Exxon Valdez Oil Spill
Restoration Project Annual Report

Ichthyophonus hoferi, Viral Hemorrhagic Septicemia Virus, and Other Causes of Morbidity in Pacific Herring Spawning in Prince William Sound in 1994

Restoration Project 94320S
Annual Report

This annual report has been prepared for peer review as part of the *Exxon Valdez* Oil Spill Trustee Council restoration program for the purpose of assessing project progress. Peer review comments have not been addressed in this annual report.

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Study History: Restoration Project 94320S was authorized under emergency conditions in April 1994, and was not part of a work plan. A detailed study plan was written after sampling was completed. Three reports contributed to this study: 1) Histopathology of Herring from Prince William Sound: April 1994 Samples, September 30, 1994; 2) Report of Laboratory Examination of Rocky Bay (PWS) Pacific Herring *Clupea harengus pallasi*, December 15, 1994; and 3) Statistical Analysis of Herring from Prince William Sound: April 1994 Samples, January 10, 1995. The project effort has continued under Restoration Project 95320S (Disease Impacts on PWS Herring Populations) as part of the Fiscal Year 1995 Work Plan.

Abstract: Pacific herring (*Clupea pallasi*) populations in Prince William Sound (PWS) declined from estimated 1.1×10^8 kg to 1.8×10^7 kg from the 1993 to the 1994 spawning seasons. In order to determine the role of disease in population decline, study of Pacific herring in PWS was initiated during April 1994. 233 fish were subjected to complete necropsy. Analysis included histopathology, plasma chemistries, virus isolation, and bacteriology. The fungus, *Ichthyophonus hoferi*, infected 62 of 112 fish (29%); lesions were often disseminated and severe. Viral hemorrhagic septicemia virus (VHSV), isolated from 11 of 233 fish (4.7%) was a secondarily significant cause of morbidity. Fish had other lesions and over 10 parasite species, but these were not significant at the population level. *Ichthyophonus* prevalence in 1994 was over twice that reported in Pacific herring annually since 1989. However, VHSV has previously been isolated from 10-80% of Pacific herring sampled throughout the Pacific Northwest. Prevalence of external gross lesions and major parasites was unrelated to fish age. No pathogens were unique, but lesions associated with *Ichthyophonus* and VHSV were severe enough to cause mortality. Study results could neither confirm nor deny a role for the spill in Pacific herring population declines.

Key Words: *Clupea pallasi*, *Exxon Valdez* oil spill, histopathology, *Ichthyophonus hoferi*, morbidity, Pacific herring, plasma chemistries, Prince William Sound, viral hemorrhagic septicemia virus (VHSV).

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Introduction

Before the 1993 spawning season, 120,000 tons of Pacific herring (*Clupea pallasi*) were forecast to arrive on the spawning grounds of Prince William Sound (PWS); only 30,000 tons appeared. Viral hemorrhagic septicemia virus (VHSV), but no other significant pathogens, was isolated from those herring. In 1994, when only 20,000 tons of an expected 30,000 tons of herring appeared on the spawning grounds, this study was initiated to investigate the cause of the herring population decline. Given the history of VHSV isolation in 1993, the study was designed to investigate the role of infectious disease in herring morbidity, with primary emphasis on the role of VHSV.

Objectives

The study described in detail herein had four objectives: (1) evaluate the general health of PWS Pacific herring; (2) determine whether certain year classes had higher disease prevalence; (3) determine the primary or secondary invader role of VHSV; and (4) determine the influence of disease on the spawning potential of herring and, conversely, the impact of spawning and other stressors on herring health. Study of PWS Pacific herring in years immediately before 1994 had revealed no evidence of ongoing hydrocarbon exposure. Based on this information, the high cost of hydrocarbon analysis, and the small chance that hydrocarbon analysis would contribute to the explanation of population decline in 1994, hydrocarbon samples were not taken. Also, the scope of this study did not include analysis of effects of environmental variables such as water temperature, currents, or food supply.

Methods

To determine disease status, 233 Pacific herring were sampled from catches on the R/V *Montague* in PWS, April 21 through 26, 1994. The complete necropsy recorded weight, standard length, and external lesions were scored. Samples were taken for histopathology (12 organs), virus isolation (head kidney and spleen), age determination (from scales), and plasma chemistry analysis (total protein, albumin, osmolality, cholesterol, glucose, total bilirubin, 5 enzymes, and 5 electrolytes). Also, blood smears were examined for the only other known herring virus, viral erythrocytic necrosis (all were negative). For histopathology, tissues from each of 212 herring were coded for blind study, and lesions were ranked on a four-point scale as none (0), mild (1), moderate (2), or severe (3). Results were analyzed statistically to determine significance. Parasites or other pathogens that were not statistically associated with lesions or alterations in plasma chemistries were not considered pathologically significant.

Results

Lesions associated with *Ichthyophonus hoferi* infection occurred in 62 of 212 (29%) Pacific herring. *Ichthyophonus*-associated granulomatous inflammation often involved multiple organs, and no lesions other than the grossly observed ulcers were consistently as severe. Viral hemorrhagic septicemia virus was isolated from 11 of 233 fish (4.7%). Abnormal findings associated with VHSV included congestion of vessels at the base of fins, inflammation of the brain (meningoencephalitis) and stomach lining (submucosal gastritis), acute focal hepatic necrosis, and low plasma albumin. External lesion scores were moderate or severe in 47 of 233 fish (20%), and they were significantly associated with VHSV but not *Ichthyophonus*.

Herring had several other lesions including inflammation, pigmented macrophage aggregates, and single cell necrosis; however, few of these other lesions were pathologically significant. Prevalence of common parasites included: (1) herring worms (Anisakidae) in the peritoneal cavity (233 of 233, 100%); (2) an intestinal coccidian (*Goussia* sp.?), not previously described (192 of 211, 91%); (3) a coccidian in the liver, *Goussia* (*Eimeria*) *clupearum* (129 of 212, 61%); (4) a myxosporean in large ducts of the kidney, *Ortholinea orientalis* (44 of 233, 19%); and (5) a myxosporean in the gall bladder, *Ceratomyxa auerbachi* (32 of 171, 19%). Prevalence of neither external gross lesions nor most major parasites was related to fish age. The gall bladder myxosporean was more common in older fish (> 6 years old) than in young (< 5 years old) or middle-aged fish (5 or 6 years old).

As expected for spawning fish, Pacific herring had several abnormal plasma chemistry values and all fish had depleted glycogen stores in their liver cells (hepatocytes). Also, females were probably in poorer physiologic condition than were males. As evidence, females had significantly lower plasma values for albumin, chloride, cholesterol, glucose, potassium, and total protein. Also, gonad weight was significantly greater in females. By comparison, males had higher plasma levels of CO_2 and potassium. The gall bladder myxosporean was more prevalent in females than in males, but gender differences were not significant for all other parasites, VHSV, or *Ichthyophonus*. Overall, however, gender differences in plasma chemistry values during spawning had little effect on disease prevalence.

Discussion

We considered whether the oil spill could have been linked to disease outbreak 4 years later. Fish that were hatched or were yearlings in 1989 at the time of the spill (1988 and 1989 year classes) might have incurred permanent damage to their ability to fight disease (i.e., irreversible immunosuppression). Under normal growth conditions, minor deficiencies in their immune system might have been insignificant. However, disease might have become a serious problem when fish experienced additional stress upon first spawning (1992 and 1993). Stress is well-documented as a cause of immunosuppression, but stress-induced changes usually are reversible if the fish survives. To address the hypothesis of age-related immunosuppression, we

examined the association of lesions with age. Several lesions were significantly associated with age (e.g., pigmented macrophage aggregates), but nearly all these lesions were more severe in older fish (i.e., fish hatched before 1988). Also, among VHSV, *Ichthyophonus*, and ten other common parasites, none were more prevalent in the 1988 and 1989 year classes than in the entire sampled population. Annual age-weight-length analysis by the Alaska Department of Fish and Game has documented that the population has decreased in the absence of abnormal changes in age distribution. Therefore, the weight of evidence suggests that the disease outbreak in PWS was not a result of permanent immune suppression caused by hydrocarbon exposure when fish were larvae or yearlings.

For all fish in the sample, the weight of evidence implicates *Ichthyophonus* as the major cause of morbidity in Pacific herring in PWS in 1994. Viral hemorrhagic septicemia virus was a secondary but significant cause of morbidity. None of the pathogens in Pacific herring in PWS were unique. Further, histologic alterations characteristic of hydrocarbon exposure were absent. In our previous studies of Pacific herring in PWS and Auke Bay, Alaska (1989-1993), *Ichthyophonus* prevalence was never more than 15%. Published studies of acute population declines in Atlantic herring (*Clupea harengus*) found that *Ichthyophonus* was the primary cause. In those studies, *Ichthyophonus* prevalence was ≥25%. Previous studies of VHSV in Pacific herring in PWS (1993) were done on samples pooled from several fish, so prevalence could not be determined. However, VHSV has been isolated from Pacific herring sampled elsewhere in Alaska, British Columbia, and Washington, where prevalence varied from 10 to 80%. Only one other report of disease-associated population decline of Pacific herring has been published; the cause was not determined, but clinical and pathological findings were more similar to our findings associated with VHSV than with *Ichthyophonus*. We established that VHSV was associated with several lesions, but determination of its role as a primary or secondary invader will require laboratory study (i.e., as proposed for 1996).

Plasma chemistries were useful for evaluating health of Pacific herring in PWS, but interpretation of results was limited by lack of reference values from healthy populations during spawning or periods of peak condition (e.g., late summer or early fall). Hence, results of this study will be re-evaluated when information from Pacific herring in Sitka Sound are evaluated in 1995. Pacific herring populations are strong in Sitka Sound, and population trends there were similar to PWS before the spill. Also, study of Pacific herring in peak condition is proposed for October, 1995.

Conclusions

Disease was probably the primary force driving population decline in 1994. As evidence, consider the assumption that all fish infected with VHSV and *Ichthyophonus* in April 1994 would have died within 6 months. This assumption is based on a combination of disease prevalence, associated lesions, and comparisons with published reports. If disease was driving population decline, then population biomass should have declined about 35% by October, 1994. Based on

the best population estimates, Pacific herring biomass in PWS dropped from 20,000 tons in April 1994, to a record low of 12,000 tons in October 1994: a 40% decline. Hence, a single but thorough disease survey in April 1994 was sufficient to explain nearly all the population decline in 1994. No other variables—food availability, predation, water temperature, currents, or recruitment—were needed to explain this significant decline. Probably, these other variable will be more important during population recovery, but disease must be ruled out as a factor limiting recovery. Pacific herring populations in PWS were not healthy in 1994, and until Pacific herring populations recover, continued study of herring morbidity is recommended.

INTRODUCTION

Pacific herring (*Clupea pallasi*) are among the most abundant fish species in coastal regions of the North Pacific, where they are important for commercial and subsistence fishing and as prey for many marine vertebrates. In Prince William Sound (PWS), Alaska, Pacific herring normally support 5 commercial fisheries, with an average annual ex-vessel value of $8.3 million. Roe fisheries, the most valuable, are harvested in April just before spawning. Pacific herring in PWS first spawn when 3 or 4 years old. They rarely live more than 12 years, and abundant year classes recruit into the fishery about once every 4 years. When the *Exxon Valdez* oil spill occurred in March, 1989, the biomass of spawning Pacific herring in PWS was the highest in 20 years of reliable estimates (about 1.1×10^8 kg; Figure 1). The population declined about 20% each of the first two years after the spill, but stabilized near 6.1×10^7 kg in 1991 and 1992.

Because toxicants such as crude oil cause relatively more severe damage in younger fish, particularly larvae (McKim 1985), long-term effects of the oil spill were thought most likely to occur in the 1988 and 1989 year classes which entered the spawning population in 1992 and 1993. Indeed, preliminary study of 4-year-old PWS Pacific herring in 1992 revealed less reproductive success in fish spawning in previously oiled sites than in unoiled sites, and fish with poor reproductive success had more severe microscopic lesions (Kocan et al. In Press). Pacific herring biomass was stable in 1992, and recruitment from the 1988 year class was expected to be excellent; therefore, fisheries biologists predicted a record spawning biomass of 1.1×10^8 kg before the 1993 spawning season (Figure 1). However, when the 1993 spawning season commenced, only 23% of the expected biomass appeared, fish were lethargic, and many had external hemorrhages. There were no reports of dead fish to explain differences in predicted and actual biomass. The North American strain of viral hemorrhagic septicemia virus (VHSV) was isolated from pooled samples of Pacific herring, but no other significant pathogens were isolated (Meyers et al. 1994). Because VHSV had not previously been isolated from Pacific herring, its role in population decline could not be determined. By 1994, spawning biomass declined to the lowest level (1.8×10^7 kg) recorded in 20 years of reliable estimates. Based on reduced biomass and the presence of external lesions, the Alaska Department of Fish and Game severely curtailed the commercial fisheries in 1993 and all Pacific herring fisheries were closed in PWS in 1994. Was VHSV the primary cause of mortality? Or, was VHSV expressed only in otherwise sick fish?

This study was initiated to determine the cause of morbidity in PWS Pacific herring. Our primary hypothesis was that VHSV was the most important cause of mortality, but the study was designed to consider and possibly rule out other pathogens. Thorough necropsy, virology, bacteriology, hematology, and histopathology were linked to traditional age-weight-length analysis to show that parasite prevalence was usually independent of age. The fungus, *Ichthyophonus hoferi*, was the primary cause of morbidity, and VHSV was a significant but less important cause of morbidity. Ten other parasites each affected more than 10% of the sampled population, but their role in population decline probably was minimal. This paper describes the pathogens and parasites of Pacific herring in PWS, discusses their role in population decline, and identifies research needs that could provide valuable information to fishery managers.

OBJECTIVES

1. Assess the general health of Pacific herring in PWS.

2. Investigate the impact of disease on population size and structure of PWS herring, and determine whether fish of a particular year class were more likely to be diseased than other year classes.

3. Assess the primary or secondary invader role of VHSV in producing disease in PWS Pacific herring.

4. Assess the influence of disease on the spawning potential of Pacific herring and, conversely, the impact of spawning and other stressors on herring health.

METHODS

Necropsy

Herring were captured in Rocky Bay of Montague Island, Prince William Sound, Alaska, from April 21 through 26, 1994, and 233 were subjected to complete necropsy on board the R/V *Montague*. To obtain a sample representative of the spawning population in PWS, fish were collected by gill net, beach seine, or purse seine in 17 different sets (8 to 18 fish per set). For this report, use of the term "prevalence" refers to the sample prevalence. Each fish was assigned a necropsy number, 94HER-1 through 94HER-233, in order of processing. After capture, fish were held in plastic containers filled with about 100 L of seawater for no more than 4 hours before necropsy. In groups of 2, herring were anesthetized in tricaine methane sulfonate (Finquel®), weighed and measured (standard length), and a scale was removed for age determination. Several diagnostic procedures were done on each fish:

1) external lesions were scored as none (0), mild (1), moderate (2), or severe (3). After lesions were scored, a summary "external lesion score" was determined for each fish. The external lesion score was the most severe score for fin base reddening, caudal fin reddening, or focal skin reddening. External lesions "iris reddening" and "caudal fin fraying" were not used for determination of external lesion score.

2) about 1.5 mL of blood was drawn from the caudal vein into 3-mL syringes that contained 0.1 mL of sodium heparin (10,000 IU/mL); a capillary tube was filled and centrifuged (5500 × *g* for 5 min) for determination of packed cell volume (PCV), a blood smear was made and air-dried, and remaining blood was centrifuged (13,600 × *g* for 5 min) and plasma was frozen for analysis by Med Veterinary Laboratory, Concord, California.

Osmolality was analyzed on a Micro Osmometer Model 3MO-plus from Advanced Instruments (Norwood, MA) using 20 µL of sample. All other analyses were done using about 200 µL of sample in a Monarch-plus analyzer from Instrumentation Laboratories (IL®) that was calibrated and run at a stabilized 25° C. Plasma was analyzed for total protein (biuret method), albumin (bromocresol green method), and CO_2 (enzymatic method); IL® substrates were used to analyze calcium, cholesterol, glucose, phosphorus, total bilirubin, alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and creatine phosphokinase (CPK); Sigma® substrates were used to analyze gamma glutamyltransferase (GGT); ion selective electrodes were used to analyze sodium, potassium, and chloride. Blood smears were stained with Diff-Quik (Dade Diagnostics, Inc., Aquada, Puerto Rico) and 30 1000x-fields were examined for cytoplasmic inclusions of viral erythrocytic necrosis (VEN).

3) for virus isolation, head kidney and spleen from each fish were pooled in a plastic bag and shipped on ice to the Alaska Department of Fish and Game Fish Pathology Laboratory in Juneau, Alaska; skin lesions, if present, were sampled and bagged separately for individual virus assay. Propagation of 2 cell lines (EPC, CHSE-214), media formulation, and tissue preparation for cell line inoculation were as described by Meyers et al. (1994).

4) for histopathology, samples of gill, liver, gonad, spleen, trunk kidney, gastrointestinal tract, heart, skin, skeletal muscle, and brain were fixed in 10% neutral buffered formalin;

5) bacterial isolation was attempted from herring with moderate or severe external lesions; kidney tissues were aseptically inoculated onto trypticase soy agar (TSA) and plates were incubated at 23° C for at least 5 days;

6) a touch preparation of kidney was air-dried, stained with Diff-Quik, and examined for pansporoblasts of the myxosporean *Ortholinea orientalis*; extent of infection was scored as for external lesions;

7) liver and gonads were weighed;

8) herring worms (Anisakidae) in the peritoneal cavity were counted;

9) archived samples (frozen) from each fish included bile (in 1-mL amber glass vials), 2 samples of liver (0.1 - 0.2 g each, in 1.5-mL plastic vials), gonad (1 to 15 g, in 20-mL scintillation vials), and a wedge of body wall, from the region of the dorsal fin (in a plastic bag).

Histopathology

Tissues from 233 herring were sent to the Aquatic Toxicology Laboratory, University of California, Davis, and randomly assigned a histopathology number (94H74-1 through 94H74-233) for blind study. Tissues from 21 herring were inadvertently put in water rather than fixative. Tissues from the remaining 212 herring were processed routinely into paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin. Tissues from each organ were read in ascending numerical order using the random histopathology number. In most cases, all tissues from one organ were read before tissues from the next organ were started. Lesions were scored using a four-point scale as none (0), mild (1), moderate (2), or severe (3). For quality control, autolysis and artifact in each organ were scored on the same four-point scale. Ranking of lesions was often based on the number of structures (e.g., *Ichthyophonus* resting spores) per 100× field; the 100× field was examined through a 10× objective lens and a 10× ocular lens on an Olympus binocular light microscope. After all organs were examined and lesions scored, data were rearranged by necropsy number and subjected to statistical analysis.

Statistical Analysis

The primary hypothesis was that fish with lesions were different from controls. In most cases, lesions with a score of none (0) were used as controls. The association of categorical variables (e.g., none, mild, moderate, and severe) with continuous variables (e.g., CPK values) was determined using one-way analysis of variance (one-way ANOVA). For example, the CPK values for fish with a liver *Ichthyophonus* score of zero were compared to livers with mild, moderate, and severe *Ichthyophonus*. When necessary, categories were combined to ensure that each group had at least 6 fish. Category-specific means and standard errors were calculated for each continuous variable and compared using Tukey's Studentized range method. Levene's test was used to evaluate the homogeneity of variance assumption for the ANOVA.

The association between 2 selected categorical variables (e.g., *Ichthyophonus* scores versus scores for hepatic focal necrosis) was evaluated using chi-square methods for categorical data analysis; comparisons were considered valid only if individual expected cell frequencies were >1. Odds ratios were calculated for standard (2x2) two-way contingency tables only. To measure the strength of the linear relationship between 2 continuous variables, the correlation coefficient r was calculated.

In the initial univariate analysis, some plasma chemistries were significantly associated with several lesions or other variables. In selected cases, multiple regression analysis was used to model a multifactor ANOVA, examining the relationships between the dependent variable (e.g., plasma albumin) and associated variables (e.g., focal skin reddening, splenic congestion, and VHSV). Lesion scores were forced into a multiple regression equation using stepwise regression to determine their joint impact in the prediction of the dependent variable (e.g., albumin level), while controlling for gender, gonad weight, hold time, and length. Criteria used for inclusion of variables in the evaluation included significance in the univariate analysis and postulated association of the equation variable with the dependent variable. Age and body weight were not used in the analyses for 3 reasons: (1) age was highly correlated with length; (2) body weight was highly correlated with both gonad weight and length; and (3) spawning fish lost up to 25% of their body weight within a few hours, so length was more constant than weight during the course of the study. The use of 2 or more highly correlated variables within the same multiple regression can result in errors in regression coefficient estimation.

To determine if certain age classes of fish were more likely to be infected by certain parasites, the association of fish age with common parasites was evaluated using the chi-square test for homogeneity. Fish were grouped into three categories for analysis: < 5 years old, 5 or 6 years old, or > 6 years old. Regardless of severity of infestation, fish with a given parasite were classified as positive, and fish without the parasite were classified as negative.

For all analyses, comparisons were considered significant when $P<0.05$ and highly significant when $P<0.01$.

RESULTS

External Gross Lesions

The summary external lesion score was moderate or severe in 47 of 233 fish (20%), and several of these fish concurrently had more than one lesion that was moderate or severe. Seven of 233 (3.0%) had ulcers (scored as severe focal skin reddening; Table 1, Figure 2). Some ulcers penetrated to underlying bone and one ulcer perforated into the peritoneal cavity, resulting in adhesions of viscera to the body wall. External lesions, especially focal skin reddening, were significantly associated with several microscopic lesions (Table 2). As scores for external lesions increased, scores for microscopic lesions usually increased. Two exceptions were (1) pigmented macrophage aggregates in the liver that were less severe in fish with increased caudal fin reddening, and (2) pigmented macrophage aggregates in the spleen that were less severe in fish with increased caudal fin fraying.

Because of the lack of published information on normal herring anatomy and histology, findings were sometimes scored without knowledge of whether they were lesions. Iris reddening is a good example. The inferior margin of the iris had a blood vessel about 3 mm long and 0.5

mm in diameter. Iris reddening occurred when the vessel contained enough blood to be detected by gross observation (Figure 2). Scores for iris reddening were assigned as follows: no reddening (0); reddening was limited to the primary vessel (1); reddening extended beyond the margins of the primary vessel, probably due to congestion of connecting venules (2); and reddening involved the entire iris (3). No fish had severe iris reddening, and mild iris reddening probably was normal. Several lesions were more prevalent in fish with no iris reddening than in fish with mild or moderate iris reddening (Table 3). For example, branchial ciliated protozoa and meningoencephalitis were more likely in fish with no iris reddening. Also, mean albumin and total protein were significantly lower in fish with no iris reddening than in fish with mild iris reddening (albumin, 0.46 vs. 0.54 g/dL; total protein, 2.0 vs. 2.3 g/dL).

Ichthyophonus

All organs contained *Ichthyophonus* (Table 1), and the multinucleate resting spore stage was the most common form. Morphology of *Ichthyophonus* and the host reaction were similar to those reported in infections in Atlantic herring (*Clupea harengus*) (Daniel 1933, Sindermann 1970). Most resting spores were surrounded by a rim of fibroblasts and maturing collagenous connective tissue, but some were surrounded by activated macrophages. Severe granulomatous inflammation, common in the heart, was usually associated with developing spores (Figure 3). Occasionally, resting spores had burst and released multinucleate endospores (Figure 4). A consistent scoring system was used for *Ichthyophonus* in each organ: no *Ichthyophonus* (score = 0); <1 resting spore per 100× field (score = 1); ≥1 but <3 resting spores per 100× field, but inflammation was limited to a thin rim of fibrous connective tissue (score = 2); or ≥1 resting spore per 100× field, with prominent granulomatous inflammation, or ≥3 resting spores per 100× field, regardless of the amount of inflammation (score = 3).

Granulomatous inflammation associated with *Ichthyophonus* had to be differentiated from other forms of macrophage aggregates. In organs such as the liver, normal parenchyma contained 1 to 7 foci of pigmented macrophage aggregates per 100× field. Foci were at least 60 μm in diameter, and pigment varied from yellow-brown to green-brown (Figure 5). Pigmented macrophage aggregates did not contain melanin. In moderate or severe cases (Figure 5), the liver contained more than 7 foci of pigmented macrophage aggregates per 100× field, and aggregates were as large as 300 μm in diameter. Aggregates of nonpigmented activated macrophages were classified as nonspecific granulomatous inflammation (Figure 5). Granulomatous inflammation was composed of activated macrophages with pale eosinophilic cytoplasm that sometimes expanded foci of pigmented macrophage aggregates. Small numbers of lymphocytes and eosinophilic granular leukocytes were scattered throughout foci of granulomatous inflammation.

Lesions associated with *Ichthyophonus* occurred in 62 of 212 (29%) fish, but no single organ had greater than 21% prevalence (Figure 6). Prevalence of *Ichthyophonus* in skin and skeletal muscle was the second highest after kidney, but most cases in skin and skeletal muscle

were mild (31 of 39, 79%). By comparison, prevalence of *Ichthyophonus* in the heart was similar to that in skin and skeletal muscle, but relatively few cases in the heart were mild (14 of 38, 37%), a higher proportion being moderate or severe.

A sum-*Ichthyophonus* (sumICH) score was calculated for each fish by adding the individual *Ichthyophonus* scores from all 10 organs for that particular fish. For example, *Ichthyophonus* scores in organs of fish #106 included spleen (score = 2), kidney (score = 1), and a combined score for skin and skeletal muscle (score = 1), but the other 7 organs had no *Ichthyophonus* (score = 0); therefore, the sumICH score for fish #106 was 4. Because the maximum *Ichthyophonus* score for each organ was 3 (severe), the maximum possible sumICH score for a fish was 30. The highest actual score was 24. SumICH scores significantly increased with increased severity of several internal lesions, but sumICH scores were not associated with any external lesions (Table 5). Lesions such as cardiac thrombosis and intestinal mesenteric steatitis were significantly associated with greater sumICH scores; however, because Levene's test for equality of variances was significant in nearly all cases, these results were interpreted with caution. Additional study on more fish infected with *Ichthyophonus* is needed to confirm the relation of these lesions to infection with *Ichthyophonus*.

Association of *Ichthyophonus* scores with plasma chemistries was variable (Table 1), but AST and CPK, enzymes commonly used in mammalian medicine as part of the evaluation of general health, were significantly associated with *Ichthyophonus* scores in every organ (univariate ANOVA). Increases in CPK in mammals result from disruption in muscle cell membranes (Willard et al. 1989). By comparison, AST is present in significant quantities in mitochondria of hepatocytes, muscle, erythrocytes, and other blood-rich organs. The most common causes of increased AST in small domestic mammals are hepatic disease, muscular disease (inflammation or necrosis), and hemolysis (Willard et al. 1989).

The significant increase in CPK and AST in every organ was inconsistent with distribution of these enzymes in mammals. Therefore, multiple regression analysis was used to model a multifactor ANOVA, examining the linear relationships between the dependent variable CPK (or AST) and *Ichthyophonus* lesion scores in 9 organs (brain, gill, heart, intestine, kidney, liver, skin/skeletal muscle, spleen, and stomach). Gonad scores were not analyzed because only 3 gonads contained *Ichthyophonus*. For CPK, brain *Ichthyophonus* status, gender, and gonad weight were the only significant predictors when all organs were included in the multiple regression equation. For AST, renal *Ichthyophonus* status and gonad weight were the significant predictors; however, in the final model, predicted values for AST decreased when a fish had renal *Ichthyophonus*.

As a relative measure of the severity of *Ichthyophonus* in individual organs, a mean sumICH score was computed as follows for each organ: all fish with *Ichthyophonus* in an organ were selected, their sumICH scores were totaled, and this sum of sumICH scores was divided by the number of fish in which the organ was infected. For example, of 212 kidneys examined, 43

had *Ichthyophonus*; the mean sumICH score for those 43 fish was 9.4; by comparison, the mean sumICH score for the 17 fish with brain *Ichthyophonus* was 14.2. Generally, organs with the lowest *Ichthyophonus* prevalence (e.g., brain) had the highest mean sumICH scores (Figure 6).

VHSV

Eleven of 233 Pacific herring (4.7%) were positive for VHSV which was isolated from 7 of 233 spleen-kidney pools and from 5 of 15 skin lesions. One fish had VHSV isolated from both the spleen-kidney pool and a skin lesion. Several lesions and alterations in blood chemistries were associated with VHSV infection (Tables 3 and 4). Among external lesions, fin base reddening was significantly associated with VHSV infection. Among chemistries, decreased plasma levels of albumin, ALP, and cholesterol were associated with VHSV infection (Table 4). Loss of albumin might have resulted from leakage from external lesions. Decreased cholesterol and ALP were probably secondary to decreased albumin, because albumin was highly correlated with cholesterol ($r = 0.895$) and ALP ($r = 0.587$) regardless of VHSV status.

Normal stomachs contained large numbers of eosinophilic granular leukocytes throughout the submucosa, but these cells did not extend into the adjacent muscularis or mucosa (Figure 2). In 53 fish, the submucosa also contained small to moderate numbers of lymphocytes and macrophages (Figure 2), and these infiltrates were significantly associated with VHSV infection (Table 3).

Sheets of mononuclear cells within gill arches were significantly associated with VHSV infection (Table 3). Gill arches normally contained scattered mononuclear cells that had densely basophilic nuclei and relatively scant basophilic cytoplasm (Figure 7). Not all cells could be identified, but they included mature inflammatory cells and hematopoietic cells in various stages of development. In 39 fish, these mononuclear cells were more abundant, but the cells did not alter tissue architecture (Figure 7).

Meningoencephalitis was significantly associated with VHSV infection (Table 3), and eosinophilic meningitis was marginally associated with VHSV infection ($P = 0.06$). In the brain, meninges usually contained 2 to 25 eosinophilic granular leukocytes in at least one 100× field, but normal meninges did not contain macrophages or lymphocytes. Forty-two fish had more than 25 eosinophilic granular leukocytes in at least one 100× field. In 7 fish, the meninges and perivascular space within the neuropile contained foci of inflammation (lymphocytes and macrophages) that were not associated with *Ichthyophonus* infection, but these foci of meningoencephalitis were <400 μm in diameter in all but one fish.

Focal hepatic necrosis was not common (6 fish affected) but was significantly associated with VHSV infection (Table 3). Broad bands of affected hepatocytes had hypereosinophilic cytoplasm and pyknotic, karyorrhectic, or karyolytic nuclei characteristic of coagulative necrosis

(Figures 4 and 5). By comparison, single cell hepatocellular necrosis was more common (16 fish affected) but was not significantly associated with VHSV infection. Individual necrotic (or apoptotic) cells had condensed hypereosinophilic cytoplasm and pyknotic nuclei. Necrotic (or apoptotic) cells were often surrounded by a pericellular clear space (Figure 5).

Focal intimal hyperplasia of arteriolar walls was relatively common and was scored in sections of intestine, skin and skeletal muscle, and spleen. In the intestine only, this lesion was significantly associated with VHSV infection (Table 3). Normal arteries and arterioles had a smooth intimal surface without valves (Figure 7). In some cases, however, the intima contained one or more foci of connective tissue that projected into the lumen from a narrow base in mild cases, and from a broad base in moderate cases (Figure 7). The origin of these foci is unknown, but they may have been sequelae to endothelial damage.

Gender-associated Lesions

Lesions significantly more frequent in ovaries included hyalinization of vessel walls and pigmented macrophage aggregates. By comparison, granulomatous inflammation was significantly more common in testes than in ovaries (Table 3). Except for one female with severe ovarian *Ichthyophonus*, germ cells were mature in all fish and lesions were not severe enough to have impaired spawning.

Gender differences were significant for several nongonadal lesions (Table 3). Myxosporeans in the gall bladder (*Ceratomyxa auerbachi*) were significantly more frequent in females. Males had a significantly greater frequency of severe intestinal mesenteric steatitis, renal proximal tubular epithelial vacuolation, and renal tubular dilation. Splenic *Ichthyophonus* prevalence was similar in males and females, but associated lesions were more likely to be severe in females. Isolation of VHSV was more frequent from males (7 of 116) than from females (4 of 117), but differences were not significant (chi-square test, 2×2 contingency table).

Intestinal mesenteric steatitis involved peritoneal fat throughout the mesenteries of the viscera. Lipid volume of adipocytes varied from moderately abundant to minimal. In moderate cases of steatitis, lipid volume was often less than the volume of adipocyte nuclei (Figure 7). Inflammatory infiltrates included macrophages, lymphocytes, and eosinophilic granular lymphocytes. All fish had at least some inflammatory cells within the peritoneal fat (Figure 7), but 19 males and 8 females had more than 30% of the volume of peritoneal fat infiltrated by inflammatory cells. The cause of these inflammatory infiltrates was not determined.

Proximal renal tubular epithelium was considered vacuolated if intracytoplasmic clear spaces were larger than adjacent nuclei. Kidneys from 9 males and one female contained vacuolated tubular epithelial cells; in only one case (a male) were more than 20% of the proximal tubular epithelial cells affected. Renal tubules were considered dilated when luminal diameter was

more than twice the thickness of tubular epithelial cells. Kidneys from 7 males and one female contained dilated tubules, but in no cases were more than 50% of the tubules dilated. Causes for these tubular changes are unknown. Although pansporoblasts of the renal myxosporean *Ortholinea orientalis* sometimes nearly filled archinephric ducts (Figure 7), only one of 44 cases was associated with dilated tubules; i.e., *Ortholinea orientalis* was not associated with dilated tubules.

In addition to these lesions, gender differences were significant for several plasma chemistries (Table 4). Compared to males, females had significantly lower values for albumin, chloride, cholesterol, CO_2, glucose, potassium, and total protein, and significantly higher values for ALP. Gender differences were not significant for other plasma chemistries.

Intraperitoneal Herring Worms (Anisakidae)

All 233 Pacific herring contained larval parasites of the family Anisakidae within their peritoneal cavities. No attempt was made to differentiate species (e.g., *Anisakis* vs. *Contracecum*), and parasite morphology and inflammatory response were consistent with previous descriptions (Hauck and May 1977). Herring worm numbers were significantly greater in females than in males, and numbers significantly increased with increasing severity of several lesions (Table 6). For example, fish with more severe hepatic cholangitis or biliary hyperplasia (Figure 4) had increased numbers of herring worms. Also, increased numbers of intraperitoneal Anisakidae were associated with increased scores for Anisakidae in the liver, intestine, skeletal muscle. Fish with renal interstitial cell necrosis had fewer herring worms than did fish without renal interstitial cell necrosis.

Other Potential Pathogens

No significant bacterial pathogens were isolated, and none of the blood smears had evidence of VEN. Ulcers often contained variable amounts of granulation tissue with a surface layer of filamentous bacteria; however, culture results indicated that the bacteria had not spread to the kidney.

Pacific herring had 10 other parasites, most of which were associated with few lesions. These parasites in descending order of prevalence included: (1) an intestinal coccidian (*Goussia* sp.?) that has not previously been described, 91%; (2) a coccidian in the liver, *Goussia* (*Eimeria*) *clupearum*, 61%; (3) a myxosporean in renal tubules, *Ortholinea orientalis*, 19%; (4) a myxosporean in the gall bladder, *Ceratomyxa auerbachi*, 19%; (5) branchial monogenetic trematodes *Gyrodactylus* spp., 13%; (6) branchial ciliated protozoans, probably *Trichodina* and *Cryptokaryon* spp., 12%); (7) renal intraductal protozoan, species unidentified, 11%; (8) branchial *Epitheliocystis*, 10%; (9) gastric intraluminal trematodes, e.g., Hemiuridae, 8.6%; and (10)

intestinal trematodes, e.g., *Lecithaster gibbosus*, 5.7%. Infestation with branchial and gastrointestinal parasites did not significantly alter plasma chemistry values or inflammatory changes.

Morphologic features and distribution of the intestinal coccidian were very similar to descriptions of *Goussia zarnowskii* in the three-spined stickleback (*Gasterosteus aculeatus*) (Jastrzebski and Komorowski 1990). In Pacific herring, the coccidians were common in small numbers throughout the intestine, including the intestinal cecae. Only 2 fish had more than 15 organisms per 400× field in several fields examined. In affected intestines, the surface of epithelial cells contained spherical to ovoid, basophilic organisms (Figure 3). Small forms of the parasite, about 8 µm in diameter and densely basophilic, were probably meronts or trophozoites. By comparison, larger forms of the organism, up to 15 µm in diameter and 20 µm long, were less intensely stained; some contained densely basophilic 1- to 2-µm-diameter spherical structures, whereas others contained eosinophilic granules that were 2 to 4 µm in diameter. The larger forms were probably microgamonts or microgametes. Oocysts were not present, and there was no inflammatory response to the forms that were present. Also, infections did not significantly alter plasma chemistry values.

Morphologic features and distribution of the hepatic coccidian were very similar to descriptions of *Goussia clupearum* in Atlantic herring (Morrison and Hawkins 1984). In Pacific herring, sporulated oocysts (about 18×12 µm) were the most abundant stage and were often in small clusters of 2 to 10 organisms, whereas unsporulated oocysts (about 35 µm in diameter) were usually solitary and rare (Figure 4). Severity scores were based almost entirely on numbers of foci of sporulated oocysts per 100× field: no parasites (score = 0); ≤2 foci (score = 1); >2 but ≤6 foci (score = 2); and >6 foci (score = 3). Despite the relatively large volume of hepatic parenchyma displaced by the parasites in severe cases, inflammation was minimal and severity of infestation did not significantly alter plasma chemistry values.

Diagnosis of the renal tubular myxosporean *Ortholinea orientalis* was less sensitive by histopathology (12 of 212, 5.7%) than by examination of kidney touch preparations (41 of 229, 18%). However, 3 cases diagnosed on histopathology were not diagnosed on touch preparations, resulting in a combined total prevalence of 19%. Pansporoblasts, the most common form, were roughly spherical, 60 to 80 µm in diameter, and were free in the lumen of the archinephric duct (Figure 7). Multiple nuclei within the pansporoblast were eccentric or polar, depending on the plane of section. Another parasite, an unidentified protozoan (ciliate?), was in the archinephric duct of 24 fish. The protozoa were unicellular and usually attached to the surface of ductular epithelial cells (Figure 7). They were 25 to 40 µm wide and 15 to 30 µm high.

For the renal myxosporean *Ortholinea orientalis*, the 5 most severely affected fish had plasma calcium levels significantly higher than other groups ($P < 0.001$, with significant Levene's test). The mean ±SE calcium value for the 5 most severely affected fish was 15.3 ± 2.0 mg/dL, whereas mean calcium values for groups of fish that were less severely affected ranged from 10.8

± 0.4 to 11.7 ± 0.14 mg/dL, and these differences were not significantly different. The proportion of fish with *Ortholinea orientalis* infection was significantly higher in fish with renal *Ichthyophonus* (chi-square test for homogeneity). Infection with the renal intraductal protozoan was not significantly associated with any changes in plasma chemistries or any other renal lesions.

The gall bladder sometimes contained large numbers of the myxosporean *Ceratomyxa auerbachi* (Figure 3). Most common were immature spores that were roughly spherical, multicellular, and 15 to 30 μm in diameter with one to 6 nuclei. Occasionally, well-developed spores had 2 polar capsules. Less common were spindle-shaped trophozoites that were 50 to 80 μm long, 15 to 20 μm in diameter, and had pale eosinophilic to vacuolated cytoplasm. Trophozoites often contained one or 2 spherical structures, 7 to 10 μm in diameter, that stained intensely eosinophilic. Severe infestations sometimes had mild mononuclear inflammation in the lamina propria of the gall bladder, but infestations were not significantly associated with liver lesions or with changes in plasma chemistries.

Age-associated Changes

The most consistent age-related change was increased severity of pigmented macrophage aggregates in older fish. Indeed, age-related changes were significant in all organs in which pigmented macrophage aggregates were scored: exocrine pancreas, liver, ovary, spleen, and trunk kidney (Table 1). Lesion scores that significantly increased with age included meningoencephalitis, epicarditis, renal tubular epithelial vacuolation, pancreatic zymogen granule depletion, and splenic ellipsoid hyalinization (Table 1). Interestingly, in the liver, scores for increased granulomatous inflammation were significantly associated with decreased age.

Among common parasites, *Ichthyophonus*, *Goussia clupearum*, and *Ortholinea orientalis* were not significantly associated with age (chi-square test for homogeneity). By comparison, *Ceratomyxa auerbachi* was significantly more frequent in older fish, and the renal intraductal protozoan was more common in younger fish (Figure 8). The number of positive VHSV cases was too small for statistical analysis of age distribution, but the 11 positive cases were distributed among 2 3-year-olds, 3 4-year-olds, 3 6-year-olds, one 9-year-old, and 2 10-year-olds. In general, VHSV-positive cases were over-represented in younger and older fish in the sample; for example, the 1988 year class (6-yr-old fish) comprised 60% of the sample but only 27% of the VHSV-positive cases.

Plasma chemistries

As hold time increased, plasma potassium and CO_2 significantly increased, but plasma glucose significantly decreased (Table 7). Changes in several other plasma chemistries were not as significant in relation to hold time ($|r| < 0.25$). A complicating factor was that hold time was significantly longer on the last day of sampling when most fish had completed spawning.

Therefore, many of the marginally significant changes might have been related to spawning condition rather than hold time. For example, using multifactor regression, hold time was not a significant predictor of albumin levels even though their values were significantly correlated in univariate analysis.

Among enzymes, AST and CPK values were most variable, and differences in lesion scores (particularly *Ichthyophonus*) could be discerned on the basis of AST and CPK (Tables 1). Variability of ALP was intermediate, and only rarely could lesions scores be differentiated on the basis of ALP values. Variability of ALT and GGT was minimal and measured values were never greater than 17 U/L (Table 8). However, correlations of $\log_e$ ALT with total bilirubin ($r = 0.493$) and gonad weight ($r = 0.335$) were highly significant..

Albumin and total protein were unusually low (Table 8) when compared to published values for other species (McDonald and Milligan 1992), and albumin was particularly low after the fish were spawned out (Figure 9). Total protein values derived from refractometer readings were consistently greater than values derived from the biuret method (mean difference = 3.1 g/dL, range = 1.6 - 4.4 g/dL); therefore, only values derived from the biuret method were used in this report.

Postspawning fish commonly had clear high-protein fluid in the peritoneal cavity (ascites). Forty-three fish had 0.1 to 2.5 mL of ascites, and no fish with gonad weight greater than 5 g had ascites (Figure 9). Ascites was more frequent in males (26 of 116, 22%) than in females (17 of 116, 17%), but differences were not significant (chi-square test, 2 × 2 contingency table). Fish with ascites had albumin levels that varied from 0.0 to 0.6 g/dL, and albumin levels in fish without ascites ranged from 0.0 to 1.1 g/dL.

Multiple regression analysis was used to model a multifactor ANOVA, examining the relationships between the dependent variable albumin and 3 variables (focal skin reddening, splenic congestion, and VHSV). Iris reddening, which was significant in the univariate analysis, was left out of the regression because fewer cases were scored on this variable, contributing to a loss of 19 cases in the analysis. Based on the responses from 205 fish, 7 factors were entered in the final model (gender, gonad weight, hold time, length, focal skin reddening, splenic congestion, and VHSV); the adjusted r^2 was 0.38. A stepwise regression equation derived from significant factors only was used to quantify the contribution of each variable to albumin levels (g/dL). The constant (0.21 g/dL) is altered as follows:

gender male =	+0.114
gender female =	+0.000
gonad weight (g) =	+0.0045×(gonad wt)
VHSV-negative =	+0.047
VHSV-positive =	−0.047
focal skin reddening, none =	+0.098

focal skin reddening, mild =	−0.006
focal skin reddening, moderate/severe =	−0.104
splenic congestion, none =	+0.046
splenic congestion, mild =	−0.008
splenic congestion, moderate/severe =	−0.038

For example, a male (+0.114) with a gonad weight of 10 g (+0.045) that was VHSV negative (+0.047) and had no focal skin reddening (+0.098) and mild splenic congestion (−0.008) would be expected to have a plasma albumin level of 0.51 g/dL. The predicted plasma albumin level in a similar male with moderate focal skin reddening would decrease to 0.30 g/dL.

Like albumin, scores for several lesions and other variables could be differentiated on the basis of PCV, and PCV was significantly associated with several plasma chemistries (Tables 1 and 7). Multiple regression analysis was used to model a multifactor ANOVA, examining the relationships between PCV and 7 variables. Based on the responses from 186 fish, 12 factors were entered in the final model (gender, gonad weight, hold time, length, osmolality, focal skin reddening, splenic *Ichthyophonus*, renal hematopoietic cells, hepatic lipidosis, cardiac thrombosis, gastric trematodes, and VHSV); the adjusted r^2 was 0.29. Because of the potential that dehydration could effect PCV, osmolality was added as a controlling variable. A stepwise regression equation derived from significant factors only was used to quantify the contribution of each variable to PCV (%). The constant (51.14 %) is altered as follows:

gender male =	+2.30
gender female =	+0.00
gonad weight (g)=	+0.1513×(gonad wt)
osmolality (mOsm/kg) =	−0.0433×(osmolality)
hepatic lipidosis, none =	+1.49
hepatic lipidosis, mild =	−0.71
hepatic lipidosis, moderate/severe =	−0.77
splenic *Ichthyophonus*, none =	+1.50
splenic *Ichthyophonus*, mild =	−2.43
splenic Ichthyophonus, moderate/severe =	−0.92
renal hematopoietic cells, none =	−2.29
renal hematopoietic cells, mild =	+1.63
renal hematopoietic cells, moderate =	+0.67
gastric trematodes, none =	+1.87
gastric trematodes, mild/moderate =	−1.87

For example, a male (+2.30) with a gonad weight of 10 g (+1.51), osmolality of 425 mOsm/kg (−18.40), no hepatic lipidosis (+1.49), no splenic *Ichthyophonus* (+1.50), mild renal hematopoietic cells (+1.63), and mild gastric trematodes (−1.87) would be expected to have a

PCV of 39.3%. By comparison, a similar male with no renal hematopoietic cells and mild splenic *Ichthyophonus* would have a predicted PCV of 31.5%.

Annual Trends in Spawning Biomass and Pathogen Prevalence

Sample prevalence of *Ichthyophonus* in this study was twice that of previous years (Table 9). During the damage assessment phase of study from 1989 through 1992, and disease studies in 1993 (Meyers et al. 1994), prevalence of *Ichthyophonus* in Pacific herring sampled from PWS was never more than 15%. By comparison, prevalence of *Goussia clupearum* has remained fairly constant between 41 and 63%, and *Ortholinea orientalis* prevalence has not exceeded 17%. The slight increase in *Ortholinea orientalis* prevalence in this study (19%) was probably at least partly due to increased efficiency of diagnosis when touch preparations were examined; previous prevalence data were derived from histopathology only. Prevalence of VHSV and other parasites was not determined in previous studies because appropriate tissues were not examined.

DISCUSSION

Ichthyophonus hoferi

Ichthyophonus was the major cause of morbidity in Pacific herring in PWS during spawning in 1994, with nearly 30% of the fish infected. *Ichthyophonus* has not previously been described as a major cause of mortality in Pacific herring, but in Atlantic herring several epidemics of *Ichthyophonus* have been linked to population decline (Fish 1934, Sindermann 1958). Indeed, *Ichthyophonus hoferi* is the most commonly reported and most severe marine fungal pathogen, and "this disease may be the most important single limiting factor to population growth of herring in the western North Atlantic" (Sindermann 1970). Outbreaks in Atlantic herring tend to begin during biomass peaks, outbreaks usually last 2 to 3 years, and recovery often takes more than 3 years (Sindermann 1970). In Pacific herring in PWS, peak biomass in 1989 did not result in a major *Ichthyophonus* outbreak, but a predicted record biomass in 1993 was followed by an *Ichthyophonus* outbreak in 1994. Previous declines in Pacific herring biomass have been recorded in PWS, but these were attributed to poor year-class recruitment and over-fishing (Rounsefell and Dahlgren 1932). In Atlantic herring in the Gulf of Maine, *Ichthyophonus* caused population declines in 1931 and 1947, and anecdotal evidence was strong for *Ichthyophonus* as the major cause of population declines in 1898 and 1916 (Fish 1934, Sindermann 1965). From 1898 to 1947, outbreaks occurred about every 16 years and this trend held for 4 cycles; however, no *Ichthyophonus* outbreaks have been documented in the Gulf of Maine since 1947. Sporadic but significant *Ichthyophonus* outbreaks have also been described in the Gulf of St. Lawrence (Sindermann 1970).

Some features of the *Ichthyophonus* epidemic in PWS Pacific herring were different from those described in wild Atlantic herring. For example, Atlantic herring with severe infections often had gross lesions in the muscle described as "rough or granulomatous skin" or "sandpaper effect" (Post 1987); associated ulcers have been termed "pepper effect," partly as a result of pigment deposition in the lesions (Fish 1934). By comparison, Pacific herring had no external lesions directly associated with *Ichthyophonus*, and microscopic lesions in the skin and skeletal muscles were usually mild. Further, Pacific herring had no pigment associated with *Ichthyophonus* resting spores. Another difference was that epidemics in Atlantic herring were always characterized by large numbers of moribund and dead fish in shallow areas (Fish 1934, Sindermann 1958), whereas there were no confirmed reports of dead fish in PWS. Not all features of *Ichthyophonus* were different in Atlantic and Pacific herring; multifocal to coalescing granulomas in internal organs of PWS Pacific herring were similar to the descriptions of gross and histologic lesions reported in Atlantic herring.

The epidemiology of *Ichthyophonus* infection in Pacific herring in PWS is still unclear with only 7 samples in 6 years from 1989 through 1994. Many questions remain unanswered: (1) what is the latency period between *Ichthyophonus* exposure and overt signs of disease? (2) when *Ichthyophonus* is diagnosed histologically, how long will the affected fish live? (3) can a fish, once infected, initiate a successful immune response and overcome the disease, or are all infected fish destined to die? and (4) does the prevalence of *Ichthyophonus* in a population change significantly over a period of weeks, months, or years? Between October 1990 and April 1991, prevalence of *Ichthyophonus* in PWS decreased from 15% to 5%; by October 1991, prevalence had decreased to 2% and remained at less that 6% in 1992 and 1993 samples. Indeed, *Ichthyophonus* prevalence remained at 5% in 1993 despite significant population decline (Meyers et al. 1994). The large spike in *Ichthyophonus* prevalence in this study (29%) was unexpected, but was consistent with infection levels of about 25% described in epidemics affecting Atlantic herring (Sindermann 1970). Sindermann (1970) stated that endemic levels were about 1%, lower than any samples from Pacific herring in PWS, but methods that Sindermann used to determine prevalence were not clearly described. Method of diagnosis can make a significant difference in the number of positive cases identified (Holst 1994), and in our study, diagnosis using only gross examination would have resulted in underestimation of the number of infected fish.

In Pacific herring from our study, CPK and AST values could be used to differentiate *Ichthyophonus* lesion scores in nearly every organ. Multifactor regression techniques identified significant associations for the brain with CPK and for the kidney with AST. Creatine phosphokinase is a dimeric enzyme with isoenzyme types CK_1 (BB, brain), CK_2 (MB, heart), and CK_3 (MM, skeletal muscle). In mammals, the brain form of CPK is not found in plasma, even during neurologic disease (Duncan and Prasse 1986); therefore, the finding that brain *Ichthyophonus* status was the best predictor for increased CPK in our study was unexpected. Brain *Ichthyophonus* was uncommon, but the high mean sumICH score for fish with brain *Ichthyophonus* (Figure 6) provided evidence that the fungus was disseminated when it appeared in the brain. That is, if *Ichthyophonus* was disseminated sufficiently that it affected the brain, then

the fish probably also had muscle *Ichthyophonus* severe enough to increase CPK. Further, 79% of muscle *Ichthyophonus* cases were mild. Although the damage caused by these muscle lesions was probably not sufficient to increase CPK, the number of mild *Ichthyophonus* lesion scores was sufficient to influence results of the multifactor regression. Alternatively, the brain form of CPK might be released during neurologic disease in Pacific herring, but isoenzymes were not determined. Isoenzyme analysis is planned for 1995 studies. Also, note that multifactor analysis treats lesion scores as categories, and this type of analysis has limited ability to account for the fact that enzyme levels in fish with severe lesions should be higher than enzyme levels in fish with mild lesions. Two things might improve the ability of statistical analysis to identify the source of enzymes: (1) increase the number of positive fish analyzed, and (2) repeat the analysis using a continuous value that measures the severity of *Ichthyophonus* in each organ. Additional study in 1995 will increase the number of *Ichthyophonus*-infected fish studied. Deriving a continuous variable for the *Ichthyophonus* response could be done with more involved techniques such as point-count morphometry, but morphometric techniques are expensive and are not planned in 1995 studies.

For AST in mammals, lesions in liver, muscle, and blood-rich organs are most highly associated with increased enzyme levels (Duncan and Prasse 1986). For Pacific herring, renal *Ichthyophonus* status was significant in all regressions. Because kidney is a blood-rich organ, this result was not completely unexpected. However, following the same line of reasoning, the values for the spleen and heart should have remained significant, but they did not. As additional *Ichthyophonus*-infected fish are studied, sources of AST might be better defined. Also, quantitative techniques might increase the power of multifactor regression.

The effects of *Ichthyophonus* infection on plasma chemistries have not previously been described in natural epidemics. In laboratory-exposed rainbow trout (*Oncorhynchus mykiss*), *Ichthyophonus* infection was associated with anemia and leukopenia, but did not change plasma chloride, creatinine, glucose, osmolarity, potassium, total protein, sodium, or T4 (Rand and Cone 1990); enzymes CPK and AST were not measured. In addition to increased CPK and AST in this study, *Ichthyophonus* infection was significantly associated with anemia and variable plasma protein levels; white blood cells were not counted. Based on the equation derived from multifactor analysis, a fish with mild splenic *Ichthyophonus* would be predicted to have a PCV that was 4% less than a similar fish with no splenic *Ichthyophonus*.

VHSV

The North American strain of VHSV was the second major cause of morbidity in Pacific herring in PWS during spawning in 1994, with nearly 5% of the fish infected. Fish from which VHSV was isolated had significantly associated gross lesions as well as microscopic lesions in the gills, liver, stomach, arteries, and heart. Most lesions were consistent with a disseminated endotheliotrophic virus, and lesions such as coagulative necrosis in the liver have been attributed

to VHSV in natural and laboratory infections in rainbow trout (Amlacher et al. 1980, Wolf 1988b). Also, VHSV in Pacific herring was significantly associated with focal skin reddening ($P = 0.03$, chi-square test for homogeneity), but the minimum expected cell frequency was <1. The low minimum expected cell frequency resulted from having only 11 positive fish out of 233 fish sampled. Because the VHSV outbreak might have been nearly over in 1994, opportunities to confirm association of lesions with VHSV by further field study may be limited. Therefore, laboratory study is planned to fulfill Koch's postulates and further define and confirm lesion association.

The only other published report of VHSV in Pacific herring was from fish sampled in PWS in 1993 (Meyers et al. 1994). In that study, VHSV was unexpectedly isolated from pooled samples, and lesions could not be directly linked to VHSV isolation. However, Meyers et al. (1994) postulated that several lesions were associated with VHSV: subdermal and renal hemorrhages, kidney tubule degeneration, and active reticuloendothelial cell foci in the kidneys. Also, active reticuloendothelial cell foci in the liver were associated with hepatocellular necrosis. In this study, we confirmed an association of VHSV with fin base reddening and focal coagulative hepatic necrosis, and we had some evidence of association of VHSV with ulcers (i.e., severe focal skin reddening). Association of VHSV with renal hemorrhage or kidney tubule degeneration could not be confirmed. In this study, "active reticuloendothelial cells" were classified as either pigmented macrophage aggregates or granulomatous inflammation, and neither was significantly related to VHSV in the liver or kidney. However, infiltrates of lymphocytes or macrophages in the gastric submucosa, gill arches, and brain were significantly associated with VHSV infection. In a study of PWS Pacific herring from 1992, granulomatous inflammation was associated with decreased reproductive success (Kocan et al. In Press), but based on our results we cannot attribute these lesions to VHSV.

Population fluctuations are well-documented in Pacific herring, but only one other population decline has been attributed to disease. 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 dying fish came to the surface and could, while still alive, be picked up by gulls or by hand." Mortality involved pre- and post-spawners, and fish continued to be lethargic and school in shallow water near shore until mid-May (Tester 1942). Diagnostic examination included gross necropsy, bacteriology, blood smears, and parasite screen, but no significant pathogens were found. Based on this level of diagnostic detail, *Ichthyophonus* can essentially be ruled out as the cause of mortality in 1942, but many features were similar to the current epidemic in PWS. Both outbreaks had lethargic fish, some of which had reddening of the fins, and both outbreaks followed an excellent fishing year. The epidemic near Vancouver Island involved a dominant 1938 year class (4-yr-olds), whereas the PWS epidemic involved a dominant 1988 year class (5-yr-olds). As a difference, the Vancouver Island outbreak had large numbers of dead fish, whereas dead fish were not reported in the PWS epidemic. One other disease, VEN, has been reported to cause significant mortality in juvenile Pacific herring when such year classes are strong. However, VEN has not been associated with

significant decline in population biomass (Meyers et al. 1986), and PWS fish in 1994 had no evidence of VEN.

As with *Ichthyophonus*, several questions about the pathogenesis of VHSV cannot be answered without the aid of laboratory study. Is exposure to VHSV sufficient to cause disease, or do many fish carry the virus and express VHSV and disease only when subjected to stress? A related virus, infectious hematopoietic virus (also in the family Rhabdoviridae), is commonly carried by salmonids. Disease is only a serious problem in fingerlings, and virus is expressed in survivors only when fish are spawning (Wolf 1988a). In a study of Pacific herring at the National Marine Fisheries Service Laboratory in Auke Bay, Alaska, VHSV was expressed in a dose-dependent manner after 17 days of exposure to weathered crude oil (Mark Carls, pers. commun.). Although the VHSV status of these fish before the study began was unknown, the study provided evidence that oil can act as a stressor that activates VHSV. Other questions of interest include: How is VHSV transmitted? What is the incubation time? Once virus is expressed, can fish mount a successful immune response and overcome the disease? And, does expression of VHSV cycle seasonally? Study proposed for fiscal year 1996 will investigate VHSV status in Pacific herring sampled in October or November.

Other Potential Pathogens

A few comprehensive reports are available on the prevalence of parasites in Pacific herring, and their potential role in stock identification (Arthur and Arai 1980, Moser and Hsieh 1992). The purpose of our study was not to repeat these studies, but to determine which of the common parasites of Pacific herring in PWS could potentially contribute to population decline. More than 30 species of parasites have been described from Pacific herring (Arthur and Arai 1980). In our study, 10 parasites occurred in prevalences sufficient to study their role in disease and population decline. Two criteria were used to determine if a parasite caused significant damage to the host: (1) Was the parasite associated with histopathologic damage, particularly inflammation? and (2) Was infection with the parasite associated with alterations in plasma chemistries? Using these criteria, *Ichthyophonus* clearly caused significant damage to herring because it was associated with histopathologic lesions and increases in plasma AST and CPK. Linkage of damage to infections by other parasite was less clear.

The intraductal renal myxosporean *Ortholinea orientalis* was not associated with morphologic lesions, nor was there metastatic calcification, but fish with large numbers of organisms had elevated plasma calcium. Because the kidney is one organ that excretes calcium (Dacke 1979), large numbers of organisms might have impaired calcium excretion. The relation of intraductal parasites and calcium levels has not previously been described, and this effect would need to be confirmed with controlled laboratory study.

Gender-associated Lesions

Lower plasma albumin levels in females than in males could partly be explained by vitellogenin synthesis in females. At the nuclear level in the hepatocyte, estradiol activates the vitellogenin gene, but production of albumin is depressed (Mommsen and Walsh 1988). Low plasma albumin is commonly associated with ascites in mammals, and Pacific herring with ascites tended to have lower albumin levels than fish without ascites; however, females were not more likely to develop ascites than were males. Several other plasma chemistries and lesion scores had significant gender differences, but little information is available to explain these differences in Pacific herring.

Age-associated Lesions

Based on the results of this study, we could critically evaluate only one hypothesis regarding the link between the oil spill and disease in 1994. Fish that were hatched or were yearlings in 1989 at the time of the spill (1988 and 1989 year classes) might have incurred permanent damage to their ability to fight disease (i.e., irreversible immunosuppression). Under normal growth conditions, minor deficiencies in their immune system might have been insignificant. However, disease might have become a serious problem when fish experienced additional stress upon first spawning (1992 and 1993). Stress is well-documented as a cause of immunosuppression, but stress-induced changes usually are reversible if the fish survives (Anderson 1990). To address the hypothesis of age-related immunosuppression, we found that several lesions were significantly associated with age (e.g., pigmented macrophage aggregates), but nearly all these lesions were more severe in older fish (i.e., fish hatched before 1988). Also, among VHSV, *Ichthyophonus*, and ten other common parasites, none were more prevalent in the 1988 and 1989 year classes than in the entire sampled population. Annual age-weight-length analysis by the Alaska Department of Fish and Game has documented that the population has decreased in the absence of abnormal changes in age distribution (Fritz Funk, unpubl. data). Therefore, the weight of evidence suggests that the disease outbreak in PWS was not a result of permanent immune suppression caused by hydrocarbon exposure when fish were larvae or yearlings.

We did not have enough cases of VHSV to statistically link age to disease, but the trend was towards a higher proportion of VHSV in fish <5 years old or >6 years old. Prevalence of VHSV was not determined before 1994 in PWS, but prevalence might have been higher in 1992 or 1993 because fish in the dominant 1988 years class were younger during those years.

Alterations in Plasma Chemistries

Analysis of plasma chemistry values was inexpensive and provided useful information for evaluating health of Pacific herring in PWS. However, interpretation of results was limited by lack of reference values. In the only published study of normal plasma enzyme values in Pacific herring (Márquez 1976), analysis of electrolytes and other nonenzyme chemistries was not included. Márquez (1976) captured 5 to 12 Pacific herring by angling, held the fish for 12 hours, and then drew blood to analyze for plasma enzymes at 30° C. His mean values for CPK (2948 U/L) and AST (1778 U/L) were more than twice the maximum values of normal ranges established in our study (Table 8). Differences between the 2 studies resulted from Márquez performing analyses at 30° C instead of the 25° C of our study. Also, the 12-hour hold time might have been sufficient for capture damage to increase enzyme levels. Hold time was <4 hours in our study, and hold time was not significantly correlated with plasma CPK or AST.

In future studies, plasma values from Pacific herring in Sitka Sound will provide reference values for comparison with this study. Pacific herring populations are strong in Sitka Sound, and population trends there were similar to PWS before the spill. Also, study of Pacific herring in peak condition is proposed for October, 1995. Reference values from fish in peak condition are needed to better interpret changes associated with spawning.

Interesting findings in plasma chemistry values included unusually low albumin levels, unusually high osmolality, and several changes associated with the spawning process. Altered plasma chemistry values have been associated with spawning in other fish species, but abnormalities were transient (McDonald and Milligan 1992). Low albumin levels in Pacific herring were associated with ascites in both males and females, but not all fish with low albumin levels had ascites. Because albumin levels were significantly decreased only at the end of spawning, and ascites occurred only in spawned out fish, development of ascites was probably related to physiologic changes at the end of spawning.

Higher total plasma protein values determined using a refractometer were higher than values determined by colorimetry. Similar differences have been documented in other fish species (Hunn and Greer 1990, Hunn et al. 1992), but the molecular cause for this difference has not been determined. Because values derived from colorimetry analysis are more accurate, future analysis of total plasma protein in Pacific herring will use only the colorimetry technique.

Plasma glucose, CO_2, and potassium were useful markers of the effects of hold time between capture and necropsy. As hold time increased, plasma glucose decreased, and plasma CO_2 and potassium increased. The increase in plasma CO_2 was indicative of respiratory acidosis, and potassium levels are expected to increase during acidemia (McDonald and Milligan 1992). Decreased glucose levels may have been associated with increased anaerobic glycolysis, but lactate levels were not determined. Normally, capture stress results in hyperglycemia (Hopkins

and Cech 1992), but lack of hepatocellular glycogen in Pacific herring during spawning might have limited the ability of the liver to increase plasma glucose levels. Future analysis of Pacific herring plasma will include lactate levels to determine the relation of hold time to metabolic acidosis and other plasma chemistry values.

CONCLUSIONS

Disease was probably the primary force driving population decline in 1994. As evidence, consider the assumption that all fish infected with VHSV and *Ichthyophonus* in April 1994 would have died within 6 months. This assumption is based on a combination of disease prevalence, associated lesions, and comparisons with published reports. If disease was driving population decline, then population biomass should have declined about 35% by October, 1994. Based on the best population estimates, Pacific herring biomass in PWS dropped from 1.8×10^7 kg in April 1994, to a record low of 1.1×10^7 kg in October 1994: a 40% decline. Hence, a single but thorough disease survey in April 1994 was sufficient to explain nearly all the population decline in 1994. No other variables—food availability, predation, water temperature, currents, or recruitment—were needed to explain this significant decline. These other variables may prove more important during population recovery, but disease may continue to be a significant factor limiting recovery. Pacific herring populations in PWS were not healthy in 1994, and until Pacific herring populations recover, continued study of herring morbidity is recommended. Continued research, to included study of fish from a reference site and during peak condition, is important for confirming the findings in this study and determining the role of seasonal cycles in disease expression.

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Table 1. Lesion severity (number of fish classified in each lesion score) and prevalence (% of sample having lesion score >0) in mature Pacific herring sampled from Prince William Sound, Alaska, during spawning, 1994. Lesions were scored as none (0), mild (1), moderate (2), or severe (3). Age, hold time, and blood values were compared for groups of fish based on lesion scores using one-way analysis of variance and Tukey's multiple comparison procedure. Significant trends were based on rank order of mean responses for fish groups classified by lesion scores. Compared to fish with the lowest lesion score, mean response for the fish group with the highest lesion score was significantly higher (↑), lower (↓), or there was no significant trend (NT) in the rank order. For comparisons in which Levene's test for equality of variance was significant (*), only comparisons with $P \leq 0.010$ are shown.

Organ - lesion or tissue type	Lesion score				Sample prevalence	Significant trends (*P*-value)
	0	1	2	3		
External gross lesions						
caudal fin fraying (n = 233)	39	177	15	2	83	↑[a]- calcium (0.005*), osmolality (0.008)
caudal fin reddening (n = 233)	127	95	9	2	45	↓[b]- ALP (0.022)
fin base reddening (n = 233)	112	89	29	3	51	↑- hold time (<0.001), osmolality (0.005) ↓- chloride (0.050)
iris reddening (n = 205)	100	100	5	0	51	↑- albumin (0.003), ALP (<0.001), Calcium (<0.001), chloride (<0.001), cholesterol (0.017), osmolality (<0.001), phosphorus (<0.001*), potassium (<0.001*), total protein (<0.001) ↓- CO_2 (0.006)

Organ - lesion or tissue type	Lesion score				Sample prevalence	Significant trends (*P*-value)
	0	1	2	3		
External gross lesions						
skin reddening, focal (includes ulcers; n = 227)	148	60	12	7	35	↑- chloride (0.002) ↓- albumin (<0.001), ALP (<0.001), calcium (0.034), cholesterol (<0.001*), total protein (<0.001*) NT- PCV (0.043)
Brain microscopic lesions (n = 212)						
Ichthyophonus	195	16	1	0	8.0	↑- AST (0.002*), $\log_e$ AST (<0.001), CPK (<0.001), $\log_e$ CPK (<0.001), potassium (0.021), total protein (0.023) ↓- PCV (0.049)
meningeal eosinophilic granular leukocytes	28	142	39	3	87	NT°- GGT (0.007)
meningoencephalitis	205	6	1	0	3.3	↑- age (0.003*)
Gall bladder microscopic lesions (n = 171)						
myxosporean (*Ceratomyxa auerbachi*)	139	31	1	0	19	↑- age (0.005*)
Gill microscopic lesions (n = 212)						
ciliated protozoa (e.g., *Trichodina* spp.)	187	25	0	0	12	↓- chloride (0.035)

Organ - lesion or tissue type	Lesion score				Sample prevalence	Significant trends (*P*-value)
	0	1	2	3		
Gill microscopic lesions (n = 212)						
Epitheliocystis	190	20	2	0	10	none
foreign body granuloma	193	19	0	0	9.0	none
gill arch inflammation or hematopoiesis	1	161	39	0	100	↓- albumin (0.003), ALP (0.004), calcium (0.011), cholesterol (0.009), osmolality (0.048)
Ichthyophonus	185	18	5	4	13	NT- AST (0.003*), $\log_e$ AST (0.003), CPK (<0.001*), $\log_e$ CPK (<0.001), total protein (0.001)
lamellar hyperplasia	204	7	1	0	3.8	↑- glucose (0.048)
monogenetic trematodes (e.g., *Gyrodactylus* spp.)	185	27	0	0	13	none
Gonad - female (n = 110) microscopic lesions						
eosinophilic granular leukocytes	38	53	19	0	65	NT - phosphorus (0.006)
granulomatous inflammation	108	1	1	0	1.8	none
hyalinization of vessel walls	43	57	10	0	61	none
Ichthyophonus	108	2	0	0	1.8	none

Organ - lesion or tissue type	Lesion score				Sample prevalence	Significant trends (*P*-value)
	0	1	2	3		
Gonad - female (n = 110) microscopic lesions						
macrophage aggregates (pigmented)	40	68	2	0	64	↑- age (<0.001)
Gonad - male (n = 102) microscopic lesions						
eosinophilic granular leukocytes	45	34	22	1	56	↓ - calcium (0.051)
granulomatous inflammation	93	8	0	1	8.8	none
hyalinization of vessel walls	102	0	0	0	0.0	ND[d]
Ichthyophonus	101	0	0	1	1.0	ND
macrophage aggregates (pigmented)	99	3	0	0	2.9	none
spermatocyte numbers (3 = abundant)	9	23	30	40	NA[e]	↑- glucose (<0.001), osmolality (<0.001), total protein (<0.001*) NT- albumin (0.001), ALP (0.001), chloride (0.021)
Heart microscopic lesions (n = 210)						
epicarditis	105	105	0	0	50	↑- age (0.017)
Ichthyophonus	172	14	12	12	18	↑- CPK (<0.001*), $\log_e$ CPK (<0.001) NT- AST (<0.001*), $\log_e$ AST (<0.001*), total protein (0.001)

Organ - lesion or tissue type	Lesion score				Sample prevalence	Significant trends (*P*-value)
	0	1	2	3		
Heart microscopic lesions (n = 210)						
leukocytes, focal, parenchymal	107	103	0	0	49	↓- glucose (0.020), total protein (0.009)
mineralization, myocardial	208	2	0	0	0.9	↑- ALT (0.003*)
thrombosis	193	16	0	0	8.1	↑- AST (<0.001*), $\log_e$ AST (0.008*), CPK (<0.001*), $\log_e$ CPK (0.004) ↓- PCV (0.026)
Intestine and intestinal cecae, microscopic lesions (n = 211)						
Anisakidae	51	137	23	0	76	none
arteriolar hyperplasia, focal, intimal	133	76	2	0	37	none
Coccidian, intraepithelial (*Goussia* sp.?)	19	190	2	0	91	↓- osmolality (0.028)
eosinophilic granular leukocytes, submucosal	0	202	9	0	100	↓- ALP (0.033)
foreign body granuloma	133	78	0	0	37	none
Ichthyophonus	193	17	1	0	8.5	↑- $\log_e$ AST (0.031), CPK (<0.001*), $\log_e$ CPK (0.008)
steatitis	0	184	27	0	100	↑- AST (<0.001*), $\log_e$ AST (0.002*)

Organ - lesion or tissue type	Lesion score				Sample prevalence	Significant trends (*P*-value)
	0	1	2	3		
Intestine and intestinal cecae, microscopic lesions (n = 211)						
trematodes (e.g., *Lecithaster gibbosus*), cecal	199	9	3	0	5.7	none
Kidney (trunk) microscopic lesions (n = 212)						
congestion, interstitial, vascular	156	55	1	0	26	↑- AST (0.008*), $\log_e$ AST (0.002)
granulomatous inflammation	139	43	15	15	34	NT- age (0.004)
hematopoietic cells (relative area)	16	156	40	0	92	NT- ALP (0.016), cholesterol (0.034)
Ichthyophonus	169	21	13	9	20	↑- $\log_e$ AST (0.028), CPK (<0.001*), $\log_e$ CPK (0.002) NT- total protein (<0.001*)
interstitial cell necrosis	194	18	0	0	8.5	none
intratubular mineral, with associated tubular hyperplasia	206	4	2	0	2.8	none
intraductal protozoan	188	23	1	0	11	↓- age (0.031)
macrophage aggregates, pigmented	0	81	110	21	100	↑- age (<0.001) NT- glucose (0.023)
Ortholinea orientalis (intraductal myxosporean)	200	6	4	2	5.7	↑- calcium (<0.001*)

Organ - lesion or tissue type	Lesion score				Sample prevalence	Significant trends (*P*-value)
	0	1	2	3		
Kidney (trunk) microscopic lesions (n = 212)						
tubular dilation (of lumen)	204	8	0	0	3.8	none
tubular epithelial vacuolation	202	9	1	0	4.7	↑- age (0.044), albumin (0.004), calcium (0.002), chloride (0.011), cholesterol (0.026), osmolality (0.004), phosphorus (0.035)
Liver microscopic lesions (n = 212)						
cholangitis or biliary hyperplasia	191	20	1	0	9.9	↓- chloride (0.004*)
Liver microscopic lesions (n = 212)						
coccidiosis (*Goussia* [*Eimeria*] *clupearum*)	83	58	43	28	61	none
eosinophilic granular leukocytes	15	187	10	0	93	↓- CO_2 (0.009) NT- AST (0.001*), $\log_e$ AST (0.003*)
glycogen depletion	0	0	2	210	100	none
granulomatous inflammation	131	57	10	14	38	↑- $\log_e$ AST (0.018), potassium (0.006) ↓- age (0.028)
Ichthyophonus	178	14	11	9	16	↑- AST (<0.001*), $\log_e$ AST (>0.001*) NT- CPK (<0.001*), $\log_e$ CPK (<0.001)
leukocytes, focal, parenchymal	119	93	0	0	44	↑- albumin (0.015), cholesterol (<0.001), glucose (0.021), phosphorus (0.003*)

Organ - lesion or tissue type	Lesion score				Sample prevalence	Significant trends (*P*-value)
	0	1	2	3		
Liver microscopic lesions (n = 212)						
lipidosis, hepatocellular	145	49	15	3	32	↑- AST (0.003*), $\log_e$ AST (0.010*), CPK (0.011), osmolality (<0.001), phosphorus (<0.001*), potassium (<0.001*) ↓- glucose (0.012), PCV (<0.001*) NT- ALP (0.039), cholesterol (0.019)
macrophage aggregates, pigmented	0	85	95	32	100	↑- age (<0.001*)
necrosis, focal	206	3	2	1	2.8	none
necrosis, hepatocellular, single cell	196	11	3	2	7.5	none
Pancreas, exocrine, microscopic lesions						
macrophage aggregates, pigmented	78	131	2	0	63	↑- age (0.018) ↓- ALT (0.006), $\log_e$ALT (0.007)
zymogen granule depletion	0	4	70	137	100	↑- age (0.045)
Skin and skeletal muscle, microscopic lesions (n = 212)						
Anisakidae	205	7	0	0	3.3	↓- potassium (0.038)
arteriolar hyperplasia, focal, intimal	82	127	1	0	61	none

Organ - lesion or tissue type	Lesion score				Sample prevalence	Significant trends (*P*-value)
	0	1	2	3		
Skin and skeletal muscle, microscopic lesions (n = 212)						
Ichthyophonus	173	31	7	1	18	↑- AST (<0.001*), log_e AST (0.001), CPK (<0.001*), log_e CPK (<0.001), total protein (0.003)
leukocytes, perivascular	27	183	2	0	87	↑- osmolality (0.002), total bilirubin (0.016) ↓- phosphorus (0.010*)
myodegeneration or myonecrosis	202	9	1	0	4.7	↑- AST (<0.001*), GGT (0.029)
myositis	193	19	0	0	9.0	↑- AST (0.008*)
Spleen microscopic lesions (n = 211)						
arteriolar hyperplasia, focal, intimal	150	57	4	0	29	↓- CO_2 (0.023)
congestion, vascular	81	90	37	3	62	↑- hold time (<0.001), CO_2 (0.045) ↓- albumin (<0.001), ALP (0.001), calcium (0.032), cholesterol (<0.001), GGT (0.010), total protein (0.006)
ellipsoid hyalinization or hypertrophy	30	147	33	1	86	↑- age (<0.001*)
Ichthyophonus	173	16	17	5	18	↑- AST (<0.001*), CPK (<0.001*), total protein (0.014) NT- PCV (0.020)

Organ - lesion or tissue type	Lesion score				Sample prevalence	Significant trends (*P*-value)
	0	1	2	3		
Spleen microscopic lesions (n = 211)						
macrophage aggregates, pigmented	0	33	122	56	100	↑- age (<0.001*)
serosal cell thickening	44	139	27	1	79	↓- ALP (0.033)
Stomach microscopic lesions (n = 210)						
eosinophilic granular leukocytes, submucosal	0	0	157	53	100	↓- albumin (0.025), ALP (0.005), cholesterol (0.020), phosphorus (0.002*)
foreign body granuloma	150	60	0	0	29	↓- osmolality (0.034)
Ichthyophonus	188	17	1	4	10	↑- AST and $\log_e$ AST (<0.001*), CPK (<0.001*), $\log_e$ CPK (<0.001)
leukocytes, focal, parenchymal	171	38	1	0	19	none
serositis	163	46	1	0	22	↑- chloride (0.015)
trematodes, intraluminal (e.g., Hemiuridae)	192	17	1	0	8.6	↑- total protein (0.014) ↓- PCV (0.025) NT- CPK (<0.001*)

[a]↑ calcium = when plasma calcium values (mg/dL) were separated into three groups based on scoring of caudal fin fraying, mean (±SE) scores increased as follows: none ($11.7^{A} \pm 0.4$), mild ($11.5^{A} \pm 0.1$), and moderate/severe ($13.1^{B} \pm 0.7$). Means with a superscript in common were not significantly different (Tukey's analysis, $P > 0.05$).

Organ - lesion or tissue type	Lesion score				Sample prevalence	Significant trends (*P*-value)
	0	1	2	3		

[b]↓ = when ALP values (U/L) were separated into three groups based on scoring of caudal fin reddening, mean (±SE) scores decreased as follows: none ($57.8^{A} \pm 1.8$), mild ($53.3^{A,B} \pm 2.0$), and moderate/severe ($41.6^{B} \pm 8.9$). Means with a superscript in common were not significantly different (Tukey's analysis, $P > 0.05$).

[c]NT = when GGT values (U/L) were separated into three groups based on scoring of meningeal eosinophilic granular leukocytes, mean (±SE) scores for the least affected group (none) were not significantly different from mean scores for the most affected group (moderate/severe) as follows: none ($6.5^{A,B} \pm 0.7$), mild ($6.5^{A} \pm 0.3$), and moderate/severe ($8.6^{B} \pm 0.6$). Means with a superscript in common were not significantly different (Tukey's analysis, $P > 0.05$).

[d]ND = not done

[e]NA = not applicable

Table 2. Other lesions associated with external lesions in Pacific herring sampled from Prince William Sound, Alaska, during spawning, 1994. Chi-square test for association. For lesions with minimum expected cell frequency <1 (*), only chi-square tests with $P \leq 0.010$ are included. Trends in the associated lesion scores were classified in comparison to an increase in the given external lesion score. As the external lesion score increased, the associated lesion score either increased (↑), decreased (↓), or changes in the associated lesion score were not linear (NL; e.g., as scores for the external lesion increased, associated lesion scores initially increased and then later decreased). Lesions not listed were not significant.

	External lesion							
	caudal fin fraying		caudal fin reddening		fin base reddening		focal skin reddening	
Associated lesion	Trend	*P*-value	Trend	*P*-value	Trend	*P*-value	Trend	*P*-value
caudal fin reddening	↑	<0.001*						
fin base reddening		NS[a]	↑	0.001				
focal skin reddening		NS	↑	<0.001*	↑	0.001		
iris reddening	↓	0.049		NS	↓	0.002		NS
gastric *Ichthyophonus*		NS		NS		NS	↑	0.027
gastric serositis		NS		NS		NS	↑	0.026
gastritis, submucosal	↑	0.044		NS		NS	↑	0.012
gill arch inflammation or hematopoiesis		NS		NS		NS	↑	0.029
gonadal pigmented macrophage aggregates		NS		NS		NS	NL	0.039

	External lesion							
	caudal fin fraying		caudal fin reddening		fin base reddening		focal skin reddening	
Associated lesion	Trend	*P*-value	Trend	*P*-value	Trend	*P*-value	Trend	*P*-value
hepatic Anisakidae		NS		NS	↓	0.006		NS
hepatic focal/multifocal parenchymal leukocytes		NS		NS		NS	↓	0.003
hepatic granulomas (or focal granulomatous inflammation)	↑	0.025		NS		NS		NS
hepatic pigmented macrophage aggregates		NS	↓	0.009		NS		NS
intestinal foreign body granulomas	NL	0.016	↓	0.042		NS		NS
intestinal mesenteric steatitis		NS		NS	↑	0.022	↑	0.003
renal congestion	↑	0.039		NS		NS		NS
renal hematopoietic cells		NS		NS		NS	↑	0.020
skeletal myodegeneration and necrosis	↑	0.008	NL	<0.001*		NS		NS
splenic macrophage aggregates	↓	0.021		NS		NS		NS

[a]NS = not significant

Table 3. Lesion frequency (%) within variables of gender, iris reddening, and VHSV in Pacific herring sampled from Prince William Sound, Alaska, during spawning, 1994. Lesions were scored as none (0), mild (1), moderate (2), or severe (3). Chi-square test for homogeneity. Lesions not listed were not significant. For some lesions, sum of individual frequencies within a category is different from 100% due to rounding differences.

Variable and lesion	Lesion score	Frequency		χ^2 *P*-value[b]	Odds ratio[a]	95% Confidence interval for odds ratio
Gender		Female (n≈110)	Male (n≈102)			
gall bladder myxosporeans (*Ceratomyxa auerbachi*)	0	73	90	0.003		
	1+2	27	10		3.5	1.5, 8.4
gonadal granulomas (or focal granulomatous inflammation)	0	98	91	0.022		
	1+2+3	2	9		0.2	0.0, 0.9
gonadal hyalinized vessel walls	0	39	100	<0.001		
	1	52	0		NC[c]	NC
	2	9	0			
gonadal pigmented macrophage aggregates	0	36	97	<0.001		
	1+2	64	3		58	17, 190
intestinal mesenteric steatitis	1	92	82	0.036		
	2	8	18		0.4	0.2, 1.0
renal proximal tubular epithelial vacuolation	0	99	91	0.007		
	1+2	1	9		0.1	0.0, 0.8

Variable and lesion	Lesion score	Frequency		χ^2 *P*-value[b]	Odds ratio[a]	95% Confidence interval for odds ratio
Gender		Female (n≈110)	Male (n≈102)			
renal tubular dilation (of lumen)	0	99	93	0.023		
	1	1	7		0.1	0.0, 1.0
splenic *Ichthyophonus*	0	83	82	0.031		
	1	4	12		NC	NC
	2+3	14	7			
Iris reddening		Mild/ Moderate (n≈93)	None (n≈94)			
branchial ciliated protozoa	0	95	82	0.007		
	1	5	18		0.3	0.1, 0.7
caudal fin fraying	0	10	22	0.049		
	1	85	73		NC	NC
	2+3	6	5			
fin base reddening	0	59	36	0.002		
	1	32	44		NC	NC
	2+3	9	20			
meningoencephalitis	0	100	96	0.044		
	1+2	0	4		0.0	NC

Variable and lesion	Lesion score	Frequency		χ^2 *P*-value[b]	Odds ratio[a]	95% Confidence interval for odds ratio
Iris reddening		Mild/ Moderate (n≈93)	None (n≈94)			
pancreatic zymogen granule depletion	1+2	27	44	0.019		
	3	73	56		2.1	1.1, 3.8
renal congestion	0	67	80	0.043		
	1+2	33	20		2.0	1.0, 3.8
splenic congestion	0	45	28	0.037		
	1	35	52		NC	NC
	1+2	20	20			
splenic ellipsoid hyalinization	0	9	23	0.013		
	1	78	60		NC	NC
	1+2	13	17			
VHSV		Positive (n=11)	Negative (n≈200)			
fin base reddening	0	18	50	0.005		
	1	36	38		NC	
	2+3	45	12			
gastritis, submucosal	2	27	77	<0.001		
	3	73	23		9.1	2.3, 36

Variable and lesion	Lesion score	Frequency		χ^2 *P*-value[b]	Odds ratio[a]	95% Confidence interval for odds ratio
VHSV		Positive (n=11)	Negative (n≈200)			
gill arch inflammation or hematopoiesis	0+1	45	83	0.002		
	2	55	17		5.7	1.6, 20
meningoencephalitis	0	82	98	0.005*		
	1+2	18	2		8.7	1.5, 51
hepatic focal necrosis	0	82	98	0.002*		
	1+2+3	18	2		11	1.8, 68
intestinal arteriolar focal intimal hyperplasia	0	27	75	0.012		
	1+2	73	35		5.0	1.3, 19
myocardial mineralization	0	90	99	0.003*		
	1	10	1		22	1.3, 380

[a]Odds ratio is defined as the ratio of the odds of a fish being at one level of a condition (e.g., having a scorable lesion) as opposed to being at another level of a condition (e.g. having no lesion) for one category of a variable (e.g., female or VHSV-positive) to the corresponding odds for the other category of the variable (e.g. male or VHSV-negative). For example, females were 58 times more likely to have pigmented gonadal macrophage aggregates than were males, fish with mild/moderate iris reddening were 2 times more likely to have renal congestion than were fish with no iris reddening, and VHSV-positive fish were 11 times more likely to have hepatic focal necrosis than were VHSV-negative fish.

[b]*P*-value. For lesions with minimum expected cell frequency <1 (*), only comparisons with $P \leq 0.010$ were considered significant. Note that for comparisons with a low expected cell frequency, the odds ratio has a wide confidence interval.

[c]NC = odds ratios were not calculated for lesions with more than 2 groups (e.g., splenic *Ichthyophonus*).

Table 4. Plasma chemistry values that were significantly different ($P < 0.05$) based on status of viral hemorrhagic septicemia virus (VHSV) or gender. Pacific herring were sampled during spawning in Prince William Sound, Alaska, 1994. One-way analysis of variance; for comparisons in which Levene's test for equality of variance was significant (*), only comparisons with $P \leq 0.010$ are shown. Plasma chemistries not shown were not significant

Plasma chemistry	Mean	SE	Mean	SE	*P*-value
	VHSV status				
	Negative (n = 222)		Positive (n = 11)		
Albumin (g/dL)	0.52	0.01	0.36	0.05	0.007
ALP (U/L)	56.1	1.4	36.6	4.5	0.002
Cholesterol (mg/dL)	221.4	4.7	156.9	21.0	0.003
	Gender				
	Female (n = 117)		Male (n = 116)		
Albumin (g/dL)	0.47	0.02	0.56	0.02	<0.001
ALP (U/L)	59.3	2.1	51.1	1.6	0.002
Chloride (mmol/L)	160.4	0.9	165.6	1.2	0.001*
Cholesterol (mg/dL)	202.1	6.3	234.8	6.6	<0.001
CO_2 (mmol/L)	5.6	0.2	6.5	0.2	0.004
Glucose (mg/dL)	75.9	2.6	90.0	4.3	0.001
Potassium (mmol/L)	2.13	0.10	2.45	0.11	0.029
Total protein (g/dL)	2.14	0.06	2.30	0.05	0.042

Table 5. Sum-*Ichthyophonus* scores (mean ± SE) for categories of fish based on non-*Ichthyophonus* lesion scores in Pacific herring sampled from Prince William Sound, Alaska, during spawning, 1994. Sum-*Ichthyophonus* scores were calculated by summing the *Ichthyophonus* scores in each organ; actual values varied from 0 to 24. One-way analysis of variance (ANOVA) and Tukey's multiple-comparison procedure. If Levene's test for equality of variances was significant (*), only comparisons with $P \leq 0.010$ are listed. Within rows, means with a superscript in common were not significantly different ($P > 0.05$); lesions not shown were not significant.

Lesion	Category based on lesion score									*P*-value for ANOVA
	A			B			C			
	mean	SE	n	mean	SE	n	mean	SE	n	
hepatic eosinophilic granular leukocytes	none			mild			moderate/severe			
	1.3^{A}	1.0	15	1.9^{A}	0.3	187	6.4^{B}	2.2	10	0.009*
cardiac thrombosis	none			mild/moderate			NA[a]			
	1.4^{A}	0.3	193	9.6^{B}	1.6	17				<0.001*
gastric foreign body granuloma	none			mild			NA			
	1.0^{A}	0.2	150	4.8^{B}	0.9	60				<0.001*
gastric focal parenchymal leukocytes	none			mild/moderate			NA			
	1.7^{A}	0.3	171	3.9^{B}	1.0	39				0.006*
intestinal foreign body granuloma	none			mild			NA			
	0.8^{A}	0.2	133	4.2^{B}	0.7	78				<0.001*

Lesion	Category based on lesion score									*P*-value for ANOVA
	A			B			C			
	mean	SE	n	mean	SE	n	mean	SE	n	
intestinal mesenteric steatitis		mild			moderate			NA		
	1.7[A]	0.3	184	4.7[B]	1.4	27				<0.001*
skeletal myositis		none			mild			NA		
	1.8[A]	0.3	193	4.4[B]	1.1	19				0.024

[a]NA = not applicable

Table 6. Number of intraperitoneal herring worms (Anisakidae) in categories based on gender or lesion scores in Pacific herring sampled from Prince William Sound, Alaska, during spawning, 1994. One-way analysis of variance (ANOVA) and Tukey's multiple-comparison procedure. If Levene's test for equality of variances was significant (*), only comparisons with $P \leq 0.010$ are listed. Within rows, means with a superscript in common were not significantly different ($P > 0.05$); lesions not shown were not significant.

	Category based on lesion score									
	A			B			C			*P*-value for ANOVA
Variable	Mean	SE	n	Mean	SE	n	Mean	SE	n	
	male			female						
gender	10.7^{A}	0.6	117	14.2^{B}	0.8	116		NA[a]		<0.001*
splenic pigmented macrophage aggregates	none/mild			moderate			severe			
	$12.6^{A,B}$	1.5	33	10.8^{A}	0.6	122	15.5^{B}	1.2	56	<0.001*
renal pigmented macrophage aggregates	mild			moderate			severe			
	10.2^{A}	0.8	81	14.1^{B}	0.8	110	$11.9^{A,B}$	1.5	21	<0.002
renal interstitial cell necrosis	none			mild			NA			
	12.8^{A}	0.6	194	7.9^{B}	1.0	18				0.010*
hepatic cholangitis or biliary hyperplasia	none			mild/moderate			NA			
	11.8^{A}	0.5	191	17.8^{B}	2.7	21				0.001*
intrahepatic Anisakidae	none			mild/moderate			NA			
	11.8^{A}	0.6	166	14.6^{B}	1.4	46				0.030

Variable	Category based on lesion score									*P*-value for ANOVA
	A			B			C			
	Mean	SE	n	Mean	SE	n	Mean	SE	n	
intestinal mesenteric Anisakidae	10.7[A]	none 0.9	51	12.4[B]	mild 0.7	137	16.9[B]	moderate 1.8	23	0.005
skeletal muscle Anisakidae	12.2[A]	none 0.5	205	19.3[B]	mild 2.8	7		NA		0.016

[a]NA = not applicable

Table 7. Linear correlations (r) of age (yr), body weight and gonad weight (g), standard length (mm), hold time (min), albumin (g/dL), sum-*Ichthyophonus* (sumICH) scores, and blood values in Pacific herring sampled from Prince William Sound, Alaska, during spawning, 1994. Significant correlations ($P < 0.05$) are denoted (*); sample size varies from 208 to 233.

	Age	Body weight	Length	Gonad weight	Hold time	sumICH	Albumin
Body weight	0.665*						
Length	0.712*	0.898*					
Gonad weight	0.356*	0.746*	0.504*				
Hold time	−0.151*	−0.242*	−0.202*	−0.157*			
SumICH	−0.062	−0.041	−0.067	0.082	0.034		
Albumin	0.130*	0.297*	0.178*	0.335*	-0.132*	0.036	
PCV (%)	0.073	0.265*	0.167*	0.289*	−0.143*	−0.159*	0.377*
Total protein (g/dL)	0.111	0.359*	0.184*	0.518 *	-0.082	0.209*	0.749*
$\log_e$ AST (U/L)	−0.041	0.093	−0.007	0.230*	−0.032	0.259*	0.166*
ALP (U/L)	0.031	0.295*	0.108	0.459*	-0.185*	0.096	0.587*
$\log_e$ ALT (U/L)	−0.029	0.022	−0.040	0.334*	0.189*	0.016	0.049
$\log_e$ CPK (U/L)	0.062	0.195*	0.137*	0.237*	−0.102	0.298*	0.336*
GGT (U/L)	0.001	0.087	0.063	0.022	-0.227*	0.091	0.155*
Calcium (mg/dL)	0.001	0.144*	0.040	0.281*	-0.130*	−0.032	0.435*
Chloride (mmol/L)	0.160	0.173*	0.243*	0.054	-0.137*	−0.032	0.088

	Age	Body weight	Length	Gonad weight	Hold time	sumICH	Albumin
Cholesterol (mg/dL)	0.102	0.268*	0.142*	0.343*	-0.166*	0.089	0.895*
CO_2 (mmol/L)	−0.018	−0.120	−0.088	-0.212*	0.443*	<0.001	-0.027
Glucose (mg/dL)	0.157*	0.215*	0.163*	0.255*	-0.328*	−0.051	0.373*
Osmolality (mOsm/kg)	0.075	0.272*	0.200*	0.282*	-0.113	−0.070	0.297*
Phosphorus (mg/dL)	0.010	0.146*	0.034	0.305*	-0.102	0.019	0.237*
Potassium (mmol/L)	−0.054	−0.034	−0.061	0.029	0.482*	0.083	0.025
Total Bilirubin (mg/dL)	−0.046	−0.028	−0.098	0.110	0.127	−0.140*	0.057

Table 8. Plasma chemistry values in 233 Pacific herring sampled from Prince William Sound, Alaska, during spawning, 1994.

Plasma chemistry	Mean	Minimum	Maximum	SD	Normal[a]	
					low	high
Total protein (g/dL)	2.2	0.2	3.8	0.6	1.0	3.1
Albumin (g/dL)	0.5	0	1.1	0.2	0.1	0.8
ALP (U/L)	55	2	116	21	13	95
ALT (U/L)	3.7	0	14	2	0	8
AST (U/L)	346	11	2590	318	0	860
CPK (U/L)	450	10	8080	705	0	1240
GGT (U/L)	7	0	17	4	0	15
Potassium (mmol/L)	2.3	0.6	7.7	1.1	0	4.4
Chloride (mmol/L)	163	141	197	12	139	184
CO_2 (mmol/L)	6	0	17	2	1.7	10.3
Phosphorus (mg/dL)	12.7	5.5	38	4.3	3.7	21.5
Calcium (mg/dL)	11.6	6.7	21	1.9	7.9	14.8
Cholesterol (mg/dL)	218	4	420	71	74	353
Glucose (mg/dL)	83	17	411	39	3	164
Total Bilirubin (mg/dL)	0.04	0	0.4	0.08	0	0.2
Osmolality (mOsm/kg)	428	374	512	24.6	378	475

[a]Normal values are the range (mean ± 2 SD; n = 140) after removal from the data set of all fish with *Ichthyophonus*, VHSV, or severe *Ortholinea orientalis*.

Table 9. Sample prevalence (%) of parasites and virus in adult Pacific herring in Prince William Sound, Alaska, 1989-1994.

Sample Date	n	*Goussia clupearum*	*Ichthyophonus hoferi*	*Ortholinea orientalis*	Viral hemorrhagic septicemia virus
1989 April[a]	40	63	13	TNE[b]	TNE
1990 October[a]	99	60	15	6.1	TNE
1991 April[a]	59	54	5.1	17	TNE
1991 October[a]	48	54	2.1	15	TNE
1992 April[c]	105	53	5.7	3.1	TNE
1993 April[d]	79	41	5.1	4.3	2 of 3 5-fish pools
1994 April	212	61	29	5.7[e]	4.7

[a]unpubl. data from G.D. Marty, M. S. Okihiro, and D. E. Hinton

[b]TNE = Tissue not examined

[c](Kocan et al. In Press)

[d](Meyers et al. 1994) and unpubl. data from T.R. Meyers

[e]Prevalence based on histopathology only; total prevalence using touch preparations and histopathology was 19%.

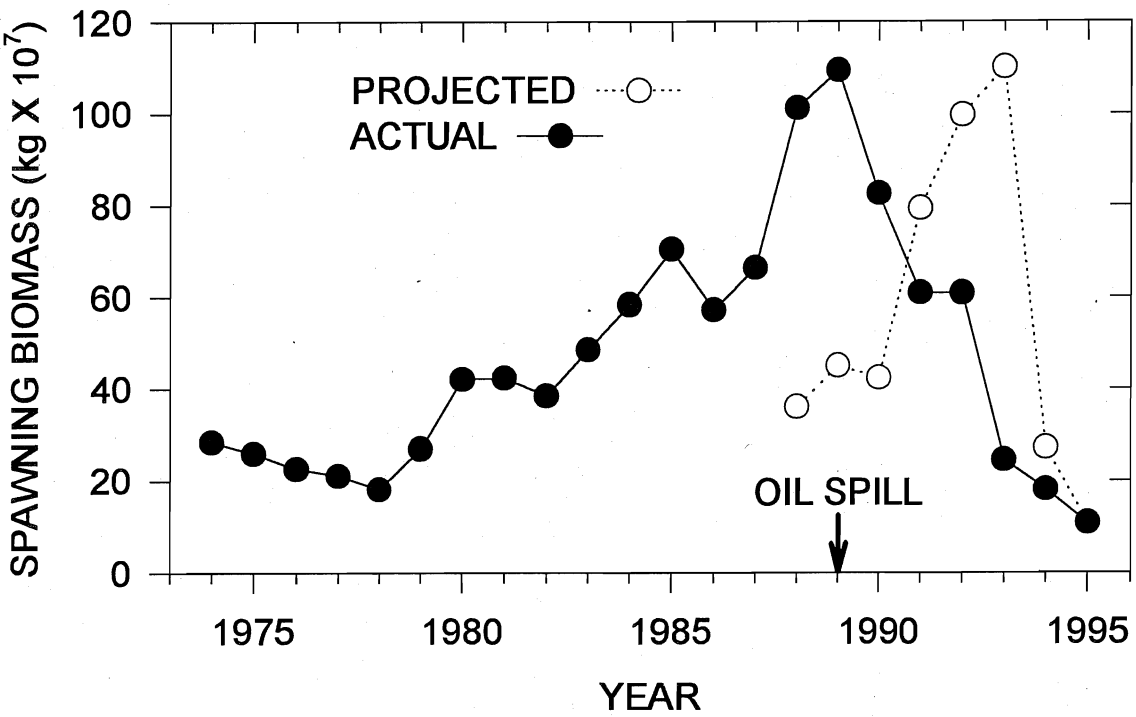

Figure 1. Biomass estimates of mature Pacific herring in Prince William Sound, Alaska. Unexploited biomass projected in the year before spawning (PROJECTED) and estimated during spawning (ACTUAL). Estimates were made by Fritz Funk, Alaska Department of Fish and Games, Juneau, Alaska; unpubl. data.

Figure 2. Gross and histologic lesions in Pacific herring sampled from Prince William Sound, Alaska, during spawning, 1994. A. The ulcer with neovascularization on the right lateral side of this 198-mm-long female was positive for viral hemorrhagic septicemia virus (VHSV). B. A similar ulcer on the dorsal caudal peduncle of a 245-mm-long female was negative for VHSV. C. Mild reddening of the ventral region of the iris (arrow) was considered normal; this fish was released and not cultured for VHSV. D. Normal gastric submucosa with large numbers of eosinophilic granular leukocytes. E. Gastric submucosa with increased numbers of lymphocytes and macrophages (i.e., submucosal gastritis). D and E - hematoxylin and eosin stain, gastric glands (g), same magnification, bar length = 100 μm.

Figure 4. Microscopic lesions in the liver and stomach of Pacific herring sampled from Prince William Sound, Alaska, during spawning, 1994; hematoxylin and eosin stain. A. Liver with a ruptured *Ichthyophonus* resting spore (arrow) that has released several multinucleate endospores; bar length = 100μm. B. Trematode (probably Hemiuridae) attached to the gastric mucosa with an oral sucker. Note the prominent acetabulum (a); bar length = 300 μm. C. Hepatic coagulative necrosis; note pyknosis and karyolysis within a broad band of hepatocytes; bar length = 40μm. D. Biliary hyperplasia at the base of the gall bladder (g); bar length = 200 μm. E. Multiple foci of *Goussia clupearum* scattered throughout the hepatic parenchyma; bar length = 200 μm. F. Sporulated oocysts and an unsporulated oocyst (arrow) of *Goussia clupearum* in the liver. Note minimal inflammation; bar length = 50 μm.

Figure 3. Internal parasites of Pacific herring sampled from Prince William Sound, Alaska, during spawning, 1994; hematoxylin and eosin stain. A. The myxosporean *Ceratomyxa auerbachi* in the gall bladder lumen; despite large numbers of organisms, inflammation in the gall bladder wall is minimal; bar length = 80 µm. Inset - trophozoites and maturing spores in the same fish; bar in larger print = 180 µm at inset magnification. B. Several stages of an unclassified coccidian (*Goussia* sp.?) in the apical margin of epithelial cells of intestinal cecae. Note different stages of development (arrows) and lack of inflammation; bar length = 30 µm. C. Forms of *Ichthyophonus* in the heart include multinucleate resting spores with minimal inflammation (arrows), remnants of ruptured resting spores (r) with small endospores, and developing spores surrounded by severe granulomatous inflammation (g); bar length = 400 µm.

Figure 5. Normal liver histology and hepatic lesions in Pacific herring sampled from Prince William Sound, Alaska, during spawning, 1994; hematoxylin and eosin stain. A. Normal liver with a small pigmented macrophage aggregate (arrow); bar length = 150μm. B. Liver with 3 pigmented macrophage aggregates; magnification same as A. C. Liver with 2 foci of granulomatous inflammation (arrows) that were unrelated to *Ichthyophonus*. Note that pale foci of activated macrophages contain scattered lymphocytes but pigment is minimal; magnification same as A. D. Severe, acute, zonal, coagulative, hepatic necrosis with small irregular foci of viable hepatocytes (e.g., v and arrows); magnification same as A. E. Severe single cell hepatocellular necrosis (apoptosis). Several hepatocytes have condensed nuclei with contracted hypereosinophilic cytoplasm (arrows); bar length = 30 μm.

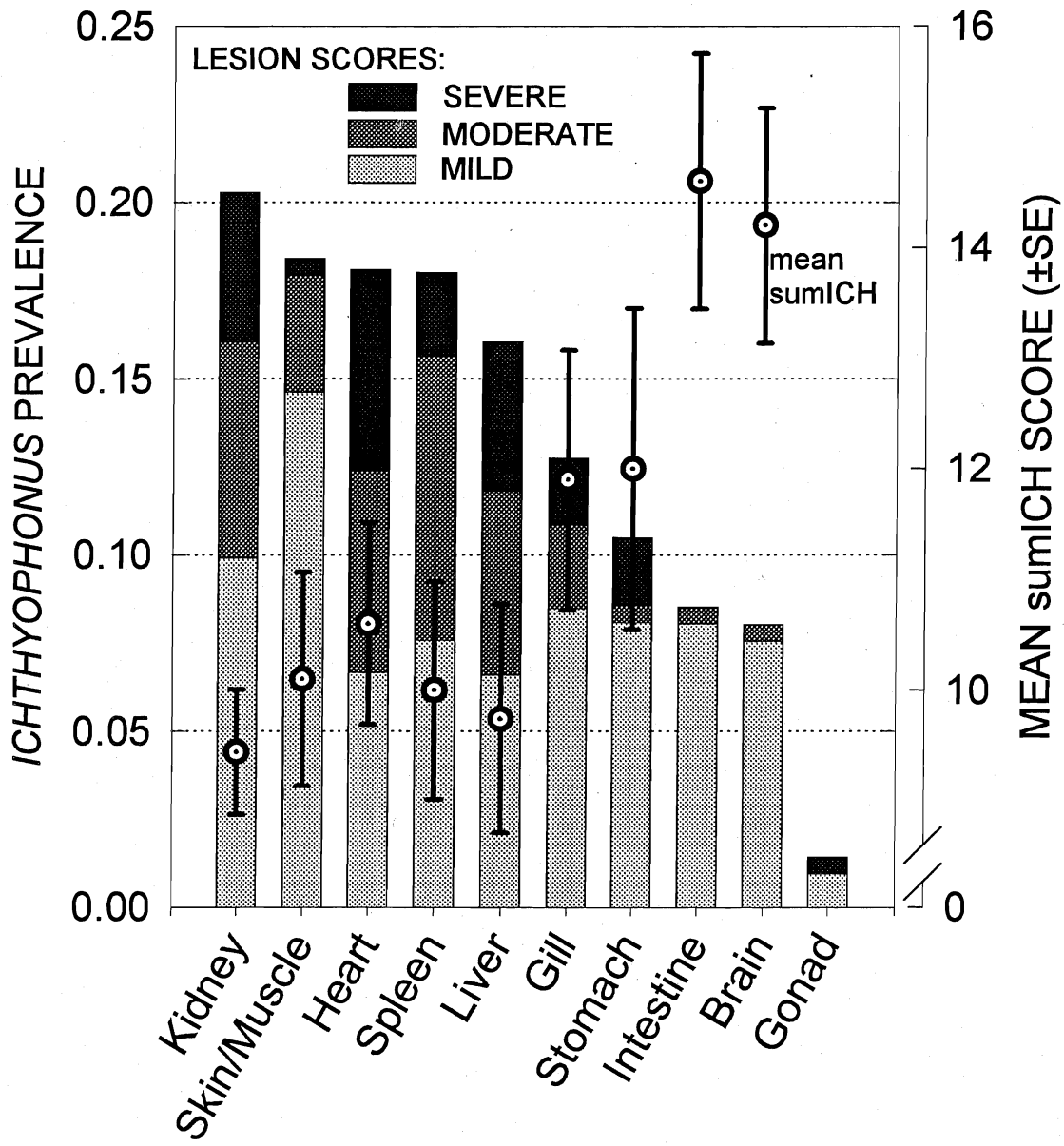

Figure 6. Sample prevalence of *Ichthyophonus* lesion scores in various organs compared with mean sum-*Ichthyophonus* (mean sumICH) score for each organ. Lesions were scored as none (0), mild (1), moderate (2), or severe (3), and the sumICH score was calculated for each fish by adding the *Ichthyophonus* score for all organs in that fish. The mean sumICH score was calculated for each organ. For example, the mean sumICH score for the brain was the average of sumICH scores for all 17 fish that had brain *Ichthyophonus*; fish without brain *Ichthyophonus* were not used for calculations of the mean sumICH score for the brain. Sample size varies from 210 to 212.

Figure 7. Microscopic lesions in various organs of Pacific herring sampled from Prince William Sound, Alaska, during spawning, 1994; hematoxylin and eosin stain. A and B. Gill arches normally contained scattered inflammatory or hematopoietic cells (A), but some fish had more abundant inflammatory or hematopoietic cells (B); same magnification, bar length = 100 μm. C and D. Small arteries and nerves were common near exocrine pancreatic tissue between intestinal cecae. Normal arteries (a) had a smooth intimal surface (C), but arteries in some fish had focal intimal hyperplasia (D) that varied from mild (f and arrow) to moderate (arrowheads); same magnification, bar length = 150 μm. E and F. Intestinal mesenteries normally had mild infiltrates of inflammatory cells and moderately sized adipocytes (E), but some fish had moderate infiltrates of inflammatory cells (steatitis) and atrophied adipocytes (F); same magnification, bar length = 40 μm. G and H. Renal archinephric ducts contained intraluminal parasites, but associated inflammation was minimal. Pansporoblasts of the myxosporean *Ortholinea orientalis* (G, arrow) were free within the lumen, whereas unidentified protozoans (H, arrow) were smaller and adhered to the luminal epithelium; same magnification, bar length = 100 μm.

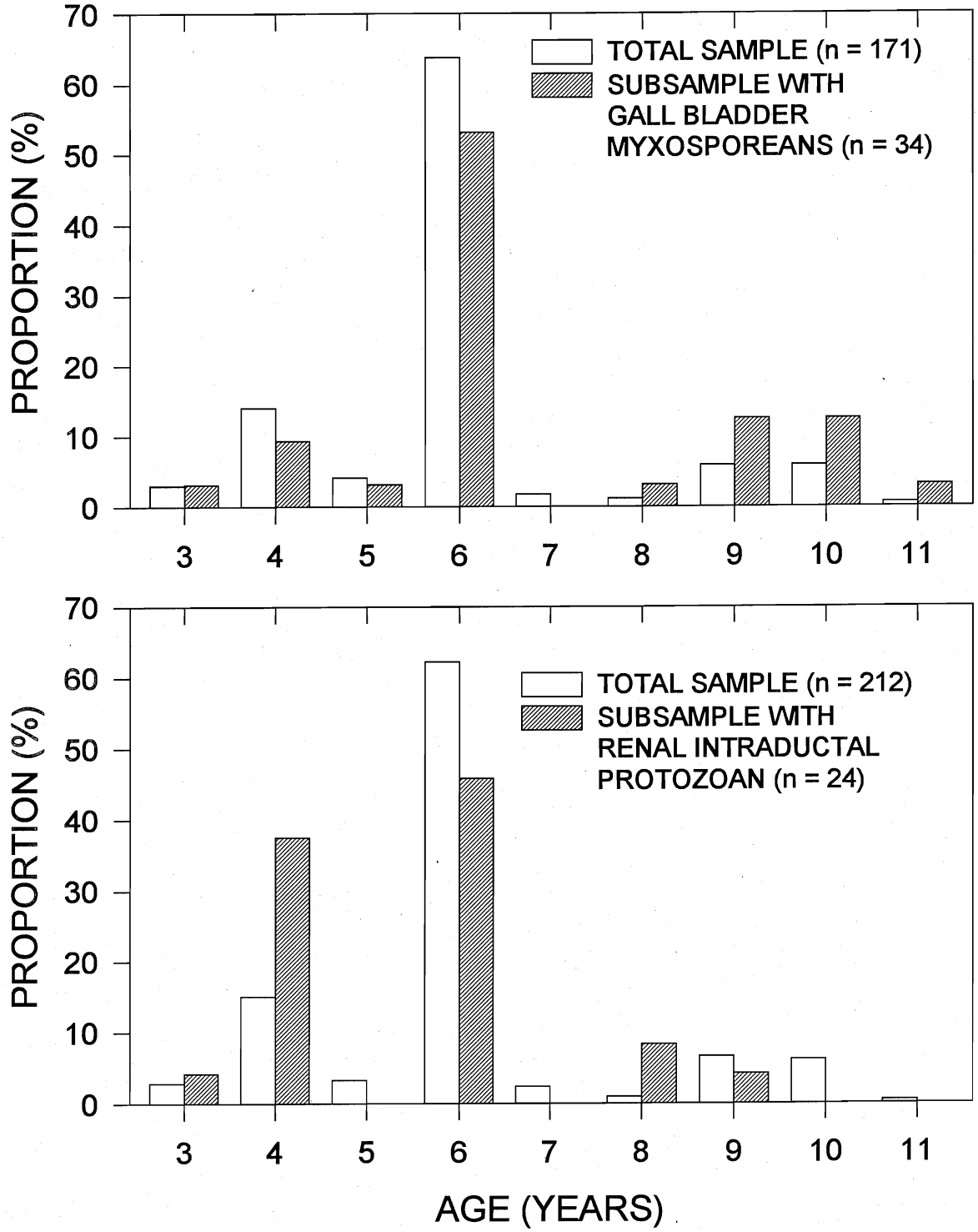

Figure 8. Age distribution of fish that had common parasites compared with the age distribution of fish that were examined for each parasite. Pacific herring were sampled from Prince William Sound, Alaska, during spawning, 1994. Top - intraluminal gall bladder myxosporean (*Ceratomyxa auerbachi*). Bottom - unidentified renal intraductal protozoan.

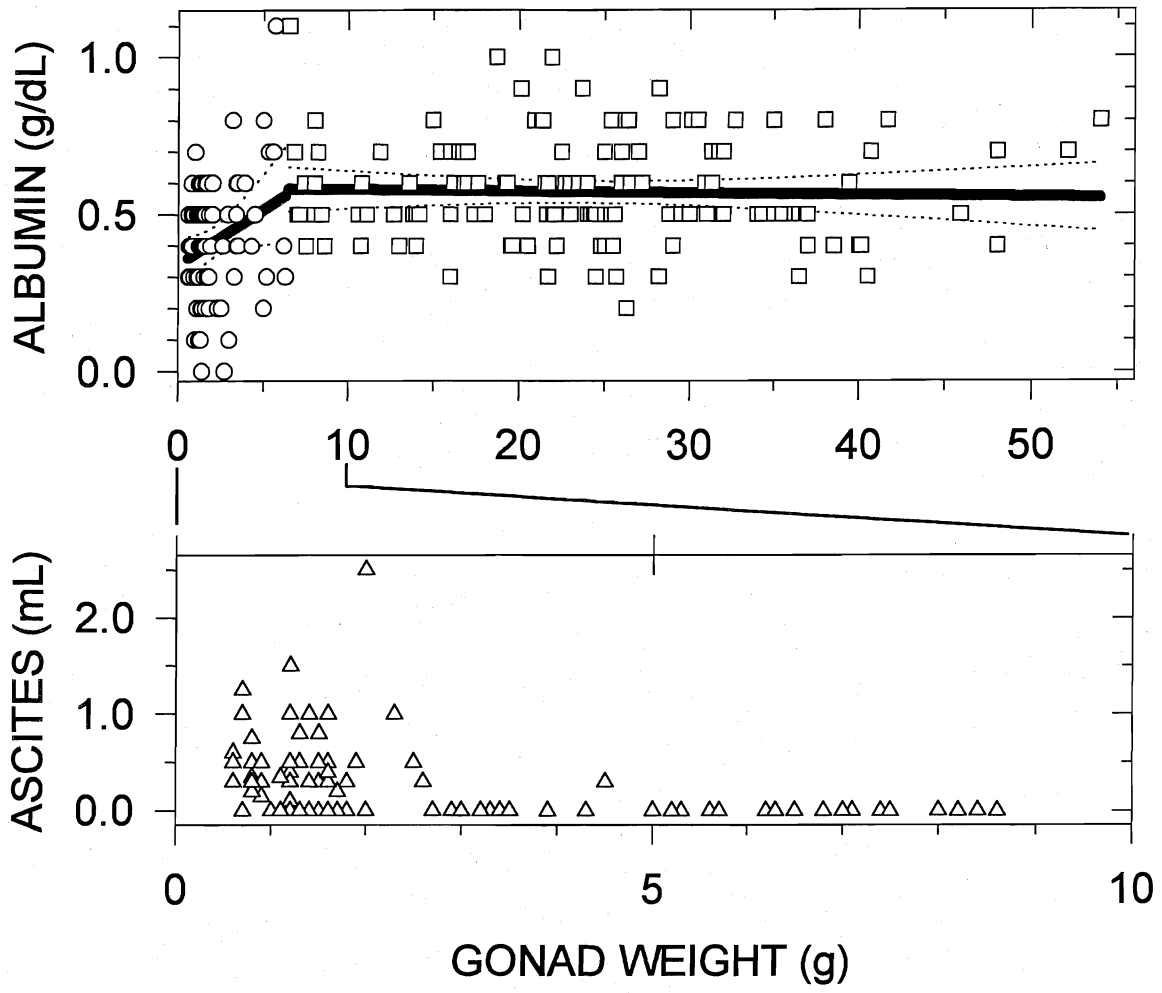

Figure 9. Effect of gonad weight on plasma albumin and ascites in Pacific herring sampled from Prince William Sound, Alaska, during spawning in 1994. Separate linear regressions of plasma albumin and gonad weight (±99% confidence intervals) are shown for spawning fish (□, gonad weight ≥ 7.0 g) and postspawning fish (○, gonad weight < 7.0 g). Ascites (△) occurred only in fish with gonad weight less than 5.0 g, and fish with gonad weight >10 g are not shown on the ascites graph.