89-e48

TOXICOPATHIC LIVER DISEASE OF PEN-REARED SALMON IN PORT TOWNSEND BAY – 1988 STUDIES

M. L. Kent
R. A. Elston

Battelle/Marine Sciences Laboratory
Sequim, Washington

January 1989

Prepared for
Washington State Department of Ecology
Olympia, Washington
under Contract 14500

Battelle
Pacific Northwest Laboratories
Richland, Washington 99352

CONTENTS

INTRODUCTION 1

METHODS 4

PULP MILL EFFLUENT BIOASSAY (TASK 1) 4

FIELD STUDIES (TASK 2) 5

RESULTS 7

PULP MILL BIOASSAY 7

FIELD STUDIES 7

Blue Water 7
Crane Point 12

DISCUSSION 14

SUMMARY 18

LITERATURE CITED 19

GLOSSARY 21

FIGURES

1. Location of salmon net pens and pulp mill in Port Townsend Bay 2

2. Normal liver of Atlantic salmon, *Salmo salar* 9

3. Liver of Atlantic salmon, *Salmo salar*, with toxicopathic liver disease ... 10

INTRODUCTION

A severe liver disease, associated with essentially 100% mortality, was observed in Sea Farm Washington's pen-reared Atlantic salmon (*Salmo salar*) in Port Townsend Bay in the summers of 1986 and 1987 (Kent et al. 1988). Mortalities associated with the disease began in August and persisted through the fall months. Fish introduced to seawater pens near Glen Cove and Crane Point in Port Townsend Bay were similarly affected in 1986. In 1987, Atlantic salmon were introduced only at Crane Point and, again, the liver disease occurred at this site. Kent et al. (1988) concluded that the cause of the disease was a waterborne toxicant. The histological appearance of the liver lesions was consistent with those of toxicopathologic diseases, and extensive examinations at Battelle Marine Sciences Laboratory and at the University of California, Davis, did not reveal an infectious agent associated with the condition. The source of the toxicant was most likely through the water column rather than the feed, because liver lesions were not detected in Atlantic salmon that were fed the same commercial diet but maintained (by the same grower) in net pens at Port Angeles. Furthermore, Atlantic salmon maintained at both locations originated from the same freshwater hatchery and were of the same genetic strains. The disease is chronic and, in both years, the prevalence and severity of the disease increased over several months until nearly all the fish died.

Though there is strong presumptive evidence that the disease is caused by a widespread waterborne toxicant in Port Townsend Bay, extensive chemical analysis of affected tissue, water, and sediment by Washington Department of Ecology (Ecology) did not reveal its source or identity (Johnson 1988a,b). The Port Townsend Paper Corporation pulp mill (Figure 1) discharges effluent at the rate of approximately 12 million gallons/day into Port Townsend Bay, and this was a

potential suspected source of the unknown toxicant. As a result, a long-term bioassay of Atlantic salmon, using mill effluent, was conducted. The only other discharge permitted is the U.S. Navy Sewage Treatment Plant in Indian Island (Figure 1), which releases 0.02 million gallons/day of effluent (Johnson 1988a).

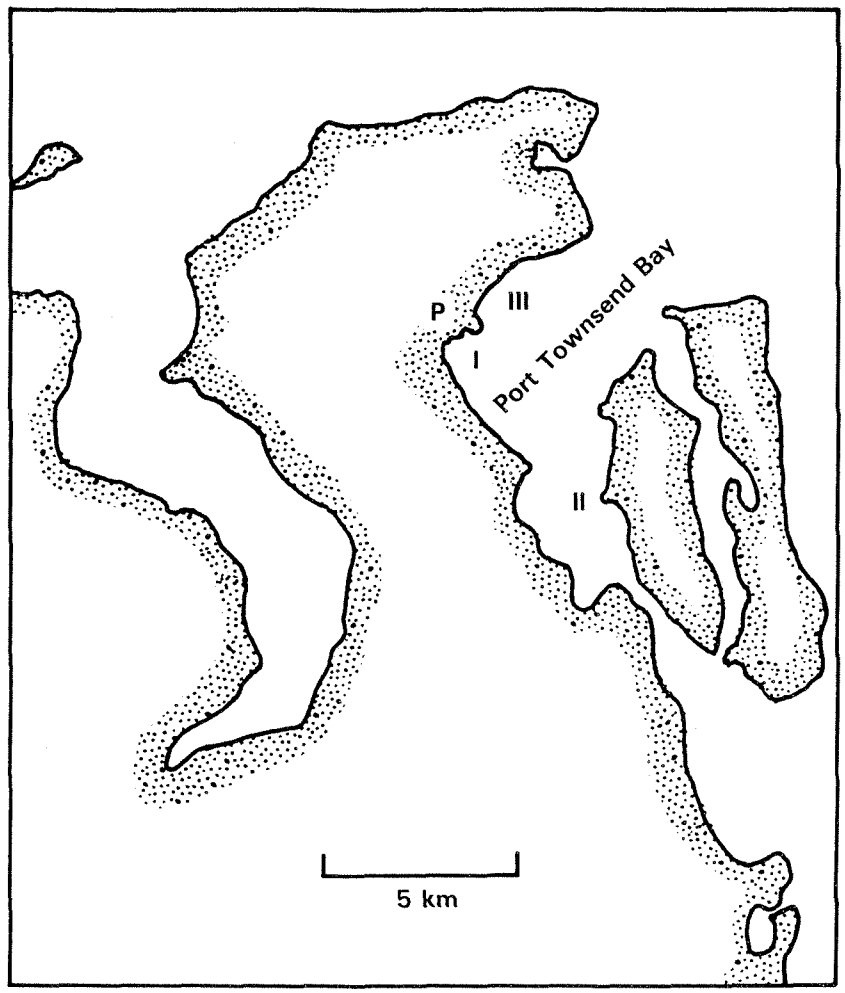

FIGURE 1. Location of salmon net pens and pulp mill in Port Townsend Bay. Site I is the location of Sea Farm's Glen Cove facility, Site II is the location of Sea Farm's Hadlock facility near Crane Point and the experimental pen. Site III is the location of Blue Water Farms. P = location of the Port Townsend Corporation pulp mill

The disease was previously detected only in Atlantic salmon. A third net-pen farm (Blue Water Farms), located near the city of Port Townsend in Port Townsend Bay, reared coho salmon (*Oncorhynchus kisutch*) in 1987, and histological examination of these fish did not reveal the disease. It was not known if the absence of the disease was due to absence of the toxicant at this site or resistance in Pacific salmon. To resolve this question, Atlantic and chinook salmon, *Oncorhynchus tshawtyscha*, were maintained at the Blue Water site and examined throughout the summer of 1988. To determine if nonsalmonid fishes were susceptible to the disease, shiner perch, *Cymatogaster aggregata*, were examined from the net pens at the Blue Water Farms site.

Sea Farm Washington did not rear Atlantic salmon in Port Townsend Bay after the 1987 epizootic. Therefore, an experimental net pen was established at the original Crane Point site to determine if the presumed toxicant and the liver disease would recur in the south part of Port Townsend Bay in 1988.

METHODS

PULP MILL EFFLUENT BIOASSAY (TASK 1)

A bioassay was conducted at the Battelle Marine Sciences Laboratory, Sequim, Washington, to determine the effects of the effluent from the mill on Atlantic salmon. Smolts from Sea Farm Washington's freshwater hatchery were acclimated to seawater at Battelle and maintained in 200-L glass aquaria with undergravel biological filters. The exposures were conducted in static systems. The temperature was maintained between 12-15°C by placing the aquaria in a water bath. Twenty fish (average wt, 50 g) were maintained in each aquarium and exposed to pulp mill effluent at 30%, 10%, 1% or 0% (control). Each exposure concentration was conducted in duplicate. Fresh effluent was collected weekly from Port Townsend Paper, and the water in each aquarium, with the appropriate effluent, was changed weekly with clean sand-filtered seawater from Sequim Bay. To insure that the previous week's effluent was removed prior to replacing the samples, the aquaria were flushed with ambient seawater for 24 h before water changes. Therefore, fish were exposed to effluent for 6 days per week. Dissolved oxygen, total ammonia, salinity, and pH in each aquarium were determined weekly at the beginning and end of each exposure cycle. Temperature in each aquarium was recorded daily. This experiment was initiated on 7 June 1988 and conducted for 4 months. Fish were fed daily to satiation with Oregon Moist Pellet feed.

Monthly samples of 10 fish from each exposure group (5 fish/aquarium) were examined by histology for 4 months. Samples of gills and visceral organs (including the liver) were fixed in Davidson's solution and processed using standard

techniques; slides were stained with Harris' hematoxylin and eosin. Mortality in all groups was recorded throughout the study.

The carcasses of the salmon in the bioassay were wrapped in aluminum foil and frozen at -70°C for future chemical analysis. Additionally, pulp mill effluent samples from each weekly collection were frozen and saved for future chemical analysis.

FIELD STUDIES (TASK 2)

Examination of fishes maintained at Blue Water Farms and in an experimental net pen near Crane Point (Sea Farm's Hadlock site) was conducted to determine the geographic distribution and species specificity of the liver disease.

Blue Water Farms received approximately 1,400 Atlantic salmon smolts (average wt, 50 g) from Scan-Am fish farm on 7 June 1988. Monthly samples of Atlantic salmon were collected and examined from Blue Water Farm and Crane Point. Atlantic salmon from Blue Water were examined on 9 June, 8 July, 8 August, 12 September, and 7 October 1988. Two year-classes of chinook salmon, reared at Blue Water, were also examined on these dates. One group of chinook (large chinook) were introduced to the Blue Water site in October 1987; they ranged in weight from approximately 400 to 8000 g at the sample times. The other group of chinook (small chinook) were introduced on 16 June 1988; these fish were 10-20 g at the time of sampling. Fifteen fish from each group were examined at each sample time unless otherwise indicated.

Donaldson rainbow-steelhead, *Salmo gairdneri*, were introduced to net pens at Blue Water in June 1988; 10 of these fish were examined on 12 September 1988.

The experimental system at Crane Point consisted of a standard fish net pen and a vinyl-coated wire cage (8 ft^3). The fish were maintained in this cage, which was anchored within the pen. One hundred twenty Atlantic salmon smolts from Scan-Am, along with 60 large chinook salmon and 120 shiner perch, were placed in the Crane Point cage on 9 June 1988. Seals and sea lions apparently ate all but 11 chinook and 2 shiner perch by the time of the first 1-month samples (11 July 1988). Therefore, an additional 120 Atlantic salmon, 60 chinook, and approximately 100 shiner perch were introduced to the system. Again, seals destroyed all the fish before samples were taken, and a third attempt was made to maintain fish at this site. A rigid net cage with a hinged lid (4 x 4 x 8 ft) was placed in the net pen, and 100 Atlantic salmon from Sea Farm Washington's freshwater hatchery were placed in this pen on 29 August 1988. The livers of 15 of these Atlantic salmon were examined on 12 September and 7 October 1988. In addition to histological examinations, carcasses of fish were wrapped in aluminum foil and frozen at -70°C for future chemical analysis.

Dr. Michael Kent examined and interpreted the histological material. Representative slides from both the bioassay and field study were examined by Dr. Jack Fournie, U.S. Environmental Protection Agency, Environmental Research Laboratory, Sabine Island, Gulf Breeze, FL 32516.

RESULTS

PULP MILL BIOASSAY

No histological signs of the liver disease were observed in any fish examined throughout the study. Except for the control aquarium, mortality was minimal, and most fish appeared generally healthy, although some fish in the control and 1% aquarium appeared thin and lethargic. Over the 4-month period, 10 fish died in the control aquaria, 1 fish died in one aquarium with 1% effluent, 1 fish died in the group exposed to 10% effluent, and no deaths occurred in the 30% effluent. The water temperature ranged from 12 to 14.5°C in all aquaria except immediately following the weekly addition of fresh effluent. At that time, the water temperature was elevated to 16-17°C in the 30% aquaria and 15-16°C in the 10% aquaria due to the warm effluent temperature. Dissolved oxygen, which was near saturation in all aquaria, ranged from 7.4 to 8.4 ppm. The pH was between 7.4 and 8.0 in all aquaria. The pulp mill effluent was freshwater, thus the salinity in the aquaria was correspondingly diluted by the amount of added effluent. Control aquaria had 28 to 30 ppt salinity; that for 1% effluent was 27 to 29 ppt; for 10% effluent, 25 to 27 ppt; and for 30% effluent, 18-20 ppt. At the end of each weekly cycle during the first month the ammonia levels in some aquaria rose to 0.25 to 5.0 ppm. Ammonia was undetectable after the first month in all aquaria.

FIELD STUDIES

Blue Water

Mortality. Mortalities in the Atlantic salmon smolts introduced to seawater in June 1988 were severe throughout the summer and, by October, cumulative mortality in these fish was approximately 60%. Mortalities in the large chinook

(introduced in October 1987) and the Donaldson rainbow trout were minimal throughout the summer and fish appeared generally healthy at all sample times. There was high mortality in the small chinook throughout the summer, with a cumulative mortality of approximately 22% by mid-October 1988. No moribund shiner perch were observed in the net pens throughout the study.

Histology. 9 June 1988. Histological examination of livers from Atlantic salmon introduced on 7 June 1988 and chinook salmon introduced in October 1987 revealed no pathological changes in the livers (Figure 2).

8 July 1988. The livers of Atlantic salmon examined after 1 month in seawater exhibited early histological changes suggestive of the liver disease. Twelve livers exhibited mild nuclear pleomorphism, eight livers exhibited individual necrotic hepatocytes, and one liver had a small ductule proliferative lesion with abundant mitotic figures. Histological examination of the two year-classes of chinook salmon showed no signs of the liver disease.

8 August 1988. Two months after introduction to seawater, a random sample of 17 Atlantic salmon smolts revealed pathological changes consistent with the liver disease in 12 fish. The livers of 12 of 17 fish exhibited nuclear pleomorphism and megalocytosis, and necrotic or vacuolated hepatocytes were scattered throughout the liver parenchyma (Figure 3). All affected livers exhibited a varying degree of diffuse proliferation of bile ductule cells and this was also observed in one liver without megalocytosis or nuclear pleomorphism. All affected livers exhibited a loss of tissue architecture corresponding to the severity of megalocytosis and nuclear pleomorphism. Though there was a high prevalence of these changes, the overall severity was less than that observed in moribund fish from Crane Point and Glen

Cove in 1986 and 1987. Examination of 10 shiner perch collected from a net pen at Blue Water on 8 August 1988 revealed no signs of the disease.

One of the livers of the 15 small chinook exhibited early, mild changes consistent with the liver disease. There was proliferation of bile ductule cells and individual necrotic hepatocytes, and some hepatocytes exhibited nuclear pleomorphism. The other fish showed focal inflammatory lesions in the liver suggestive of bacterial kidney disease. None of the livers of the large chinook showed indications of the liver disease.

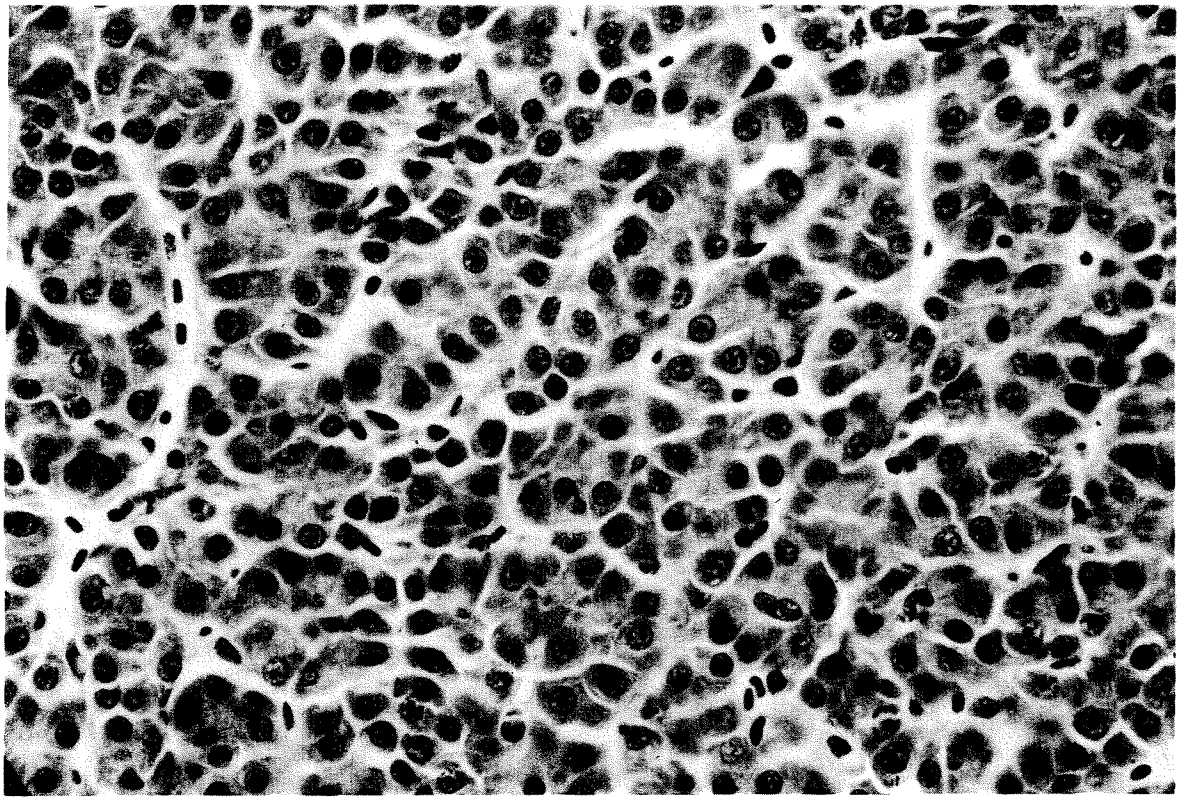

FIGURE 2. Normal liver of Atlantic salmon, *Salmo salar*. Note well defined tubulosinusoid architecture and homogeneous size of hepatocyte nuclei. Hematoxylin and eosin. X 600.

12 September 1988. All 15 Atlantic salmon examined exhibited the liver disease. The disease ranged from mild to prominent, and the histopathological changes were characterized by proliferation of ductule cells and nuclear pleomorphism, leading to frank megalocytosis in severely affected fish. In contrast to livers of moribund fish, there was minimal necrosis and vacuolation of the hepatocytes. Multifocal granulomas consistent with bacterial kidney disease were observed in one liver.

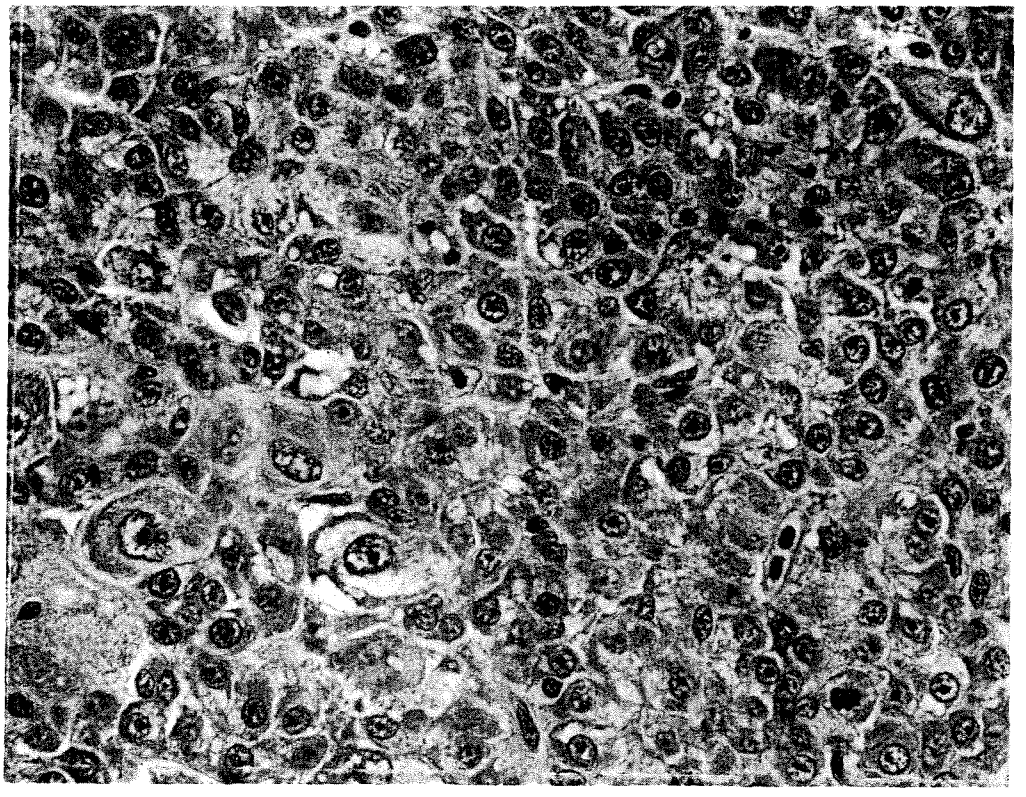

FIGURE 3. Liver of Atlantic salmon, *Salmo salar*, with toxicopathic liver disease. Note nuclear pleomorphism, megalocytosis, and loss of normal architecture. Hematoxylin and eosin. X 600.

Some of the small chinook (introduced in June 1988) exhibited the liver disease. Of the 15 fish examined, 4 exhibited severe lesions, 2 had moderate involvement, and the remaining 9 fish had very mild to nonexistent changes. Severely affected livers exhibited massive, diffuse necrosis and hypertrophy of hepatocytes, prominent megalocytosis, and complete loss of the normal liver tubulosinusoid architecture. Mildly affected livers had essentially normal parenchymal architecture, nuclear pleomorphism of hepatocytes, and mild bile ductule proliferation. The large chinook (introduced in October 1987) exhibited no signs of the liver disease except that one liver showed mild bile ductule cell proliferation.

Examination of nine apparently healthy Donaldson rainbow trout revealed histological changes consistent with the liver disease. The livers of two fish exhibited moderate megalocytosis, bile ductule cell proliferation, and perivascular cuffing. The liver of a third affected fish showed severe perivascular and peritubular cuffing, and proliferation of the bile ductule cells. Examination of 15 shiner perch revealed no signs of the disease.

7 October 1988. All the Atlantic salmon examined exhibited histological signs of the liver disease. One fish exhibited mild changes and had essentially intact liver architecture. Livers of the remaining 14 fish showed moderate to severe involvement typical of the disease: prominent perivascular cuffing, diffuse necrosis and vacuolation of hepatocytes, megalocytosis, and proliferation of bile ductule cells. Examination of small chinook revealed the liver disease in 14/15 fish; however, only one fish exhibited severe pathological changes, three fish were moderately affected, and 10 fish showed only mild changes. No large chinook or shiner perch were examined at this time.

Crane Point

13 July 1988. Examination of the remaining 11 chinook and 2 shiner perch placed in the net pen on 9 June 1988 revealed no histological signs of the liver disease.

12 September 1988. Examination of the livers of 15 Atlantic salmon smolts introduced into the cage on 29 August 1988 revealed the liver disease in 14 of the 15 fish. However, the changes were mild in all but three fish. Livers of mildly affected fish were characterized by nuclear pleomorphism, hypertrophy of hepatocytes, and occasional necrotic hepatocytes, with the histological architecture essentially intact. The most obvious change in all livers was bile ductule cell proliferation. The two more severely affected fish exhibited prominent megalocytosis. One fish exhibited multifocal granulomas consistent with bacterial kidney disease.

7 October 1988. All the fish examined exhibited the liver disease, with lesions that ranged from moderate to severe. All livers showed the characteristic perivascular cuffing, megalocytosis, and loss of liver architecture as the most prominent changes. One liver showed a large thrombus in a hepatic vein, and three livers showed focal granulomas suggestive of bacterial kidney disease.

Dr. Fournie concurred with Dr. Kent's histological interpretations of tissues from both the pulp mill bioassay and the field study.

DISCUSSION

The complete absence of the liver disease in Atlantic salmon smolts exposed to effluent from the pulp mill indicates that this was not the source of the hepatotoxicant. Water-quality parameters were acceptable in the aquaria throughout the 4-month bioassay, except for elevated ammonia levels before weekly water changes during the first month. This was likely due to inadequate establishment of nitrifying bacteria in the biological filters in the aquaria. Only one fish died in the groups exposed to 10 and 30% effluents, which further demonstrates that the effluent was not harmful to the fish.

Though no liver disease was observed in the pulp mill bioassay under laboratory conditions, the disease occurred in fish maintained at both Blue Water Farms and Crane Point. This indicates that, though the mill was not the source, the toxicant was again present throughout Port Townsend Bay in the summer of 1988. Previous studies in 1986 and 1987 indicated that only Atlantic salmon were susceptible (Kent et al. 1988), but identical liver lesions were observed in both chinook salmon and steelhead-rainbow trout. Of the two groups of chinook salmon maintained at Blue Water Farms, only the small-fish group was affected. This indicates that, at least for chinook salmon, smaller fish are more sensitive to the disease.

The disease has not been observed in nonsalmonid fishes. Shiner perch from the Blue Water net pens in 1988 were negative for the disease. Furthermore, histological signs of the disease were not observed in 25 shiner perch and 15 herring, *Clupea harengus*, collected from within net pens at the Crane Point site during an epizootic on 1 October 1987. Nor was the disease detected in 16 English

sole, *Parophrys vetulus*, collected by otter trawl near the Crane Point site on 21 August 1987.

Atlantic salmon introduced to the experimental net pen on 29 August 1988 showed early signs of the disease when examined 15 days later. This suggests that the presumed toxicant was present throughout Port Townsend Bay in the summer of 1988 and was still present at the end of August.

The histological changes in the initial phase of the disease were similar to those reported previously (Kent et al. 1988). In contrast, in the study reported here, vacuolation and severe necrosis of hepatocytes were not always predominant features, and proliferation of biliary ductules was often observed. This difference may have been due to the health status of the fish in study. Whereas livers of moribund fish were usually examined during the 1986 and 1987 epizootics, apparently healthy fish were examined in our study. It is likely that the severe necrotic changes in the livers of moribund fish from the previous years masked the proliferative changes in biliary ductules.

The source and identity of the presumed waterborne toxicant have yet to be determined. The Port Townsend pulp mill is the only source of significant discharge into the bay, and the pulp mill effluent bioassay indicated that the mill was not the cause of the disease. The Washington State Department of Ecology conducted extensive chemical analyses of affected Atlantic salmon tissues, water, and sediment from around the pens and found no indication of chemical contamination (Johnson 1988a,b).

In Washington, the liver disease has been observed only in fish reared in Port Townsend Bay, but recently we have detected it in pen-reared Atlantic salmon at four separate locations in British Columbia (Kent 1988). These sites are located in apparently unpolluted areas. This observation, with the recent findings from studies of Port Townsend Bay, suggests that the cause of the disease may be a natural toxin.

Our present hypothesis is that the agent may be a phytotoxin from algae. Algal blooms are common at the affected sites, and the disease invariably occurs during summer, when these blooms are most prevalent. There have been several reports of algal blooms associated with mortalities in fishes (Gaines and Taylor 1986). Other than investigations of gill lesions caused by *Chaetoceros* spp. (Bell et al. 1974; Ferrington 1988), little is known of the pathological effects of high concentrations of algae on pen-reared fish.

There are several similarities between the salmon liver disease and liver disease caused by pyrrolizidine alkaloids (PA). These compounds occur in several terrestrial and marine plants, and PA toxicity is widespread in domestic animals (McLean 1970). Hendricks et al. (1981) experimentally induced liver lesions in rainbow trout, *Salmo gairdneri*, with PA. As with the net-pen salmon liver disease, megalocytosis, hepatic necrosis, and bile duct proliferation are characteristic of PA toxicity in both mammals and rainbow trout. Furthermore, similar to our observations in Atlantic salmon, rainbow trout fed PA began dying a few months after exposure, and at necropsy the livers often appeared shriveled, mottled, and yellow (Hendricks et al. 1981). Natural PA toxicity has not been reported in fishes, and PA have not been associated with marine algae.

Further research on the Atlantic salmon liver disease should be directed toward determining the source and identity of the presumed toxin. This would include comparing algae that occur at affected and unaffected sites, which should identify algae correlated with the liver disease. Likely candidates would be grown in culture and Atlantic salmon experimentally exposed to high concentrations of the algae to determine if they cause liver lesions. Pyrrole metabolites of PA can be identified in affected livers (Maddocks 1986), and we have saved several grams of affected livers for this analysis.

SUMMARY

Investigations of the Atlantic salmon liver disease in Port Townsend Bay in 1988 indicated that the pulp mill was not the likely source of the presumed toxicant. The disease has also occurred at several net-pen sites in British Columbia but does not appear to be associated with chemical contamination. The disease could be caused by a natural toxin, possibly an algal toxin related to pyrrolizidine alkaloids, but this hypothesis would have to be tested with further research. Field studies verified that Atlantic salmon are the primary species affected, but rainbow trout-steelhead and chinook salmon are also susceptible. The disease was not detected in shiner perch, herring, or English sole. Chemical analysis of affected liver and muscle tissues, sediments, effluents, and the water column in separate but coordinated studies did not identify toxicants of likely significance. The occurrence and lethality of the liver disease in at least three salmonid species at several sites in the Pacific Northwest indicate that it may be a significantly limiting factor to successful net-pen culture in some Pacific Northwest locations.

LITERATURE CITED

Ferrington, C. W. 1988. Mortality and Pathology of Juvenile Chinook Salmon (*Oncorhynchus tschawytscha*) and Chum Salmon (*Oncorhynchus keta*) Exposed to Cultures of the Marine Diatom Chaetoceros Convolutus. Master's Thesis, University of Alaska-Juneau. 80 pp.

Gaines, G., and Taylor, F. J. R. 1986. "A Mariculturist's Guide to Potentially Harmful Marine Phytoplankton of the Pacific Coast of North America." Mar. Res. Sec., Fish. Branch, B.C. Ministry of Environ., Info. Rep. 10. Ministry of Environment, Victoria, BC, Canada.

Hendricks, J. D., Sinnhuber, R. O., Henderson, M. C. and Buhler, D. R. 1981. "Liver and Kidney Pathology in Rainbow Trout (*Salmo gairdneri*) Exposed to Dietary Pyrrolizidine (Senecio) Alkaloids." Exp. Mol. Pathol. 35:170-183.

Johnson, A. 1988a. Port Townsend Pen-reared Salmon Mortality; Results of Screening Surveys for Toxic Chemical in Tissues, Sediments, Seawater, and Effluents October-December 1987. Washington State Dept. Ecology, Olympia, Washington, Seg. No. 09-17-01, 33 pp.

Johnson, A. 1988b. Chemical Analyses of August 1988 Port Townsend Bay Seawater Samples. Washington State Department of Ecology Memorandum to B. Yake. Washington State Department of Ecology, Olympia, WA.

Kent, M. L. 1988. "Geographic Distribution of, and Species Suspectibility to, a Toxicopathic Liver Disease." Am. Fish. Sec./Fish Health Sect. Newsletter 16(4):5.

Kent, M. L., Myers, M. S., Hinton, D. E., Eaton W. D. and Elston, R. A. 1988. "Suspected Toxicopathic Hepatic Necrosis and Megalocytosis in Pen"Reared Atlantic Salmon, *Salmo salar*, in Puget Sound, Washington, USA." Dis. Aquat. Org. 4:91-100.

Maddocks, A. R. 1986. Chemistry and Toxicology of Pyrrolizidine Alkaloids. Academic Press, Hardcourt Brace Jovanovich, Montreal.

McLean, E. K. 1970. "The Toxic Actions of Pyrrolizidine (*Senecio*) Alkaloids." Pharmacol. Rev. 22: 429-483.

GLOSSARY

Granuloma – Focalized inflammatory response characterized by the presence of macrophages clustered in an elliptical formation around the causative agent or around a central necrotic area.

Hepatocytes – Liver parenchymal cells.

Histology – The study of the structure of tissues.

Hypertrophy – Enlargement.

Megalocytosis – Hypertrophy of the nucleus and cytoplasm. In this report it specifically refers to hepatocytes.

Necrosis – Death of a cell or group of cells as a result of injury, disease, or other pathologic states.

Nuclear pleomorphism – Significant variation in the size and shape of nuclei.

Parenchyma – The specialized epithelial portion of an organ, as contrasted with the supporting connective tissue.

Perivascular cuffing – Accumulation of inflammatory cells around blood vessels.

Thrombus – A blood clot occurring in the wall of a blood vessel where the endothelium is damaged.

Tubulosinusoid – Referring to the tubular arrangement of the liver parenchymal cells.

Vacuolation (of hepatocytes) – Vacuolation is caused by dilation of the endoplasmic reticulum cisternae, the presence of lipid droplets, or glycogen.