Exxon Valdez Oil Spill
Restoration Project Annual Report

Effect of Disease on Recovery of Pacific Herring in Prince William Sound, Alaska, Fall 2000 and Spring 2001

Restoration Project 01462
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: This project continues the field component of project 98162 (the final report for 98162 has been approved). Results for this project were reported in two annual reports: 1) 99462, "Effect of Disease on Recovery of Pacific Herring in Prince William Sound, Alaska, Spring 1999"; and 2) 00462, "Effect of Disease on Recovery of Pacific Herring in Prince William Sound, Alaska, Fall 1999 and Spring 2000." Detailed histopathological examination, blood analysis, and a modeling component were supported by a grant from the National Science Foundation (project #9871982, "Role of parasites and disease in health and population abundance of adult Pacific herring"). Most results specific to the National Science Foundation part of the study are not reported here.

Abstract: Due to disease-related declines in population biomass, Pacific herring (*Clupea pallasi*) fisheries in Prince William Sound have been closed since 1999, with no evidence of population recovery. Detailed disease study of Pacific herring sampled in November 2000 (n = 100) and April 2001 (n = 300) used the same methods as used every year since 1994. During the first 7 years of study (1994-2000) spring sample prevalence of *Ichthyophonus hoferi* varied little (16-24%) and prevalence was highly correlated with fish age. In spring 2001, however, prevalence of *Ichthyophonus hoferi* (38%) was 50% greater than it had been in any of the previous 7 years, despite a decrease in mean fish age from 6.2 years in 2000 to 5.7 years in 2001. Other potential causes of mortality were at relatively low prevalence in spring 2001: ulcers (0.7%), and viral hemorrhagic septicemia virus (1.7%). Study from fall 2001 provides preliminary evidence that the unprecedented increase in *Ichthyophonus hoferi* in spring 2001 was associated with increased mortality of older fish during the summer of 2001; however, fall samples are not always characteristic of the population, and confirmation of the effects on population age structure awaits collections and analysis of spring 2002 samples.

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

Project Data: Data include date, location, and time of capture; sex, age, standard length, body weight, gonad weight, and liver weight; gross necropsy findings; and results from virus analysis (viral hemorrhagic septicemia virus, VHSV). All project data are stored in an Excel spreadsheet (188 columns and 3276 rows). The spreadsheet is stored and maintained by Gary D. Marty, VM:APC, Univ. of CA, 1 Shields Ave., Davis, CA 95616; 530-754-8062; e-mail: gdmarty@ucdavis.edu. Data are available on a case-by-case basis.

Citation:

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Executive Summary

Introduction

The estimated spawning biomass of Pacific herring (*Clupea pallasi*) in Prince William Sound, Alaska, decreased from about 22,000 metric tons in 1999 to only 8,000 metric tons in 2000, and there was no evidence of population recovery in 2001. Due to low population biomass, Pacific herring fisheries in Prince William Sound have been close since 1999. Studies of the Prince William Sound Pacific herring population since 1994 provided evidence that disease is a significant variable in population fluctuations. Before 2001, ulcers and viral hemorrhagic septicemia virus were associated with acute mortality that was significant on a population scale (Quinn et al. 2001). The other major disease—caused by the choanoflagellate *Ichthyophonus hoferi*—is chronic and probably decreases the life span of affected fish, but it did not play a significant role in unexpected population fluctuations before 2001 (Quinn et al. 2001). In 2001, however, the prevalence of *Ichthyophonus hoferi* (38%) was 50% greater than it had been in any previous year of study. This report describes this and other major disease-related findings in Pacific herring sampled from Prince William Sound during the fall of 2000 and spring of 2001.

Objectives

Our study had three objectives: 1) determine the prevalence of major diseases in Pacific herring; 2) determine the interaction of gender, age, and season on disease prevalence; 3) determine if disease prevalence correlates with population trends.

Methods

Adult Pacific herring from Prince William Sound were sampled at random and subjected to complete necropsy in November 2000 (n = 100) and April 2001 (n = 300). Fall samples were all sampled from near the north end of Montague Island (Zaikof Bay). During the spring of 2001, fish numbers were more evenly distributed between the Northeast and Montague areas of PWS (unpublished ADFG population estimates); therefore, sampling was split between the Northeast region (near Gravina Bay; n = 220) and the north end of Montague Island (Rocky Bay; n = 80). Analysis of all fish included determination of age, weight and length, gross examination, and culture of head kidney and spleen for virus isolation. In fish with severe external lesions, kidney was cultured for bacteria (all were negative). A project supported by the National Science Foundation included analysis of blood, complete histopathology, and mathematical modeling of the role of disease on population biomass. Results from study supported solely by the National Science Foundation are not reported here, except for total prevalence of *Ichthyophonus hoferi*, which was determined by histopathological examination.

Results

Disease prevalence in the Pacific herring population increased significantly from spring 2000 to spring 2001. Prevalence of *Ichthyophonus hoferi* increased to 38% in 2001: more than 50% greater than in any year since population-level disease study began in 1994. Prevalence of viral hemorrhagic septicemia virus increased slightly from 0% in 2000 to 1.7% in 2001. Prevalence of moderate+severe focal skin reddening increased from 1.0% in 2000 to 2.3% in 2001, although prevalence of ulcers was the same in 2000 and 2001 (0.7%). None of the fall samples had evidence of viral hemorrhagic septicemia, and this finding is consistent with fall samples in all other years studied.

Discussion

Disease associated with the unprecedented increase in prevalence of *Ichthyophonus hoferi* in spring 2001 is the most important identifiable variable limiting recovery of the Pacific herring population of Prince William Sound, Alaska. Ulcers and viral hemorrhagic septicemia were the major diseases limiting population recovery in the late 1990s, but they have not been significant at the population level since 1998. Because *Ichthyophonus hoferi* disseminates in multiple organs, and causes a chronic disease that may last several years, the population impact of the outbreak of *Ichthyophonus hoferi* in 2001 cannot be determined until samples from spring 2002 are collected and analyzed (collection is part of project 02462, and funds for analysis are being requested as part of close-out for //462 in FFY03).

Conclusion

Further study, scheduled for April 2002 (project 02462) will provide more information on the effects of disease on herring population recovery. Because population biomass remains at historically low levels, and recruitment of the 1998 year class was relatively poor, it will be many years before the population recovers. According to the restoration objectives, a large year class must fully recruit into the fishery before a population can be reclassified as "recovered." Pacific herring do not fully recruit into the fishery until they are 5 years old. If recruitment of the 1999 year class is strong, which might occur based on numbers of these fish in fall 2001 samples and preliminary spring 2002 acoustic surveys, population recovery based on restoration objectives cannot be confirmed until at least the year 2004.

Introduction

When the *Exxon Valdez* oil spill occurred in March 1989, the biomass of spawning Pacific herring (*Clupea pallasi*) in **Prince William Sound (PWS)**, Alaska, was the highest in 20 years of reliable estimates (about 100×10^6 kg; Figure 1), and the population remained near record levels through 1992. Pacific herring in PWS first spawn when 3 or 4 years old. They rarely live more than 12 years, and before the spill abundant year classes recruited into the fishery about once every 4 years. In 1993, recruitment from the 1988 year class was expected to be excellent, and fisheries biologists predicted a near-record spawning biomass of 110×10^6 kg before the spawning season (Figure 1). However, when the 1993 spawning season commenced, only 17% of the expected biomass appeared, fish were lethargic, and many had external hemorrhages. Therefore, PWS Pacific herring fisheries were severely curtailed in 1993, and were never opened in 1994, 1995, or spring 1996. In PWS before 1993, Pacific herring supported 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.

Disease study supported by the Trustee Council from 1994-1998 identified a virus (viral hemorrhagic septicemia virus, VHSV) and a choanoflagellate parasite (*Ichthyophonus hoferi*) that were important causes of disease on a population scale (Marty et al. 1998). Virus prevalence was highly variable and associated with ulcers, acute disease, and unexpected population decline (Quinn et al. 2001). By comparison, *I. hoferi* prevalence was fairly constant and associated with chronic disease that probably decreased the life span of affected fish, but *I. hoferi* was not correlated with unexpected population decline (Quinn et al. 2001).

Pacific herring population biomass increased enough in PWS so that roe fisheries were re-opened in 1997 and 1998. However, in 1998 high virus prevalence (14%) coupled with high ulcer prevalence (3.2%) provided evidence that the population was at risk of another disease-related decline. Therefore, this project was initiated to determine the effect of disease on recovery of Pacific Herring in PWS.

The estimated spawning biomass of Pacific herring in PWS, Alaska, decreased from about 36,000 metric tons in 1998 to only 8,000 metric tons in 2000. Pacific herring fisheries in PWS were severely curtailed in 1999, and all Pacific herring fisheries have been closed since 1999. This report describes the major disease-related findings in Pacific herring sampled from PWS during the fall of 2000 and spring of 2001. Of greatest importance is the unprecedented increase in the prevalence of *I. hoferi* in 2001. Before 2001, prevalence of *I. hoferi* was highly correlated with age of the sampled population, but the prevalence of *I. hoferi* nearly doubled from 2000 to 2001 despite a slight decrease in mean age of the sampled population during the same time. This report describes this and other major disease-related findings in Pacific herring sampled from Prince William Sound during the fall of 2000 and spring of 2001.

Objectives

Our study had three objectives:
1) determine the prevalence of major diseases in Pacific herring;
2) determine the interaction of gender, age, and season on disease prevalence; and
3) determine if disease prevalence correlates with population trends.

Methods

Necropsy

Pacific herring were captured using a commercial purse seine in 5 sets of 20 fish each in November 2000, and by purse seine or cast net (15 groups of 20 fish each) in April 2000. Fall samples were all collected from near the north end of Montague Island (Zaikof Bay). During the spring of 2001, fish numbers were more evenly distributed between the Northeast and Montague areas of PWS (unpublished ADFG population estimates); therefore, sampling was split between the Northeast region (near Gravina Bay; n = 220) and the north end of Montague Island (Rocky Bay; n = 80). Fish were subjected to complete necropsy on board a contracted vessel at the site of capture. After capture, fish were held in plastic fish totes filled with about 300 L of seawater for no more than 5 hours before necropsy. Herring were anesthetized in tricaine methane sulfonate (Finquel®), assigned a unique necropsy number, 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). For spring samples, gonadal fullness was estimated and scored as 3 (75-100% full), 2 (50-74% full), 1 (25-49% full), or 0 (0-25% full).

2) about 1.5 mL of blood was drawn from the caudal vein into 3-mL syringes that contained 0.1 mL of lithium heparin (1,000 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 immediately decanted and frozen. Analysis of these samples was not part of this project, and results will not be reported here.

3) for virus isolation, head kidney and spleen from each fish were pooled in a plastic bag (one fish/bag) and shipped on ice to the Alaska Department of Fish and Game's Fish Pathology Laboratory in Juneau, Alaska; skin lesions, if present, were sampled and bagged separately for individual virus assay. Propagation of 1 cell line (EPC), media formulation, and tissue preparation for cell line inoculation were as previously described (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.

Analysis of these samples was not part of this project, and results will not be reported here (except for the overall *I. hoferi* prevalence scores, which are reported here).

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

6) liver and gonads were weighed; and

7) herring worms (Anisakidae) in the peritoneal cavity were counted.

Statistical Analysis

Detailed statistical analysis will be included as part of the final report. Here, basic summary statistics such as sample size, sample mean, and standard error are reported for major findings. For major parasites, standard error was calculated from the mean prevalence of each organism within each of the 20-fish sets.

Results

Another major epizootic, this time caused by *I. hoferi*, affected the health of Pacific herring in PWS beginning in spring 2001. Prevalence of *I. hoferi* in spring 2001 (38%) was more than 50% greater than it had been in any previous year of study (Fig. 2), or in any other Alaskan population studied in the past decade (e.g., populations from Sitka, Craig, and Auke Bay; Carls et al. 1998, Davis et al. 1999; G.D. Marty, unpublished observations). Spring 2001 prevalence of *I. hoferi* was greater in fish from the NE area of PWS than in fish from Rocky Bay (Montague Island), but most of the difference probably was a result of older fish comprising the samples from the NE area of PWS (Fig. 2). At both sites, the prevalence of *I. hoferi* was significantly greater than in any PWS spring sample on record.

Sample prevalence of viral hemorrhagic septicemia virus and skin ulcers in spring 2001 was slightly higher than in 2000, but still at historically low levels (Figure 3). All 5 VHSV-positive fish came from the 220 fish sampled from the NE area of PWS: 3 VHSV+ fish from 1 set, and 2 VHSV + fish from another set; site differences were probably not significant, because only 80 fish were sampled from Rocky Bay. None of the 100 fish sampled from Zaikof Bay in November 2000 was positive for VHSV.

Prevalence of cysts of unknown etiology in the gills in Nov. 2000 and April 2001 was not different from the previous year's samples (Figure 4). Also, Prevalence of copepod parasites (on the medial operculum) in Nov. 2000 and April 2001 was not different from the previous year's samples (Figure 5).

Discussion

Spring 2001 marked the beginning of the third major disease epizootic in the PWS Pacific herring population in the last decade. In the early and late 1990s, high prevalence of VHSV was associated with significant decline in population biomass (Quinn et al. 2001). By comparison, the epizootic of 2001 was associated with unusually high prevalence of *I. hoferi*. The cause of the increase in prevalence of *I. hoferi* is unknown, but it might be associated with high mortality in previous years. As evidence, prevalence of *I. hoferi* in 1993—at the height of the most severe epizootic in the past decade—was only about 5% (Marty et al. 1998), but *I. hoferi* prevalence increased to 23% in 1994 (Quinn et al. 2001). We also have good evidence of VHSV-related population decline from 1998-2000 that predated the 2001 increase in *I. hoferi* by one to three years.

How and at what age Pacific herring are naturally infected with *I. hoferi* is not known. Prevalence within a year class consistently increases with age over time, and minimal change in age-class distribution over time is consistent with infected fish living for several years with *I. hoferi*. This pattern of increased prevalence with age could be a result of small numbers of fish in a year class being infected every year. Alternatively, large numbers of fish might be infected when young, but growth of *I. hoferi* in the host increases to diagnosable levels in only a small percentage of the fish each year; the relative number of fish reaching diagnosable levels of infection each year varies, perhaps as a result of different environmental conditions.

I. hoferi is currently the most important identifiable variable limiting recovery of the Pacific herring population of Prince William Sound, Alaska. Ulcers and viral hemorrhagic septicemia were the major diseases limiting population recovery in the late 1990s, but they have not been significant at the population level since 1998. Because *I. hoferi* disseminates in multiple organs, and causes a chronic disease that may last several years, the population impact of the outbreak of *I. hoferi* in 2001 cannot be determined until samples from spring 2002 are collected and analyzed. Collection of these samples is part of project 02462, but histopathological analysis of the samples for prevalence of *I. hoferi* was part of the NSF renewal proposal submitted 8-15-01 that was not funded. Because of the importance of this epizootic in limiting recovery of the PWS Pacific herring population, additional funds for histopathological analysis of samples collected in fall 2001 and spring 2002 are being requested as part of close-out for //462 in FFY03).

Conclusions

Further study, scheduled for April 2002 (project 02462) will provide more information on the effects of disease on herring population recovery. Because population biomass remains at historically low levels, it will be many years before the population recovers. According to the restoration objectives, a large year class must fully recruit into the fishery before a population can

be reclassified as "recovered." Pacific herring do not fully recruit into the fishery until they are 5 years old. Recruitment of the 1999 year class looks fairly good based on numbers of these fish in fall 2001 samples and preliminary spring 2002 acoustic surveys by ADFG. Even if this year class remains healthy, population recovery based on restoration objectives cannot be confirmed until at least the year 2004.

Acknowledgments

We thank N.J. Del Pesco, R. Dunbar, F.C. Teh, and W.A. Smith for technical assistance. D. Janka captained the *Auklet* (fall 2000) and R. Sanford captained the *Alutiiq Sea* (spring 2001). J.S. Mukai performed necropsies in spring 2000. Fish were captured by the skipper and crew of ADFGs *R/V Montague*. Histopathological analysis for determination of *I. hoferi* prevalence was supported by U.S. National Science Foundation grant #9871982 to G.D. Marty.

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Figures

Figure 1. Biomass estimates of adult prespawning Pacific herring in Prince William Sound, Alaska. Unexploited spawning biomass projected in the year before spawning (Projected) and calculated after spawning (Best estimate) using the age-structure assessment model; for 2001 and 2002, the best estimate is based entirely on acoustic data. Estimates were made by coauthor S.D. Moffitt, and by Fritz Funk, Alaska Department of Fish and Games, Juneau, Alaska. Biomass estimates were not projected before the 2001 or 2002 spawning seasons.

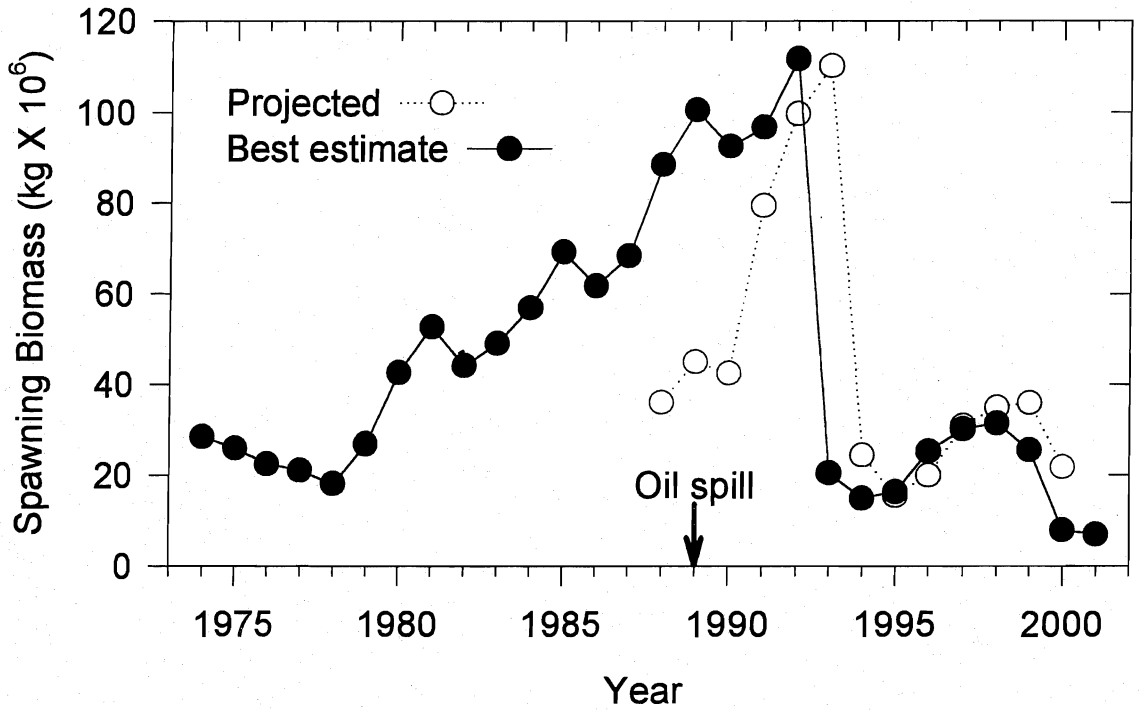
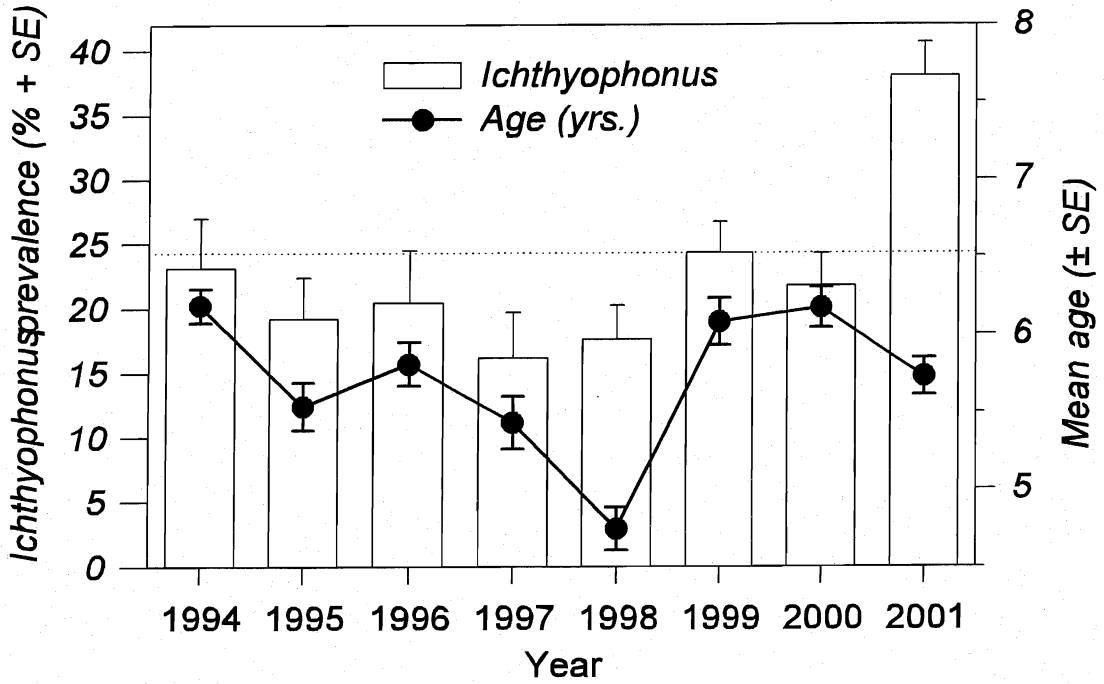


Figure 2. Mean fish age and prevalence of *Ichthyophonus hoferi* in Pacific herring sampled from Prince William Sound, Alaska. Top figure includes all data (n = 233-300 per year); bottom figure includes 2001 data only (n = 80 for Rocky Bay, 220 for the Northeast region).



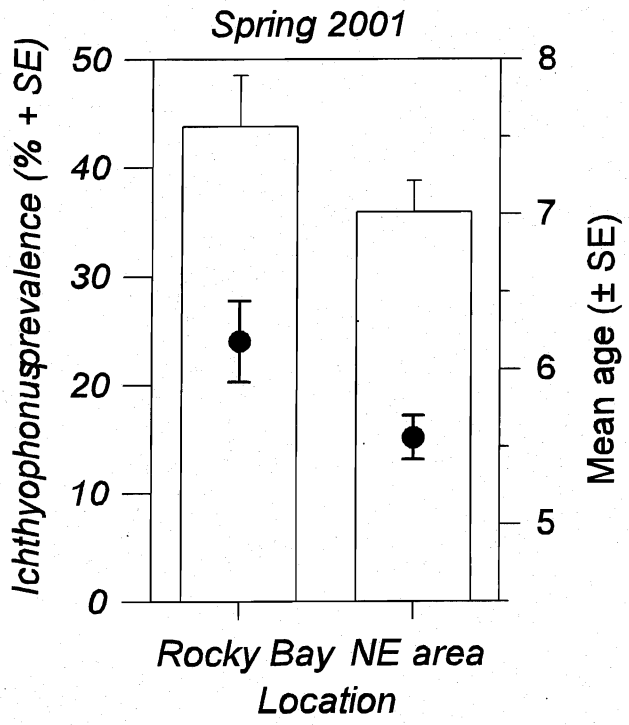


Figure 4. Prevalence of "cysts of unknown etiology" in the gills of adult Pacific herring sampled from Prince William Sound, Alaska.

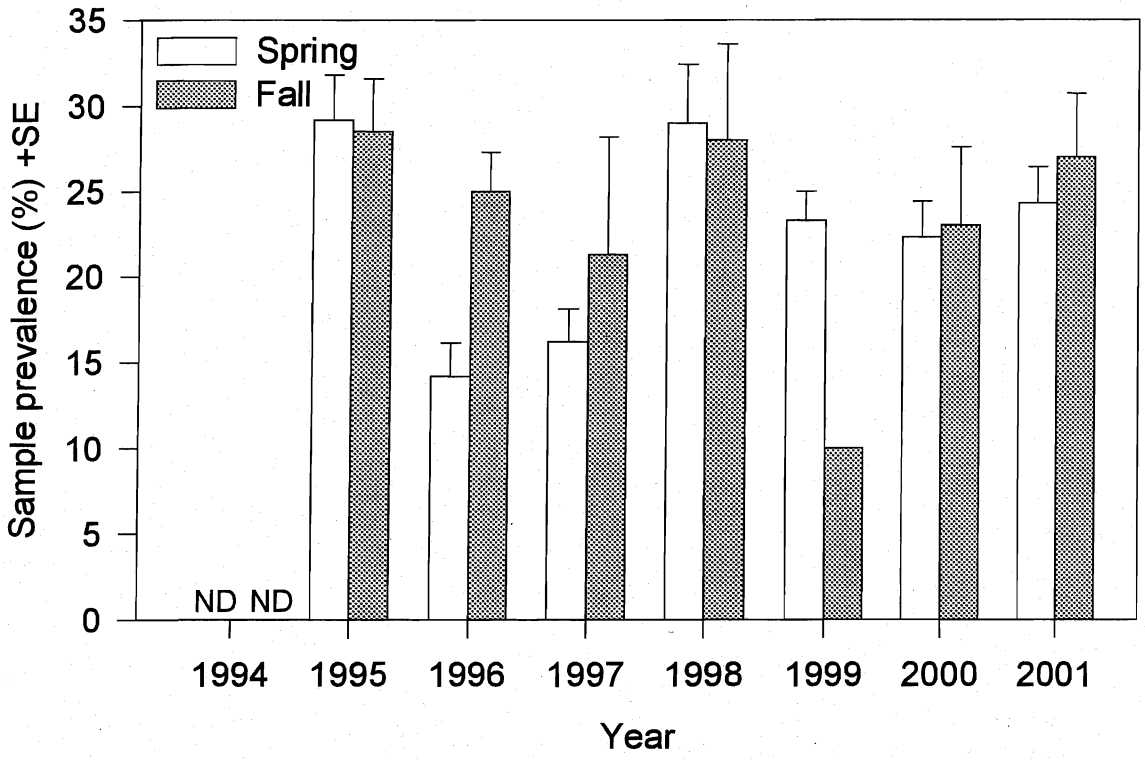


Figure 5. Prevalence of copepod parasites on the medial operculum (gill covering) of adult Pacific herring from Prince William Sound, Alaska.