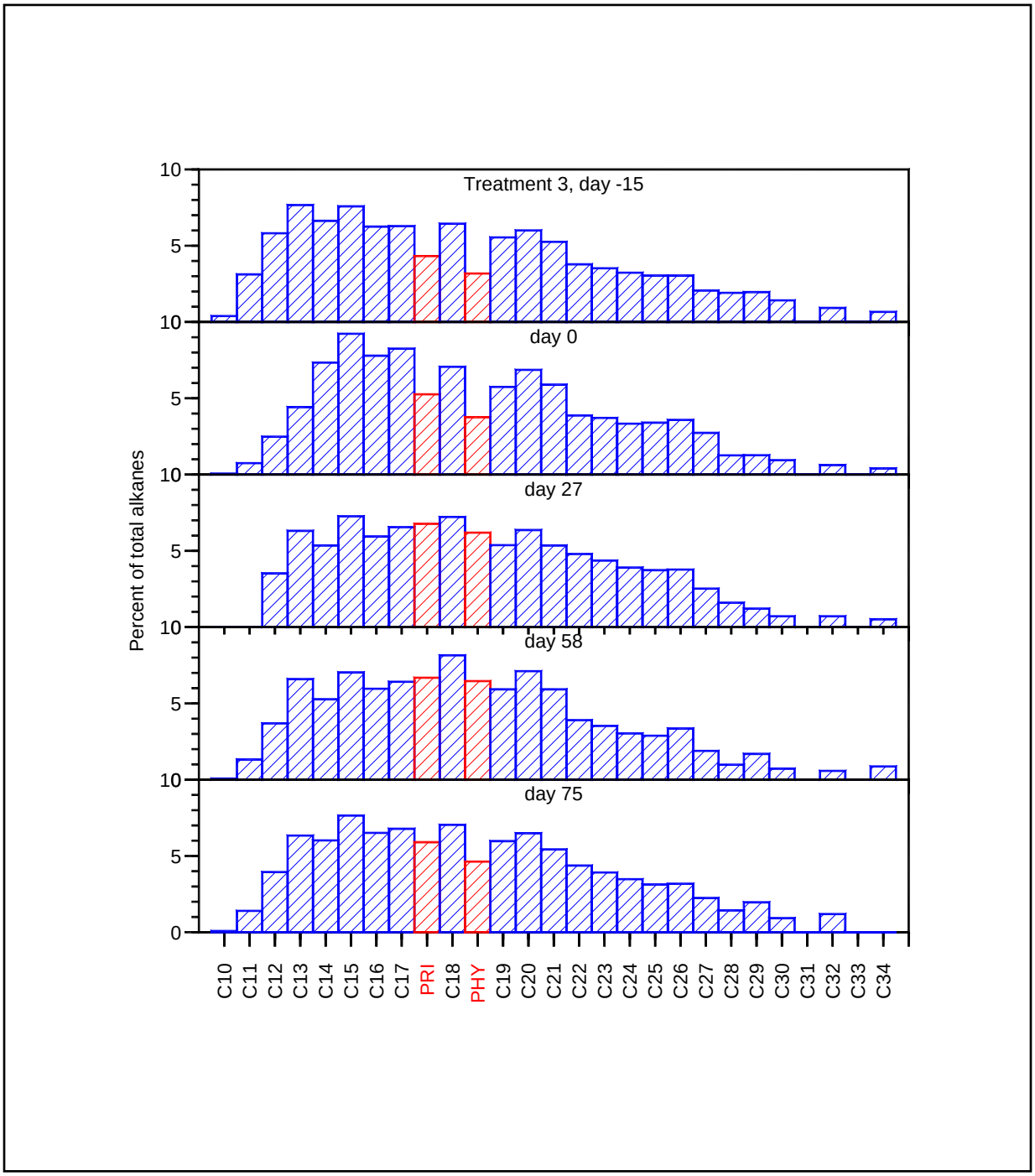
Before metabolism.



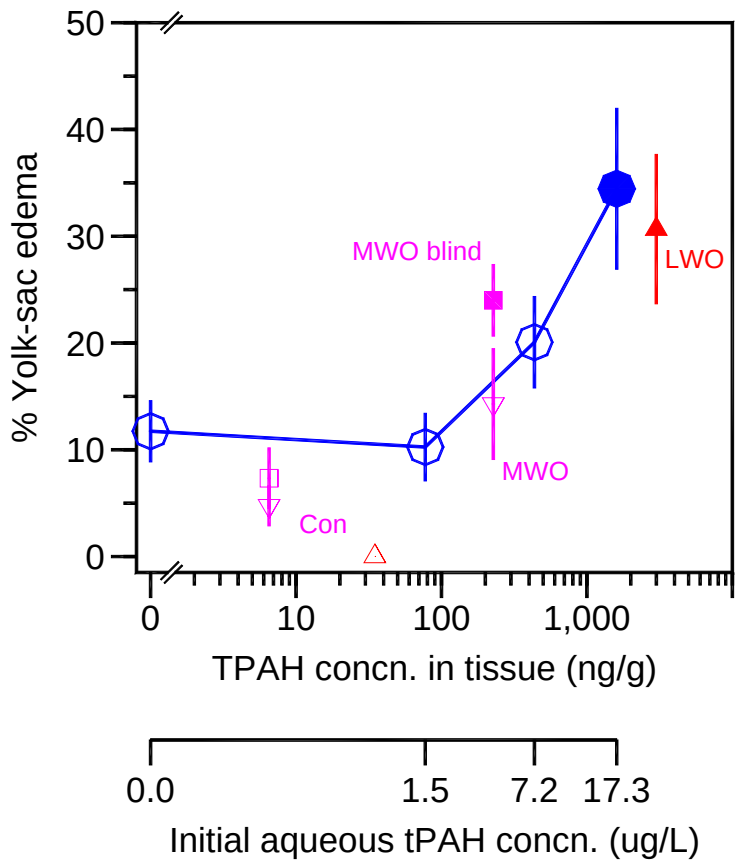
After metabolism.



In contrast to the microbial attack illustrated above, microbial growth in ORC assays was slight or negligible, as illustrated in the example below.



Biological evidence also suggests little or no microbial toxicity in ORC assays. The response of Pacific herring embryos to water-accommodated fractions (WAF) of oil were highly similar to those exposed to ORC effluent. There was very little opportunity for microbial growth in the WAF assays; test solutions were prepared and renewed daily. At low temperatures (as in ORC assays), without nutrient amendment, and knowing that significant colonization may require days, the chance of microbial metabolite toxicity in the WAF assay is very low. That toxicity in corresponding ORC assays was about the same is biological evidence that metabolite toxicity was not significant (see figure).



Comparison of embryo response in the presence of particulate oil and in oiled rock column (ORC) effluent (redrawn from Barron et al. 2003). Circles represent mean percentage (± standard error) of yolk sac edema in herring embryos exposed for 4 d to water-accommodated fractions of oil (particulate rich) versus TPAH concentration in eggs (ng/g wet weight). Triangles represent 4 d exposures to less weathered oil (LWO) in ORC effluent and squares are 4 d exposures to more weathered oil (MWO), also ORC effluent. ORC effluent has little phytane and phytane, hence particulate oil, was never observed associated with embryos in ORC assays. Solid symbols indicate significant differences from corresponding controls.

There was no evidence of time-dependent toxicity increases due to metabolic byproducts from a hydrocarbon-degrading bacterial population on week-length time scales in our apparatus. 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 laboratory 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).

Conclusion

Microbial metabolites do not contribute significantly to effluent toxicity in ORC assays. This conclusion is supported by microbial nutrient requirements, growth rates, and assay temperatures, lack of chemical evidence of microbial degradation in ORC, and consistency of embryonic response in ORC assays to those in entirely different assay conditions (WAF).

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Appendix 4

Toxicity testing

To adequately assess risk, set discharge limits, and devise environmental regulations, substances must be appropriately tested with realistic conditions and appropriate test subjects. The purpose of this appendix is to highlight important assay factors, including exposure and observation time, types of organism responses (lethal and sublethal), assay mechanics (static, static renewal, and flowthrough), source of toxin(s), life stage, and species. This discussion focuses on aromatic hydrocarbons, the principal source of petroleum toxicity.

Methods of study and exposure can critically influence assessment of PAH toxicity. For example, methods that focus on short-term acute narcotic response miss the importance of chronic exposures and long-term implications. The toxicity mechanisms are very different: narcotic effects are rapid, affecting neural function, while chronic toxicity mechanisms can affect a variety of processes in many different tissues, resulting in effects that take time to manifest and be visible. In this appendix we discuss how oil-water preparation methods, assay procedures, choice of test species, life stage, and source oil can influence conclusions regarding toxicity.

Oil preparation methods

Methods used to mix oil and water strongly influence hydrocarbon composition in water. Standard procedures designed to produce water-soluble fractions of oil, for example typically yield water contaminated almost entirely with the lightest aromatics present, i.e., monoaromatic hydrocarbons if the oil has not been weathered and the smallest PAHs. For example, when water-soluble fractions were generated by actively pumping oil onto a column of water and dripping water through the oil layer, 95% of the hydrocarbons detected in effluent water were monoaromatic, the remaining 5% were naphthalenes (N0 to N2) (Moles et al. 1985, Carls 1987). Consumption of oil in these assays was measured by the barrel. Similarly, standard oil-water mixing procedures in sealed containers or at low energy yield effluent laden with primarily low molecular weight aromatics (e.g., Singer et al. 2000). Conversely, proportions of higher molecular weight PAHs increase in water-accommodated fractions of oil when the system is not sealed and mixing energies and times increase (e.g., Fig. A4.1). Consumption of oil in the latter assays was measured by μL.

Multiple exposure procedures complicate comparison of dose among experiments and investigators; improved reporting would improve understanding. At least three procedures are commonly used, aqueous exposure (as such as detailed in Appendix 2), dosed (or otherwise contaminated) sediment, and direct injection. Sediment-oriented studies typically report PAH concentrations in the sediment (e.g., Couillard 2002, Colavecchia et al. 2004) but without other measures, actual exposure and uptake remain unknown. Estimation of equilibrium concentrations might be used in lieu of measured aqueous concentrations under these circumstances but unverified estimates should be treated cautiously. The direct injection method allows accumulation of PAHs in passive samplers to later be tested for biological activity (Springman et al. 2007) or extraction of PAHs from sediment so that testing can be independent of sediment. In an elegant experiment, Sundberg et al. (2005) extracted PAHs and other compounds (such as polychlorinated biphenyls, PCBs) from polluted sediment and fractionated the extract into multiple subfractions; the fraction mainly composed of PAHs was more teratogenic than the fraction containing dicyclic aromatics and PCBs when injected into trout

embryos. Explicit inclusion of whole body total PAH estimates in injection studies would improve comparison with other dosing methods.

Assay procedures

Also critical to understanding toxicity is how assays are performed. Assays are typically divided into two categories, acute and chronic. Acute assays focus on rapidly acting toxins, such as the narcotic monoaromatic hydrocarbons, and mortality as the endpoint (e.g., Di Toro et al. 2000). Such tests are typically 4 d or less, a convention that may have more to do with a 5 d work week than subject response. Mononuclear aromatic hydrocarbons act as narcotics in aquatic organisms, a reversible effect caused by the partitioning of hydrophobic chemicals into cell membranes and nervous tissue, disrupting the central nervous system (Barron et al. 2004). Relatively high aqueous concentrations (about 1 mg/L or more) are required to elicit these effects. Because acute toxicity tests are standardized and yield data quickly, predictive models based on narcosis are available and remain in use by regulatory agencies (e.g., (Di Toro et al. 2000, Long et al. 2000, Fairey et al. 2001, Hansen et al. 2003). Although the narcosis "toxic units" approach has validity in some instances (Di Toro et al. 2000), non-narcotic toxins with specific modes of action may be present, playing important or even dominant roles in organism responses in natural ecosystems. The importance of slower-acting toxins can be overlooked by traditional acute toxicity test. Acute assays are best suited to test rapidly acting toxins that cause mortality.

Acute assays may completely miss slower, sublethal responses that ultimately have equally devastating consequences. For example, <10% of Pacific herring embryos died when exposed to 7.6 µg/L total weathered PAH, yet >90% developed yolk sac edema (Carls et al. 1999). None of the embryos with edema were expected to survive. After 2 mo exposure, oiled pink salmon embryo survival was not statistically different than controls, yet the growth these same pink salmon fell progressively behind controls in the 6 mo interval after removal from dosing (Carls et al. 2005) and significantly fewer similarly exposed fish released into the wild returned to spawn (Heintz et al. 2000) (Fig. A4.2). Thus, acute bioassays are not accurate predictors of long term toxicity.

Chronic assays typically measure a broader range of organism responses over longer time periods than do acute assays and estimated toxicity is greater in longer assays. Comparing 10 marine species [pink salmon (*Oncorhynchus gorbuscha),* starry flounder *(Platichthys stellatus),* amphipod *(Boeckosimus nanseni),* coonstripe shrimp *(Pandalus hypsinotus),* king crab *(Paralithodes camtschaticus),* shore crab *(Hemigrapsus nudus),* ocher starfish *(Evasterias troschelii),* pink scallop *(Chlamys hericus),* file periwinkle *(Nucell lima),* and blue mussel *(Mytilus trossulus*)], Moles (Moles 1998) found that 4 to 28 day flow-through LC50 ratios averaged 2.0 (range 1.0 to 2.5). The 4 d to 16 d LC50 and sublethal response ratios in Pacific herring averaged 2.5 (range 1.1 to 8.7, $n = 18$; derived from Carls et al. 1999). Long-term observation of animal response well after exposure may provide different estimates of toxicity than at the end of dosing. For example, Heintz et al. (2000) observed pink salmon exposed to oil as embryos were apparently healthy at release but had significantly poorer ocean survival than controls. Acute to chronic toxicity ratios average about 5 (range 1.2 to 96; Di Toro et al. 2000), a relationship that must be considered when comparing various assay results. Mean acute to chronic ratios ranged from 1.5 to 2.3 in Pacific herring (derived from Carls 1987; Carls et al. 1999). However, we have observed that the toxic units model (Di Toro et al. 2000) does not adequately describe chronic response of Pacific herring and pink salmon embryos (Barron et al.

2004) and that the apparent relationship between response and molecular size (or octanol-water partition coefficients, Kow) is steeper than described by this model. Thus, acute to chronic ratios (which are constants) may not adequately adjust for the difference between acute and chronic responses. This leads to the conclusion that chronic toxicity cannot simply be predicted from short-term assays corrected by some theoretical acute to chronic ratio.

Dosing technique strongly influences assay results; estimated toxicity is greater in flow-through test than in static tests. Using the water-soluble fraction of Cook Inlet Crude oil, Moles (1998) compared static (4 d) and flow-through (4 d) assays of the same 10 marine species discussed previously. Static to flow-through median lethal toxicity (LC50) estimates averaged 2.0 (range 1.4 to 2.8). A novel partition-controlled delivery method may provide a more sensitive and realistic assessment of the chronic toxicity of nonpolar compounds than static or semi-static assays (Kiparissis et al. 2003) and should produce results similar to flow-through assays. Polydimethylsiloxane (silicon rubber) and PAHs were dissolved in hexane and dried as films in test chambers (Brown et al. 2001; Kiparissis et al. 2003). The PAHs (log K_{ow} range 2.8 to 6.1) rapidly equilibrated between the film and the water, establishing a constant dose, unlike static assays where concentration declines exponentially or semi-static assays with repeating concentration spikes and declines.

Measuring the dose

What was the exposure dose? This is a critical question in any assay, be it acute or chronic, embryo or adult. With embryos, exposure dose is sometimes difficult to determine and many studies rely on "dose added" rather than measured dose. An extreme example would be an embryo carried internally by a female, where the female is exposed to a given concentration, but quantifying embryo exposure is complicated. Measurement of PAHs in tissue may provide clues, but embryos (and certainly adult fish) have varying capacities to transform PAHs and rid their tissues of metabolites. Some metabolites are toxic, most are more easily excreted than parent PAHs, and all are more difficult to measure and quantify. Doses in many assays decline with time; static tests typically change the most, and exposure is complicated by factors such as biodegradation volatility, and uptake rate. Flow-through exposures minimize the variance of some of these factors but achieving a stable constant dose can be difficult. Quantitatively relating sediment loads to exposure dose is difficult; PAHs must dissolve in water to cross the chorion, relegating PAH concentrations in sediment to a measure of potential, rather than actual exposure. Measurements within a study certainly provide relative exposure doses but measurements between studies should be viewed with caution. Measurements of PAHs in sediment, water, and tissue are not equal and can not be directly compared between matrices.

Species and life-stage sensitivity

Inter-species differences to toxicants are obvious and should be considered when using model organisms to predict ecosystem impacts. For example, trout were all considerably more sensitive (about 10 times) to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) than zebrafish, the most resistant of 10 freshwater fish species (lake trout (*Salvelinus namaycush*), brook trout (*Salvelinus fontinalis*), rainbow trout (*Oncorhyncus mykiss*), fathead minnow (*Pimephales promelas*), channel catfish (*Ictalurus punctatus*), lake herring (*Coregonus artedii*), Japanese medaka (*Oryzias latipes*), white sucker (*Catastomus commersoni*), northern pike (*Esox lucius*), and zebrafish; (Elonen et al. 1998); Fig. A4.3). Toxicity of PAH also varies among species (Rice et al. 1979) and presumably differences in species sensitivity extends to embryo exposures.

Early life stages of organisms are generally more sensitive to toxins than adult forms (Moore & Dwyer 1974; Weis & Weis 1989). Egg or larval to adult LC50s averaged 73% in water-soluble fraction assays (range 39 to 100%; Carls 1987). Greater sensitivity of early life stages to pollutants than adults may be due in part to smaller size, hence more rapid equilibration with surrounding media but the full reason likely involves disruption of developmental processes as described in Chapter 1.

Comparison among hydrocarbon sources and laboratories

Aromatic hydrocarbon composition varies among oils, refined products, other petrogenic sources, and pyrogenic sources (Neff 2002; Wang & Fingas 2003a), consequently, the potential for damage varies. For example, a crude oil (Mesa light) with twice the PAH content of another crude oil (Alaska North Slope) was more toxic (Couillard 2002), thus illustrating the clear need to quantify specific composition in assays, including that in tissue, to allow intra- and inter-experiment comparisons. Fortunately such measures are now frequent; the nominal measures of oil quantity in early research are of limited value. However, we urge researchers to go beyond the 16 Environmental Protection Agency 'priority pollutants' and quantify alkyl-substituted homologues as well (e.g., Table A4.1). The alkyl-substituted compounds are more persistent in the environment and more toxic than parent compounds, therefore they should not be overlooked. Obviously, how many PAHs are actually quantified influences inter-laboratory measures of total PAH, complicating comparisons.

Summary of methods issues

Ecologically relevant information is lost when exposure duration is fixed (e.g., 4 day assays) and only mortality is observed; survival models that include latent mortality produce more ecologically meaningful results than conventional acute assays (Zhao & Newman 2006). In other words, the design of traditional aquatic toxicity tests has hampered recognition of the true toxicity of the most toxic components known in oil, the PAHs and related heterocyclic compounds, such as dibenzothiophenes.

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Table A41. Polynuclear aromatic hydrocarbons (PAHs) routinely analyzed by our group. The EPA "priority pollutants" are marked with asterisks; environmentally persistent PAH are marked with "p."

	*	naphthalene
		C-1 naphthalenes
		C-2 naphthalenes
		C-3 naphthalenes
		C-4 naphthalenes
		biphenyl
	*	acenaphthylene
	*	acenaphthene
	*	fluorene
		C-1 fluorenes
		C-2 fluorenes
p		C-3 fluorenes
p		C4 fluorenes
		dibenzothiophene
		C-1 dibenzothiophenes
		C-2 dibenzothiophenes
p		C-3 dibenzothiophenes
p		C4 dibenzothiophenes
p	*	phenanthrene
		C-1 phenanthrenes/anthracenes
		C-2 phenanthrenes/anthracenes
p		C-3 phenanthrenes/anthracenes
p		C-4 phenanthrenes/anthracenes
	*	anthracene
	*	fluoranthene
	*	pyrene
p		C-1 fluoranthenes/pyrenes
p		C-2 fluoranthenes/pyrenes
p		C-3 fluoranthenes/pyrenes
p		C-4 fluoranthenes/pyrenes
p	*	benzo(a)anthracene
p	*	chrysene
p		C-1 chrysenes
p		C-2 chrysenes
p		C-3 chrysenes
p		C-4 chrysenes
p	*	benzo(b)fluoranthene
p	*	benzo(k)fluoranthene
p		Benzo(e)pyrene
p	*	Benzo(a)pyrene
p		Perylene
p	*	indeno(1,2,3-cd)pyrene
p	*	dibenzo(a,h)anthracene
p	*	benzo(ghi)perylene

Fig. A4.1. Aqueous PAH composition in water-accommodated fractions (WAF) of Alaska North Slope crude oil mechanically mixed with water where mixing energy (panels a-c) and mixing time (panels c-f) were varied. Printed inside each panel are experimental conditions, aqueous (aq.) yield of total polynuclear aromatic hydrocarbons (TPAH), and relative percentages of the following homologous groups: naphthalenes, fluorenes, dibenzothiophenes, phenanthrenes, and chrysenes.

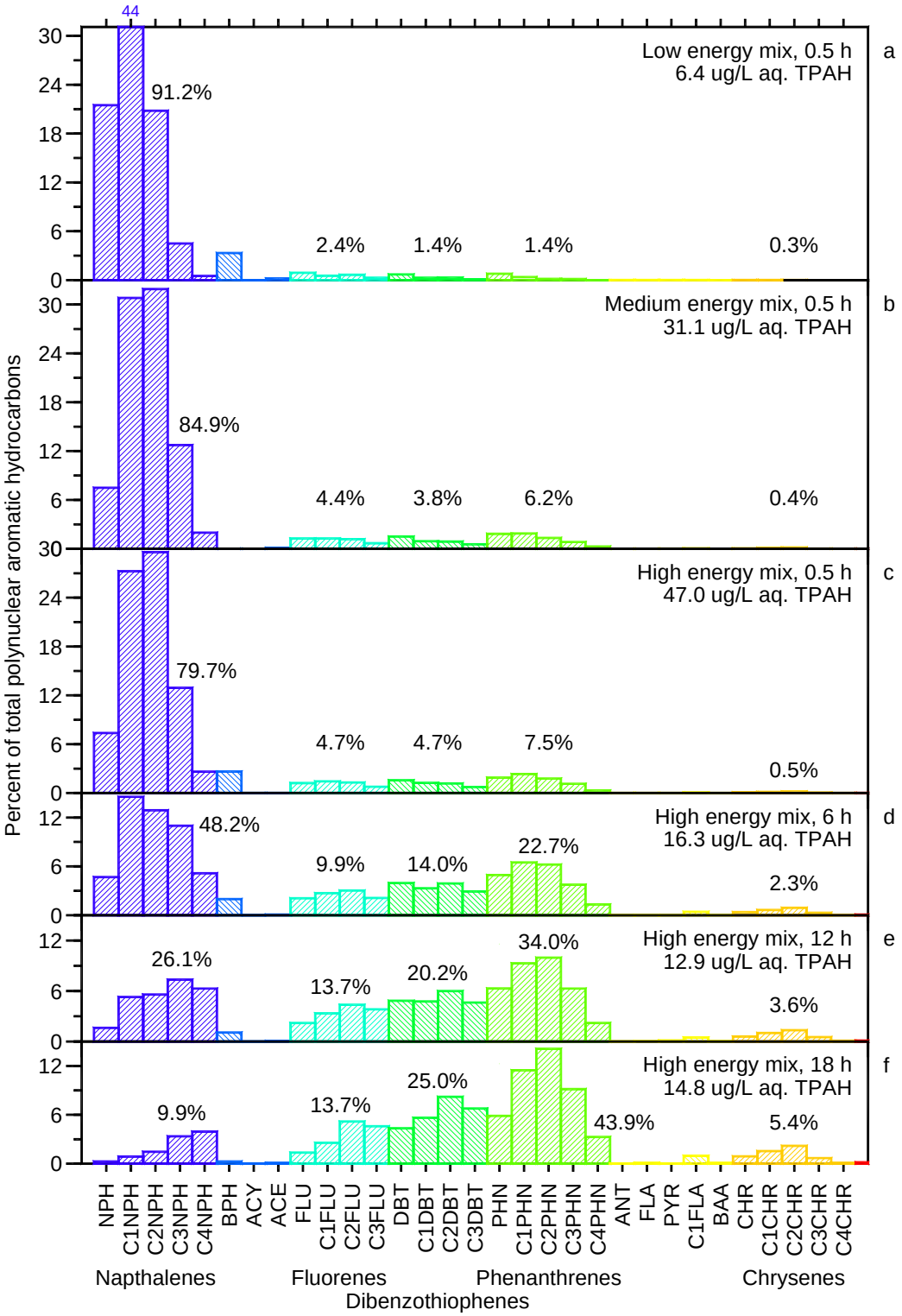


Fig. A4.2. Marine survival of adult pink salmon exposed to oiled rock column effluent as embryos (Heintz et al. 2000).

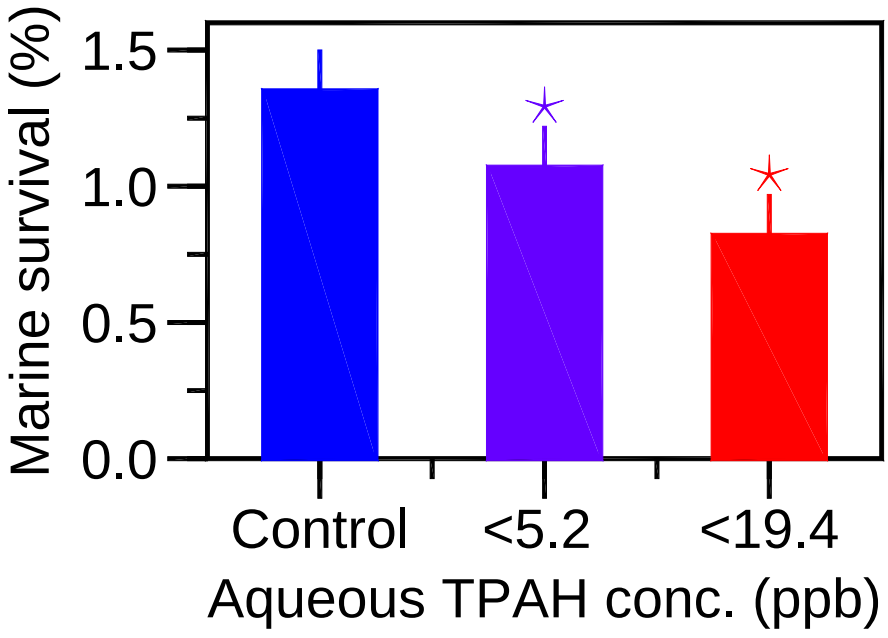
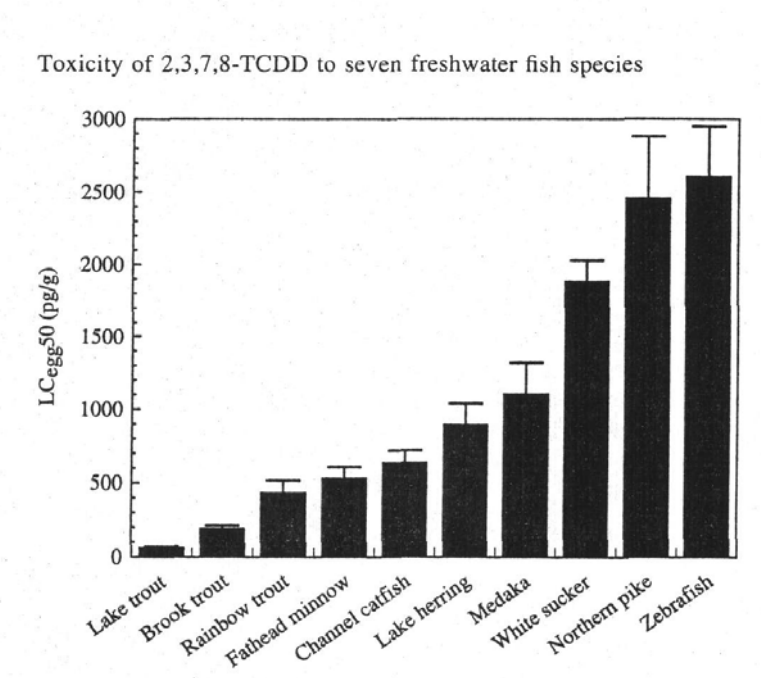


Fig. A4.3: Sensitivity of fish species to TCDD (Elonen et al. 1998).



Appendix 5

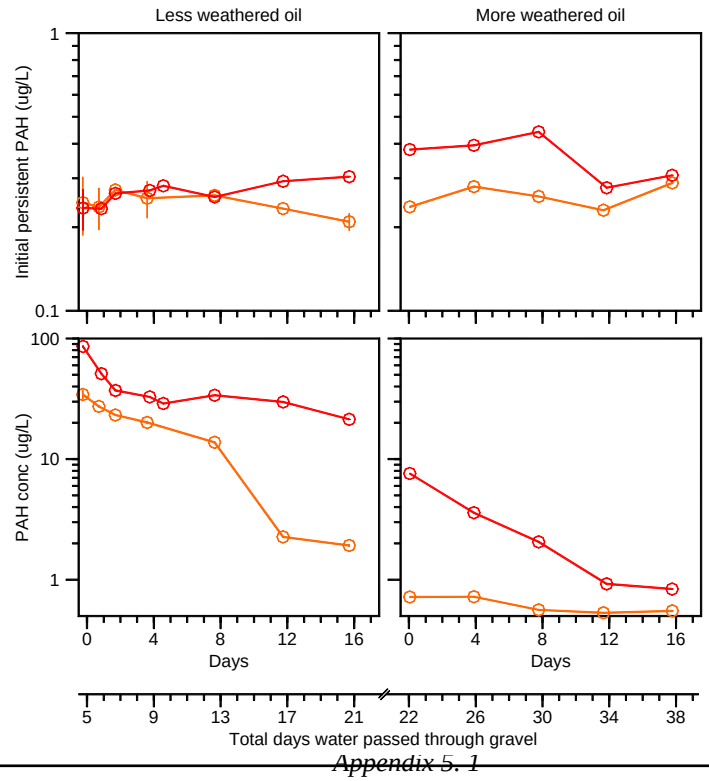
Does the Di Toro model accurately predict PAH toxicity?

In this appendix, we examine the ability of a narcotic model (based on short-term acute lethality assays; Di Toro et al. 2000) to predict responses of Pacific herring exposed to dissolved PAH (Carls et al. 1999). We agree with Di Toro et al. (2000, 2007) that toxicity varies among PAH; the argument is whether the additive model proposed by Di Toro et al. accurately predicts the toxicity of PAH mixtures or not. The Pacific herring data of Carls has become a focal point for argument; Di Toro argues that the experiment is flawed because the model does not accurately predict the outcome, Carls argues that the experiment is sound, hence the model needs revision or replacement. The following analysis is graphic-based; for a more rigorous analysis, see Barron et al. (2004).

Herring embryos were incubated in oiled rock column effluent. After 16 d exposure, embryos were moved to clean water to await hatch; this was the less-weathered oil (LWO) group. The oiled rock columns were drained; water flow was restarted 1 d before exposure of a second group of embryos, the more weathered oil (MWO) group. Because total PAH concentrations decayed exponentially, all treatments in the MWO group were exposed to lower concentrations than corresponding treatments in the LWO group. Nonetheless, adverse embryo responses were observed in both groups and MWO proved more toxic per unit mass when expressed as total polynuclear aromatic hydrocarbons (TPAH; Carls et al. 1999).

The most straight-forward explanation for this result is that the more persistent, higher molecular weight PAH are more toxic than the more volatile, lower molecular weight PAH. Consider, for example, aqueous PAH concentrations in the upper two treatments of the two experiments [high (H) and mid (M); Fig. 1]. Total PAH concentrations fell exponentially, thus concentrations in the MWO treatments were lower. However, persistent PAH concentrations were essentially constant across time.

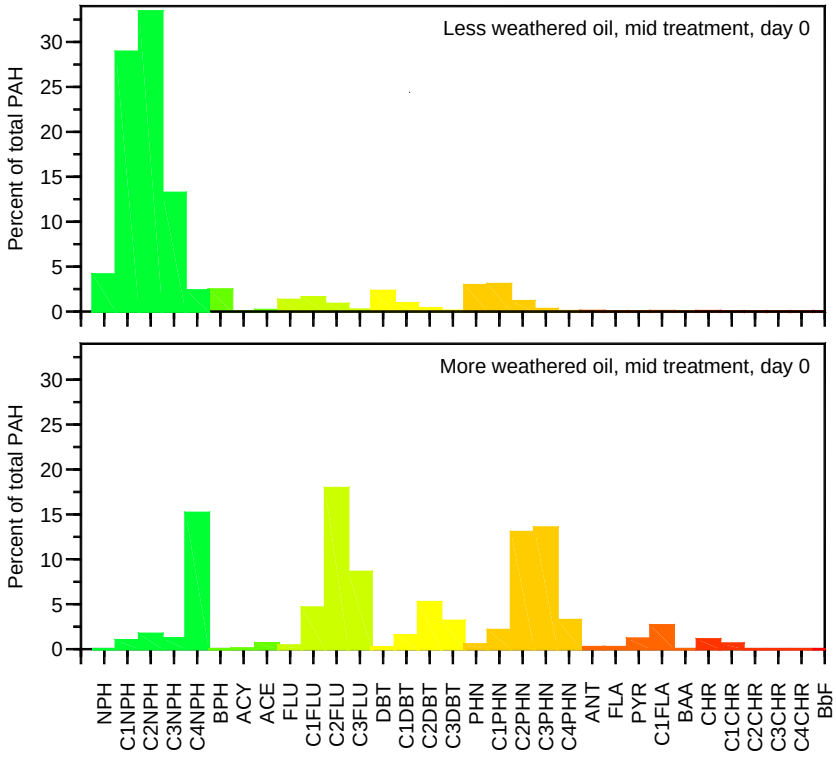
Fig. 1.



Persistent PAH were defined by Short and Heintz (1997) in conjunction with a first-order loss-rated PAH source identification model. They are simply the PAH which persisted longest in the environment (Prince William Sound) and in experimental assays. This group was used without modification in the assessment presented here: c3- and c4-naphthalenes, c2- and c3-fluorenes, c1- to c3-dibenzothiophenes, c1- to c4-phenanthrenes, and c0- to c2 chrysenes).

Comparison of PAH composition across time demonstrates which compounds were most volatile and were lost before MWO treatments. In particular, naphthalenes were lost from solution, leaving the higher molecular weight PAH (Fig. 2). Particularly abundant in the MWO treatment were the 3-ring compounds, fluorenes, dibenzothiophenes, and phenanthrenes.

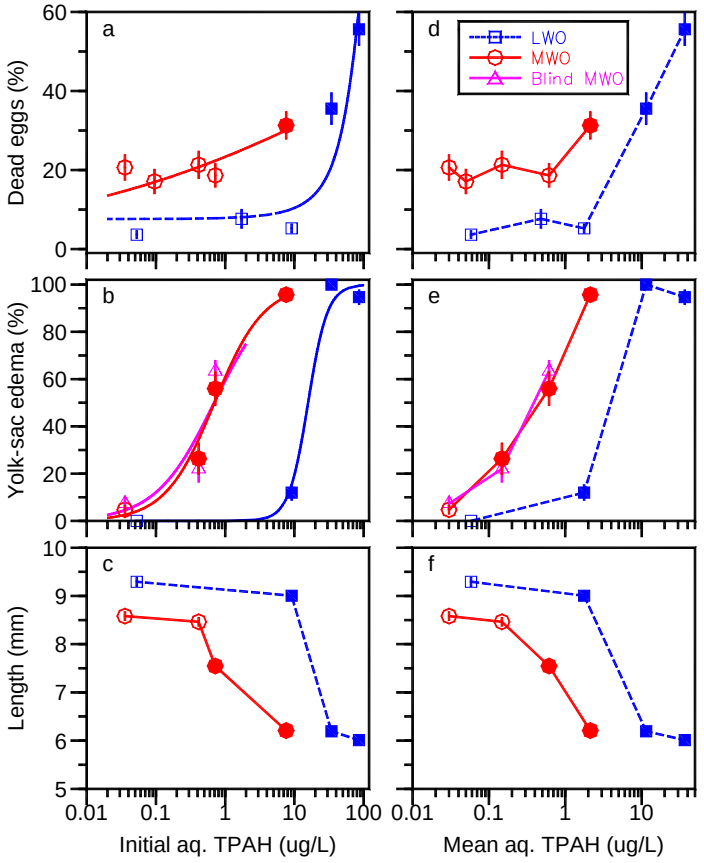
Fig. 2.



Embryos were adversely affected by both LWO and MWO. Plotted against initial TPAH concentration, embryos in the MWO group reacted to lower concentrations than those in the LWO group, hence the conclusion of Carls et al. (1999) that MWO is more toxic per unit mass (in effluent water; Fig 3a-c). Differences in response can be partially, but not entirely, explained by accounting for the exponential declines in TPAH concentration (Fig. 3d-f). The implication is that not all PAH are equally toxic. Because the 2-ring PAH account for most of the

concentration decline and were far less prevalent in the MWO group, toxicity must be caused principally by 3-ring or heavier PAH.

Fig. 3.



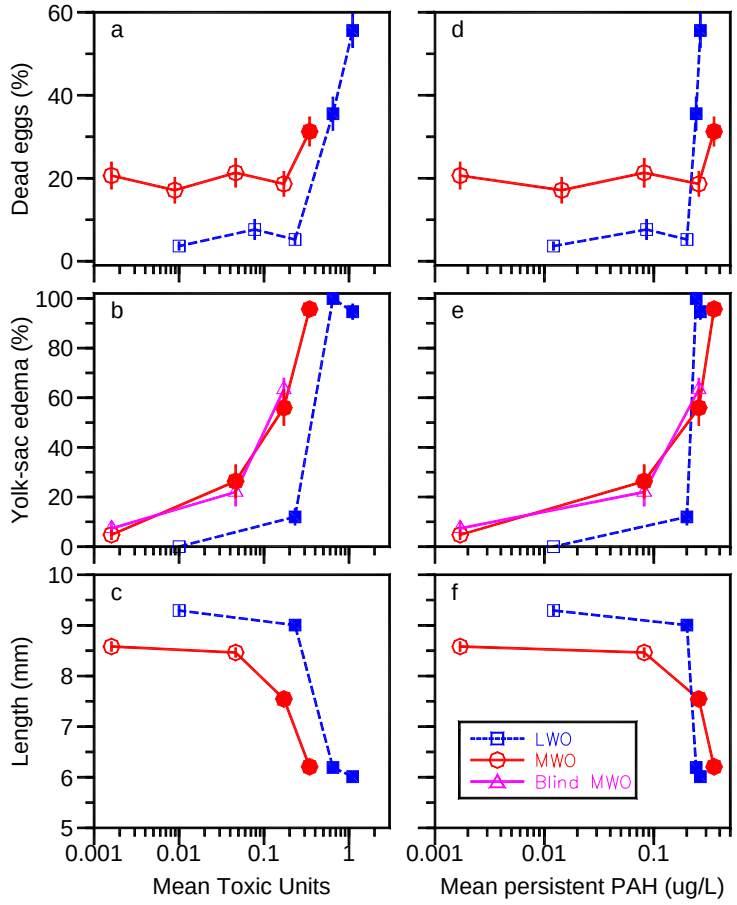
The Di Toro narcosis model (which is based on acute toxicity assays) drives responses between LWO and MWO experiments somewhat closer together than can be explained simply by exponential loss (Fig. 4a-c). The Di Toro model concept is quite simple; divide the observed aqueous concentration (µmol/L) of the i^{th} PAH by the estimated median lethal response (µmol/L) and sum all of these to get toxic units (TU). If TU = 1, then toxicity is expected (about half the animals will die). However, the narcosis model fails to adequately explain embryo responses because the 50% responses should occur at toxic unit 1. Rather, median responses occur well below this level.

The assumption that persistent PAH are the principal cause of toxicity, primarily the 3-ring compounds, provides a better explanation for embryo responses in both LWO and MWO

treatments. Responses to LWO and MWO more closely overlap under this assumption (Fig. 4d-f). Median responses are highly similar for both groups, but some overcorrection is apparent, suggesting not all contributing toxins were included in this group.

Demonstration that the 3-ring PAH were likely the most important contributors to embryo toxicity is consistent with Incardona et al. (2004) who found that toxicity varies among PAH, that 3-ring PAH are highly toxic, and that 3-ring PAH toxicity may be the principal reason for cardiac problems, which are coincident with edema and likely explain many secondary abnormalities.

Fig. 4.



Although accepted in the literature that PAH toxicity increases with molecular weight and alkyl substitution (e.g., Anderson et al. 1974; Moore and Dwyer 1974; Rice et al. 1977; Hutchinson et al. 1980; Black et al. 1983; Neff 1985; 2002), modelers Di Toro et al. (2000) dispute the conclusions of Carls et al. because they cannot explain the result with their model. The principal reason for this is likely that the Di Toro model is predicated on the lethal outcomes of rapid-acting toxins, while the chronic assays of Carls et al. (1999) measure response to slower-acting larger molecular weight PAH. Other modelers (e.g., Barata et al. 2005) continue to demonstrate that acute PAH toxicity is related to Kow (Fig. 4). However, this experiment also

focused on acute toxicity and TUs based on these data also fail to resolve the difference between the less weathered and more weathered assays of Carls et al. (Fig. 5).

Another alternative, a steeper relationship between response and molecular size (expressed as log Kow) may even better explain the seemingly disparate toxicity curves. To review, toxic units (TU) estimated with the Di Toro et al. (2000) model (Fig 5a-c) do not cause curve convergence and underpredict toxicity in both experiments. Similarly, TU estimated with data from Barrata et al. (2005) underpredict toxicity and fail to merge the curves (Fig. 5d-f). However, if we assume the relationship between chronic toxicity and Kow is twice that of acute toxicity (as estimated by Barrata et al 2005; Fig. 6), then the two toxicity curves merge (Fig. 5g-i) and the hypothetical TU correctly predicts response (response is centered about a TU of 1). We recommend this hypothesis be tested but are pessimistic that it will work in all cases because mechanism-specific toxicity is not considered additive.

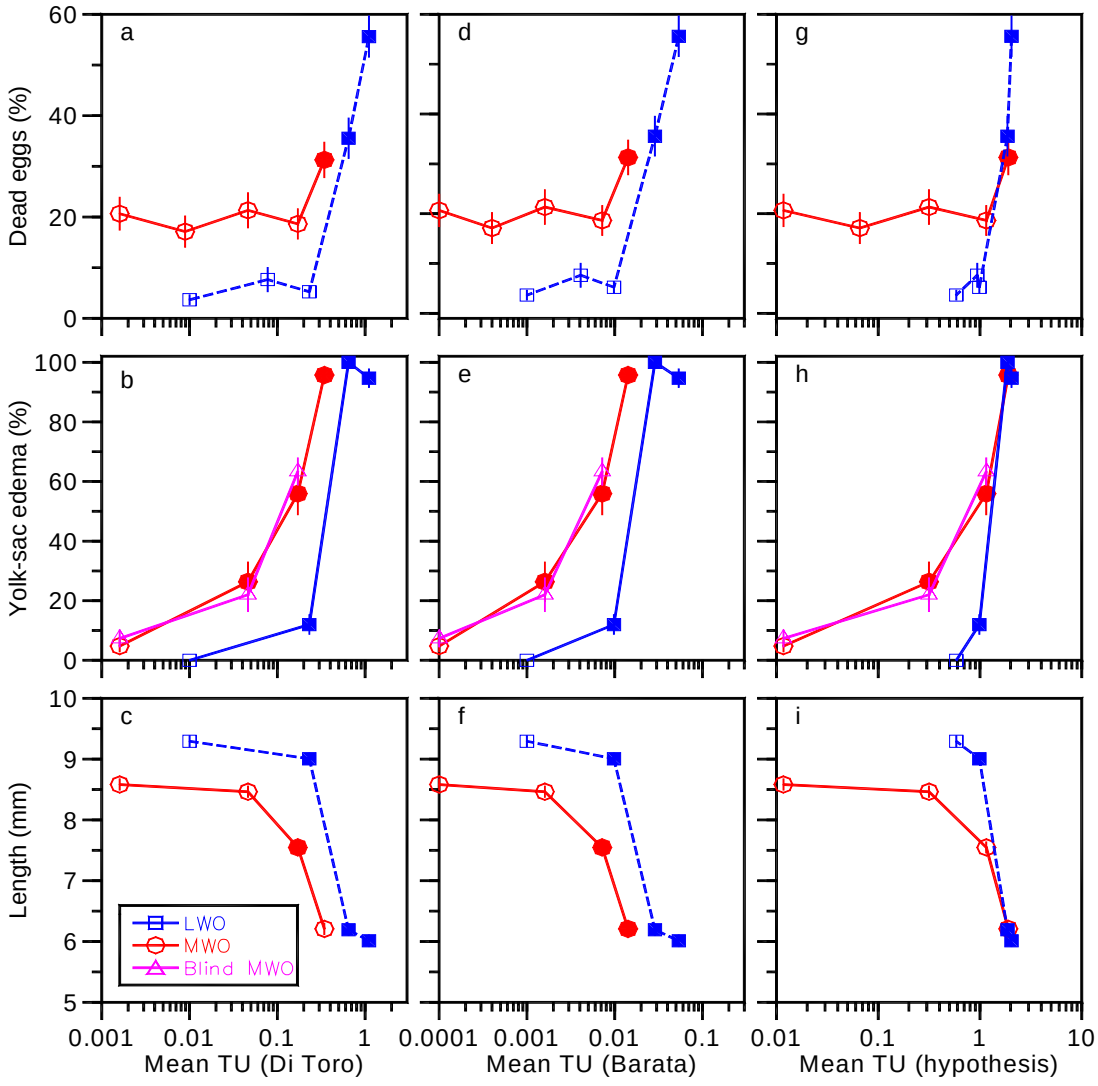


Fig. 5.

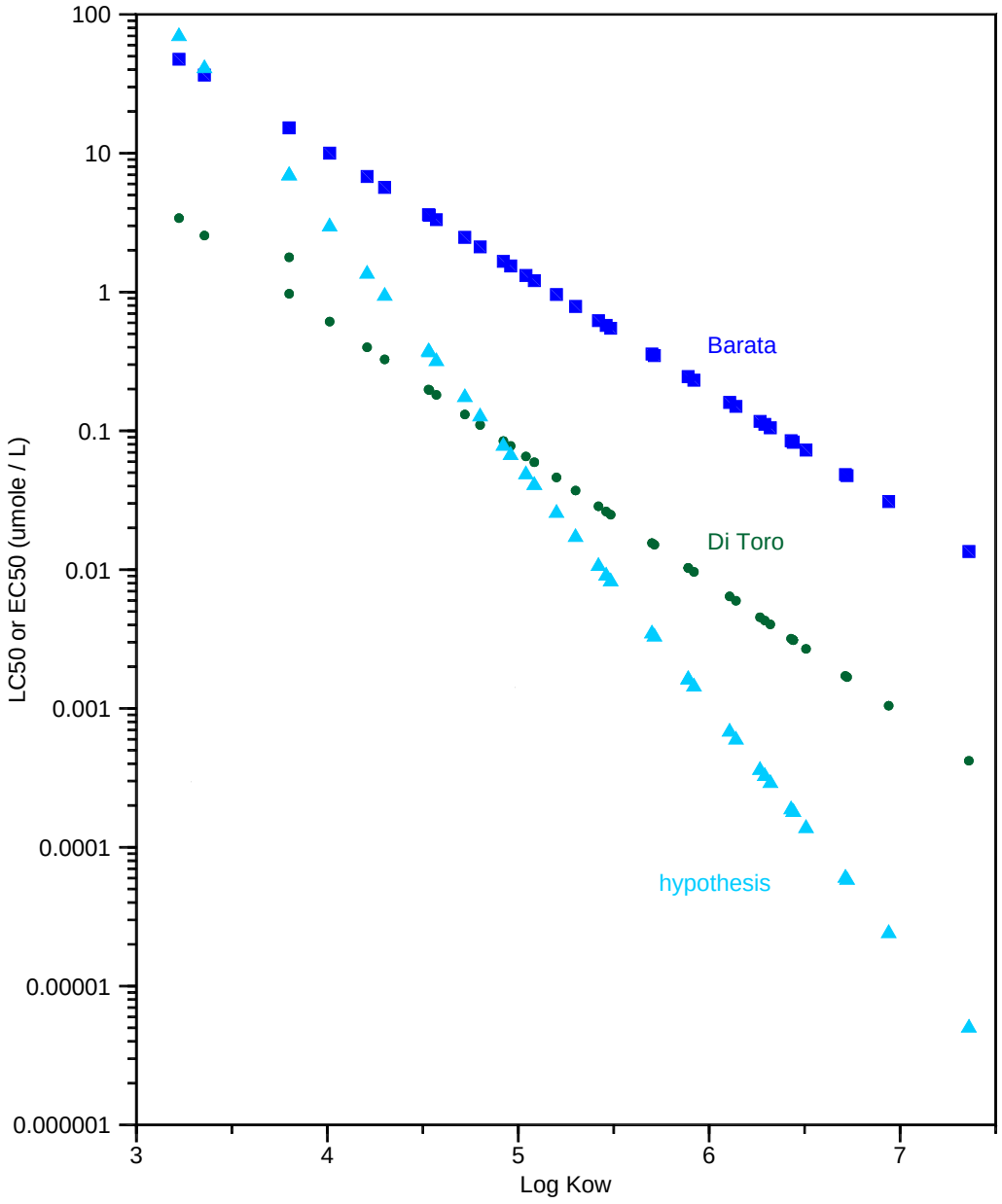

100
10
1
0.1
0.01
0.001
0.0001
0.00001
0.000001
LC50 or EC50 (umole / L)
3
4
5
6
7
Log Kow
Barata
Di Toro
hypothesis

Fig. 6.

Multi-ring PAH have specific pharmacological toxicity, thus simple additive models can not adequately explain the toxicity of PAH mixtures. Damage caused by PAHs may be very site-specific, or may be generalized, such as when enzymatically produced oxygen-free radicals randomly react with proteins or DNA. Activation of AHR, cytoplasmic receptors (AHR1 or AHR2), initiates transcription of a battery of genes including CYP1A (there are at least two forms in fish, CYP1A1 and CYP1A2) that convert PAHs to water-soluble metabolites (Wassenberg and Di Giulio 2004; Incardona et al. 2006). For some PAHs (e.g., benzo[a]pyrene), as with the halogenated planar hydrocarbon dioxin (tetrachlorodibenzo-*p*-dioxin), these metabolites (such as epoxides, quinones, and peroxides) can cause cell damage (Livingstone 1991; Wassenberg and Di Giulio 2004). Reactive metabolites can covalently link with DNA to form mutagenic adducts, compromising replication fidelity and resulting in mutations (Perlow and Broyde 2001). The toxicity of dioxin is mediated by AHR and may be caused by CYP1A activity, demonstrated with knockout studies in mice where either AHR or CYP1A were rendered inoperative (Wassenberg and Di Giulio 2004).

The PAHs interact with specific receptors in fish embryos, thus at sub-narcotic doses at least three non-narcotic mechanisms prevail. Some of these require AHR and CYP1A activation, others do not (Wassenberg and Di Giulio 2004; Incardona et al. 2004, 2005, 2006). Cardiac damage caused by tricyclic PAHs are independent of the AHR, and properly functioning AHR pathways provide modest protection against these molecules (Incardona et al. 2004, 2005). In contrast, some tetracyclic PAHs (pyrene and benz[a]anthracene) cause toxicity through the AHR pathway but the mechanisms of AHR-dependent toxicity vary (Incardona et al. 2006). Effects of benz[a]anthracene on cardiac function and morphogenesis are AHR2-dependent and CYP1A-independent [53,54]. For pyrene, products from hepatic metabolism apparently circulate to cause damage or systemic effects result from altered liver function secondary to CYP1A-mediated hepatotoxicity (Incardona et al. 2006).

In addition, PAHs are likely to interfere with steroid metabolism because CYP1A is involved in the oxidative metabolism of endogenous compounds such as arachidonic acid, prostaglandins, and steroids (Wassenberg and Di Giulio 2004). Thus, induction of CYP1A by PAHs can suppress steroids; 3-methylcholanthrene and β-naphthoflavone, for example, reduced vitellogenisis in juvenile rainbow trout (Navas, J.M., Segner 2000). Effects of steroid suppression by PAHs in fish embryos is apparently unknown; however, embryo-larval exposure to estradiol can induce vitellogenin production and bias sex-ratios toward females (Edmunds et al. 2000; Koger et al. 2000; Brion et al. 2004), demonstrating hormones can be influenced with discernable results during embryonic development.

Multiple PAH toxicity mechanisms suggest that additive toxicity models are not appropriate predictors of toxicity (Wassenberg and Di Giulio 2004; Incardona et al. 2006). This is the most likely reason the toxic units approach of Di Toro et al. (2000) fails to accurately predict PAH toxicity in Pacific herring and pink salmon assays (Carls et al. 1999; Heintz et al. 1999; Barron et al. 2004). Although toxic equivalency may be used to predict carcinogenic potential (which is mediated by AHR activation and CYP1A metabolism), this approach fails to predict early life stage toxicity in fish. This type of model predicts that chrysene is more toxic than pyrene, yet despite robust CYP1A induction, chrysene has no discernable impact through at least the first 5 d post-fertilization in zebrafish, a time period where other PAH, such as phenanthrenes, cause considerable damage (Incardona et al. 2006). The specific tissues impacted by AHR activation are apparently more relevant to toxicity than the overall levels of pathway activation. Patterns of CYP1A induction are tissue-specific and may depend on active

circulation. Incardona et al. (2006) conclude that these data do not support models that assume lipids are non-specific targets for PAHs, resulting in narcotic-like toxicity (Di Toro et al. 2000). Rather current models of PAH toxicity in fish are greatly oversimplified; the biological effects of PAHs cannot be predicted simply by quantitative measures of AHR activity or a compound's hydrophobicity. Individual PAHs are pharmacologically active compounds with distinct and specific cellular targets, thus, they should be considered as individual compounds or subfamilies of compounds with specific activities (Incardona et al. 2006). Thus, the Di Toro approach, which is predicated on additive toxicity of non-specific narcotic compounds, is fundamentally flawed and is unlikely to succeed.

Successful models accurately predict experimental outcomes. When model predictions fail to predict experimental results, either the model is in error and needs to be replaced or the experiment is flawed and should be discarded or redone. Review of current literature (main text) demonstrates that PAH are highly toxic to a variety of fish species exposed to PAH in a variety of ways by many investigators. Coupled with this graphic-based explanation and a basic understanding of the difference between non-specific additive toxicity and specific modes of PAH damage, we submit that the experimental observations are not in error, rather they are consistent with the results of other contemporary research (e.g., Brand et al. 2001; Rhodes et al. 2005). Thus, the narcotic model cannot adequately explain chronic sublethal, yet ultimately lethal PAH toxicity and should be reserved only for the acute lethal responses for which it was developed. This conclusion should not come as a surprise. Relatively few herring embryos reacted within 4 d; only sometimes were these reactions significant, far below median lethal or median effective levels (Carls et al.1999). Narcotic responses are reversible; observed embryo responses were not. Increasing proportions of embryos exposed for variable periods of time and incubated in clean water for approximately 12 to 18 before examination and quantification of abnormalities clearly demonstrates this point (Carls et al. 1999).

Exclusive use of 4 day acute toxicity data to build the narcotic model completely misses non-narcotic and latent responses (Zhao and Newman 2004). The narcotic model should be restricted to description of acute lethal responses. Because PAH have specific modes of action, and simple additive models are not applicable, adequate description of the toxicity of PAH mixtures continues to require empirical study and determination of the specific toxicity of many of these chemical species remains to be completed.

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Appendix 6

Disease index

VHSV disease index for ages 3-4 and *I. hoferi* index for ages ≥5. The disease index for VHSV on age 3-4 fish was determined by the prevalence of not only VHSV but also with corresponding ulcers (Marty et al. 2003). This disease index for VHSV was computed following:

$$\text{(A1)}\quad \text{DzIndex}_{\text{VHSV}} = \frac{\text{VHSVprev} \cdot \text{FSR(3)bounded}}{1000}, \text{ in which}$$

$$\text{(A2)}\quad \text{VHSVprev} = \frac{\#\,\text{VHSV}+}{\#\,\text{examined}} \cdot 100 \quad \left[\begin{array}{l}\text{proportion of sample infected with}\\ \text{VHSV}\end{array}\right] \text{ and}$$

$$\text{(A3)}\quad \text{FSR(3)} = \frac{\#\,\text{FSR(3)}+}{\#\,\text{examined}} \cdot 100 \quad \left[\text{proportion of sample infected with ulcers}\right].$$

FSR(3) bounded is just the FSR(3) values but with a lower bound of 0.5%, which is the proportion of the population that have ulcers along with VHSV. The lower bound allows for the inclusion of some VHSV positive fish even when the ulcer prevalence is zero.

The disease index for *I. hoferi* prevalence was calculated in the same manner, where

$$\text{(A4)}\quad \text{DzIndex}_{\text{ICH}} = \frac{\#\,\text{ICH}+}{\#\,\text{examined}} \cdot 100$$

This was further modified to use the prevalence of sum *I. hoferi* score>10. The consistency of *I. hoferi* over the years might be one of the driving forces keeping mortality fairly constant from year to year. *I. hoferi* only causes unexpected mortality when its prevalence increases above endemic levels. Only counting fish as affected if they have a sum of *I. hoferi* score greater than ten, instead of greater than zero, eliminates the mild cases and only considers the fish that will most likely die within the year.

Appendix 7
Alleles and heterozygosity

Appendix 7a. Number of alleles (N) and Heterozygosity (H_e, in parentheses) for Alaskan stations by locus. Number of alleles from Wright and Dillon (1997), H_e from O'Connell et al. (1998b). PWS = Prince William Sound overall, SMB=St. Matthew's Bay, FB=Fish Bay, RB= Rocky Bay, PC=Port Chalmers; KI = Kodiak Island; TB = Togiak Bay, Bristol Bay; Norton Sound, Bering Sea.

			Locus			N	H_e
Site	**Cha 17**	**Cha 20**	**Cha 63**	**Cha 113**	**Cha 123**	**Mean (SD)**	**Mean (SD)**
1995							
PWS-SMB	22 (0.911)	13 (0.890)	21 (0.917)	21 (0.892)	23 (0.939)	19.6 (7.3)	0.910 (0.020)
PWS-FB	24 (0.938)	12 (0.886)	20 (0.896)	19 (0.822)	22 (0.847)	19.4 (4.6)	0.878 (0.045)
PWS-RB	26 (0.940)	15 (0.896)	19 (0.897)	21 (0.918)	22 (0.929)	20.6 (4.0)	0.916 (0.019)
PWS-PC	21 (0.914)	15 (0.847)	18 (0.883)	16 (0.856)	25 (0.931)	15.0 (8.7)	0.886 (0.036)
KI	26 (0.927)	15 (0.906)	15 (0.894)	17 (0.851)	26 (0.951)	19.8 (5.7)	0.906 (0.038)
TB (1991)	20 (0.888)	14 (0.841)	17 (0.934)	15 (0.745)	24 (0.947)	18.0 (4.1)	0.871 (0.082)
NS (1991)	23 (0.912)	14 (0.887)	17 (0.859)	13 (0.770)	31 (0.957)	19.2 (7.2)	0.877 (0.070)
1996							
PWS-SMB	26 (0.937)	15 (0.916)	25 (0.942)	17 (0.931)	32 (0.959)	23.0 (7.0)	0.937 (0.016)
PWS-FB	26 (0.938)	14 (0.890)	22 (0.923)	17 (0.861)	31 (0.932)	22.0 (6.8)	0.909 (0.032)
PWS-RB	30 (0.935)	13 (0.902)	20 (0.923)	18 (0.887)	29 (0.957)	22.0 (7.3)	0.921 (0.027)
PWS-PC	27 (0.936)	16 (0.897)	20 (0.940)	21 (0.884)	34 (0.959(	23.6 (7.0)	0.923 (0.031)
KI	28 (0.924)	16 (0.899)	18 (0.925)	20 (0.918)	28 (0.946)	22.0 (5.7)	0.922 (0.017)
TB	24 (0.920)	18 (0.890)	18 (0.909)	16 (0.831)	30 (0.942)	21.2 (5.8)	0.898 (0.042)
NS	23 (0.912)	14 (0.887)	17 (0.862)	16 (0.785)	29 (0.936)	19.6 (6.1)	0.876 (0.058)

Appendix 7b. M values calculated for Prince William Sound stations by locus (Wright and Dillon 1997). Abbreviations as above.

			Locus			
Site	**Cha 17**	**Cha 20**	**Cha 63**	**Cha 113**	**Cha 123**	**Mean (SD)**
1995						
PWS-SMB	0.81	0.93	0.91	0.91	0.72	0.86 (0.09)
PWS-FB	0.60	0.92	0.80	0.90	0.73	0.79 (0.13)
PWS-RB	0.81	0.88	0.76	1.00	0.67	0.82 (0.12)
PWS-PC	0.54	0.79	0.95	0.80	0.71	0.76 (0.15)
KI	0.79	0.68	0.71	0.85	0.60	0.73 (0.10)
TB (1991)	0.80	0.74	0.81	0.75	0.62	0.74 (0.08)
NS (1991)	0.70	0.88	0.95	0.76	0.86	0.83 (0.10)
1996						
PWS-SMB	0.76	0.60	0.71	0.89	0.63	0.72 (0.12)
PWS-FB	0.84	0.82	0.88	0.81	0.69	0.81 (0.07)
PWS-RB	0.77	0.93	0.91	0.86	0.68	0.83 (0.10)
PWS-PC	0.84	0.89	0.69	1.00	0.79	0.84 (0.12)
KI	0.88	0.57	0.72	0.87	0.78	0.76 (0.13)
TB	0.59	0.64	0.64	0.80	0.77	0.69 (0.09)
NS	0.66	0.70	0.77	0.89	0.74	0.75 (0.09)

Appendix 8
Allelic diversity and heterozygosity

Appendix 8a. Allelic Diversity (N) and Heterozygosities (H_e) from 11 Microsatellite Loci (Small et al. 2004). Site followed by year. Alaska sites: PWS=Prince William Sound, NS=Norton Sound, Bering Sea; British Columbia: SG=Northumberland, Strait of Georgia; Washington: CP=Cherry Point, PS=Puget Sound, SE=Semiahmoo, SP=Squaxin Pass, PG=Port Gamble 1999+2002; F=Fidalgo Bay; California: SF=San Francisco Bay.

Collection	Cha 27	Cha 107	Cha 113	Cha 134	Cpa-D	Cpa-K	Cpa-A	Cpa-H	Cpa-172	Cpa-6	Cpa-130	Mean N (SD)	Mean H_e (SD)
PWS96	12	20	14	27	14	16	42	20	18	15	16	19.5 (8.5)	0.833 (0.086)
NS96	13	23	16	24	14	20	42	18	17	18	15	20.0 (8.1)	0.832 (0.102)
CPNS99	12	17	15	20	11	19	32	22	15	14	12	17.2 (6.0)	0.832
CP99	11	22	14	19	11	19	35	19	17	16	14	17.9 (6.7)	0.833
CP00	11	19	15	23	11	18	35	19	16	17	16	18.2 (6.6)	0.823
CP02	11	23	19	24	12	13	42	18	15	17	15	19.0 (8.7)	0.833
CP03	11	20	17	25	10	17	30	18	16	15	12	17.4 (6.0)	0.826
PSSE99	13	23	15	24	11	17	35	19	16	13	12	18.0 (7.1)	0.832
PSSE02	11	20	15	25	13	18	34	20	15	16	11	18.0 (6.8)	0.833
PSSP99	12	22	15	23	11	18	35	20	17	16	13	18.4 (6.8)	0.830
PSSP02	11	20	15	22	10	18	38	21	15	17	10	17.9 (7.9)	0.830
PSPG	13	20	17	25	11	17	38	18	15	17	12	18.5 (7.6)	0.831
PSF99	13	19	15	18	11	17	32	21	14	15	11	16.9 (5.9)	0.833
SG	13	20	14	26	13	16	35	19	18	15	10	18.1 (7.1)	0.833 (0.099)
SF	12	17	12	17	12	17	32	17	15	13	12	16.0 (5.8)	0.830 (0.121)

Appendix 8b. M Values calculated from 11 Microsatellite Loci (Small et al. 2004). Abbreviations as above.

Collection	Cha 27	Cha 107	Cha 113	Cha 134	Cpa-D	Cpa-K	Cpa-A	Cpa-H	Cpa-172	Cha-6	Cpa-130	Mean (SD)
PWS96	0.86	0.87	1.00	0.69	0.93	0.67	0.62	0.95	0.78	0.83	0.67	0.81 (0.13)
NS96	0.68	0.89	0.84	0.57	0.74	0.87	0.64	0.86	0.77	0.90	0.58	0.76 (0.12)
SG	0.81	0.77	0.93	0.65	0.81	0.94	0.64	1.00	0.95	1.00	0.83	0.85 (0.13)
CPNS99	0.75	0.68	0.88	0.49	0.73	0.83	0.70	1.00	0.79	0.88	0.75	0.77 (0.13)
CP99	0.79	0.88	0.74	0.83	0.73	0.86	0.67	0.95	0.74	0.94	0.52	0.79 (0.13)
CP00	0.85	0.73	1.00	0.77	0.69	0.78	0.64	0.95	0.73	0.90	0.59	0.78 (0.13)
CP02	0.79	0.79	0.86	0.69	0.75	0.81	0.71	1.00	1.00	0.94	0.58	0.81 (0.13)
CP03	0.85	0.77	0.85	0.71	0.77	0.85	0.59	1.00	0.94	0.75	0.80	0.81 (0.11)
PSSE99	0.76	0.82	1.00	0.65	0.69	0.85	0.52	1.00	0.76	1.00	0.71	0.80 (0.16)
PSSE02	0.92	0.80	0.88	0.56	0.65	0.78	0.61	0.91	0.88	0.89	1.00	0.81 (0.14)
PSSP99	0.71	0.79	0.94	0.68	0.92	0.86	0.66	0.91	1.00	0.94	0.54	0.81 (0.15)
PSSP02	0.85	0.80	0.73	0.49	0.91	0.95	0.64	0.91	0.79	1.00	0.71	0.80 (0.15)
PSPG	0.72	0.80	0.81	0.60	1.00	1.00	0.67	1.00	1.00	0.85	0.48	0.82 (0.18)
PSF99	0.81	0.76	0.79	0.43	0.69	0.85	0.56	1.00	0.82	0.88	0.67	0.75 (0.16)
SF	0.80	0.65	1.00	0.59	0.92	0.94	0.49	0.94	0.75	1.00	1.00	0.83 (0.18)

Appendix 9

Mitochondrial DNA haplotypes and haplotype diversity

Appendix 9a. Number of mitochondrial DNA haplotypes (H) and haplotype diversities (h) from Pacific herring collected in 1995. H from Bentzen (1997), h computed using BOTTLENECK. PWS=Prince Willam Sound overall, SMB=St. Matthew's Bay, FB=Fish Bay, RB= Rocky Bay, PC=Port Chalmers

Location	BanII	CfoI	HinfI	RsaI	BanII/CfoI	BanII/RsaI	BanII/CfoI/RsaI	Mean H (SD)	Mean h (SD)
PWS	5.8	5.5	2.5	4.5	12.2	9.5	12.3	7.5 (3.9)	0.536 (0.254)
SMB	5.0	4.0	2.0	5.0	9.0	7.0	5.0	5.3 (2.2)	0.474 (0.270)
FB	5.0	6.0	2.0	4.0	11.0	9.0	16.0	7.6 (4.8)	0.524 (0.276)
PCh	6.0	6.0	3.0	5.0	14.0	11.0	19.0	9.1 (5.8)	0.544 (0.256)
RB	7.0	6.0	3.0	4.0	15.0	11.0	20.0	9.4 (6.2)	0.571 (0.264)
KI	5.0	2.0	2.0	6.0	7.0	13.0	12.0	6.7 (4.4)	0.576 (0.296)
TB	5.0	3.0	2.0	1.0	10.0	4.0	9.0	4.9 (3.4)	0.496 (0.344)
NS	4.0	3.0	2.0	4.0	5.0	5.0	5.0	4.0 (1.2)	0.417 (0.295)

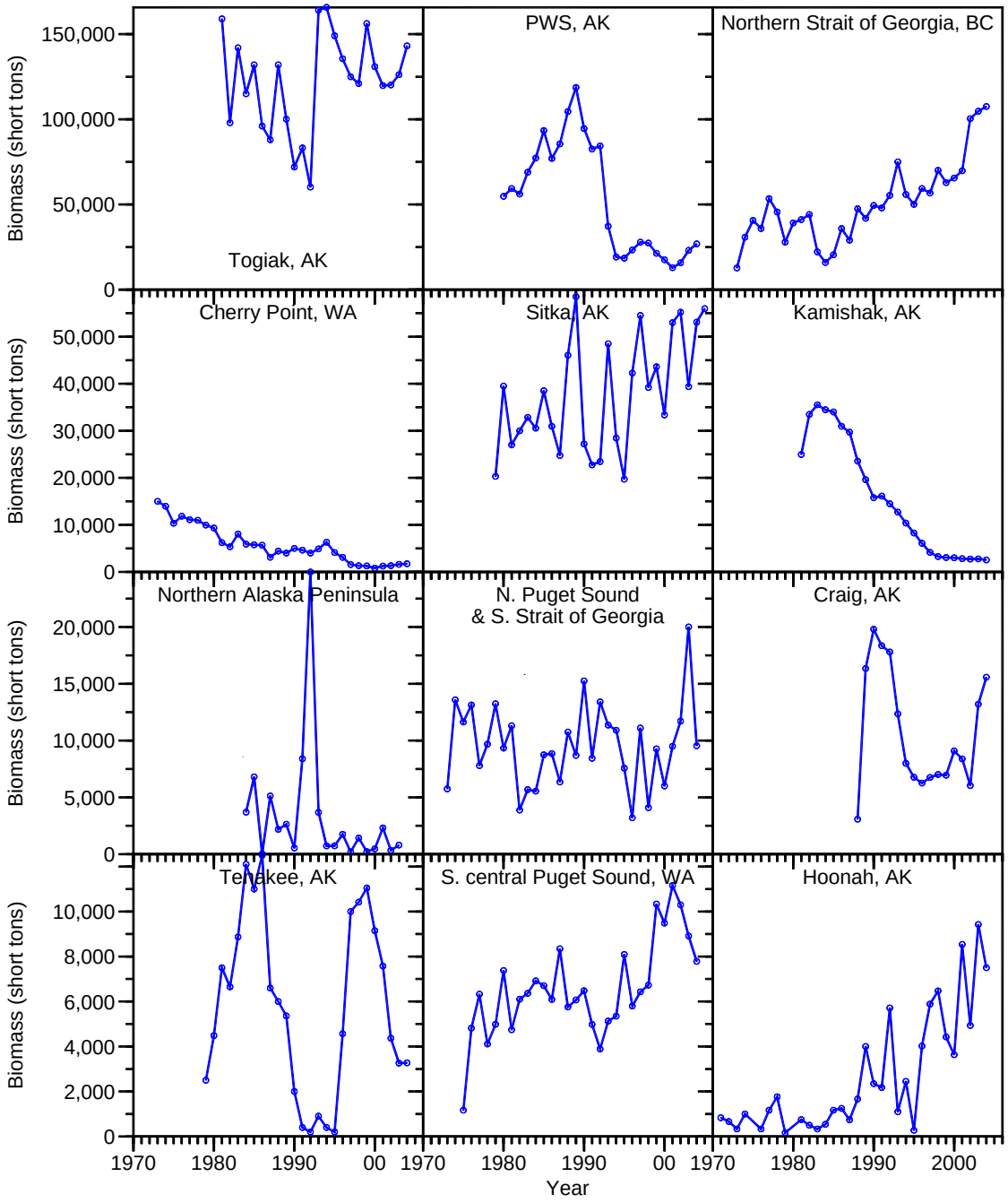
Appendix 9b. M values calculated for 1995 mtDNA from Pacific herring. Abbreviations as above.

Location	BanII	CfoI	HinfI	RsaI	BanII/CfoI	BanII/RsaI	BanII/CfoI/RsaI	Mean (SD)
PWS	0.79	0.93	0.94	0.61	0.58	0.41	0.40	0.67 (0.23)
SMB	0.83	1.00	1.00	0.50	0.47	0.32	0.17	0.61 (0.33)
FB	0.71	0.85	1.00	0.67	0.52	0.41	0.43	0.66 (0.22)
PCh	0.75	1.00	1.00	0.71	0.64	0.48	0.49	0.72 (0.21)
RB	0.88	0.86	0.75	0.57	0.68	0.44	0.50	0.67 (0.17)
KI	1.00	0.50	1.00	0.50	0.37	0.56	0.34	0.61 (0.28)
TB	0.83	1.00	1.00	1.00	0.50	0.19	0.24	0.68 (0.36)
NS	0.80	1.00	1.00	0.50	0.33	0.24	0.17	0.58 (0.35)

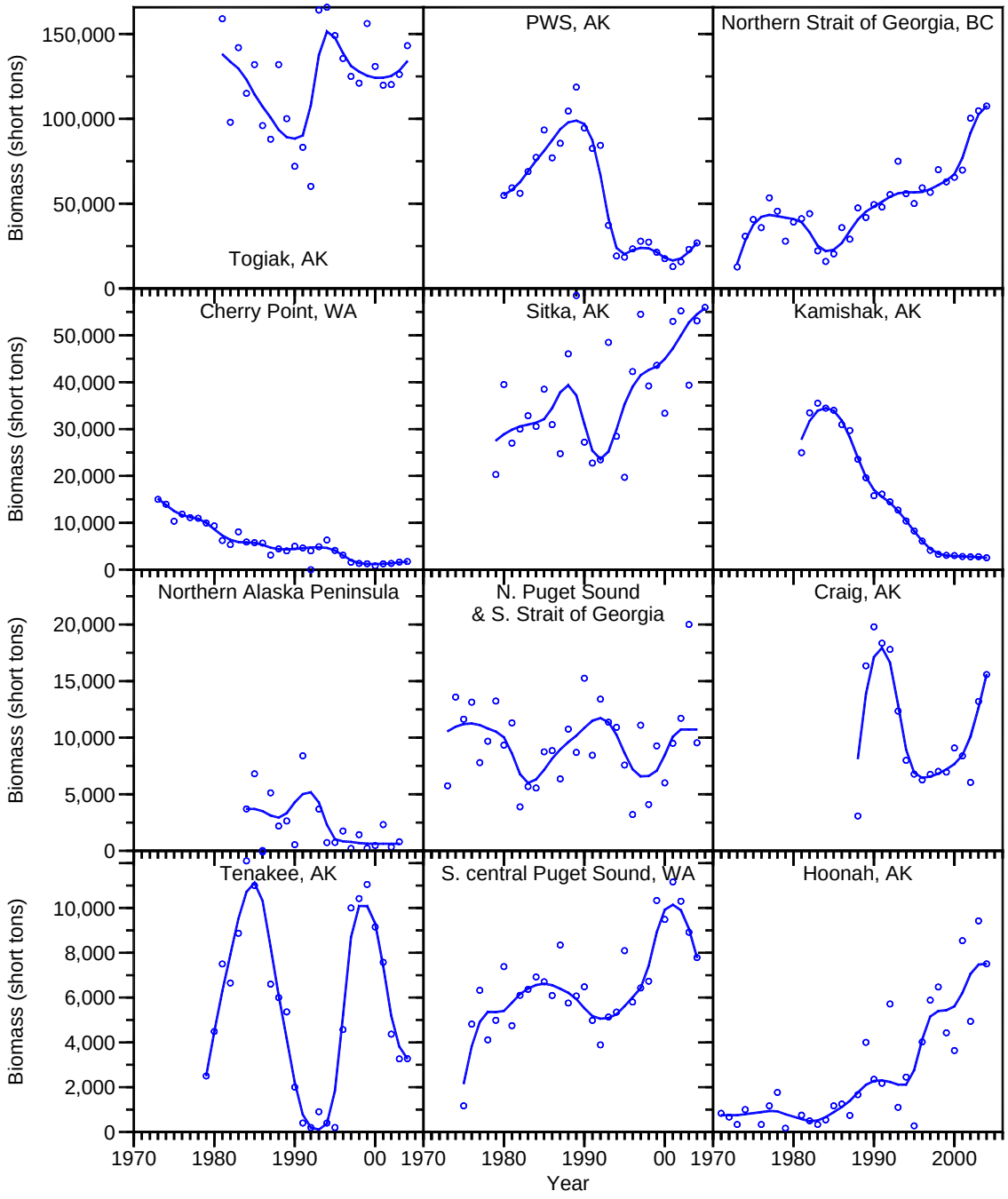
Appendix 10

Estimated herring biomass, 1970 to present, of Alaskan and other west coast stocks.

Historical trends for the 12 most abundant Pacific herring populations from Washington, British Columbia, and Alaska as reported by the Alaska Department of Fish and Game and the National Marine Fisheries Service).



Historical biomass trends, smoothed to identify long-term population trends.



Biomass trends in Prince William Sound (PWS) relative to other adjacent Alaskan Pacific herring stocks.