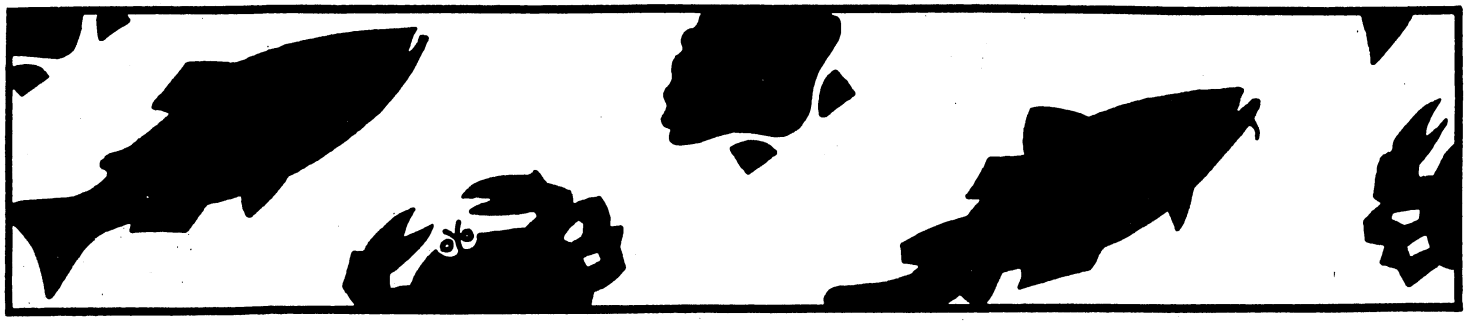

State of Washington Department of Fisheries

RESULTS OF THE GRAYS HARBOR COHO SURVIVAL INVESTIGATIONS, 1987-1990

Edited by
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Kurt Fresh

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Washington State Department of Ecology
Washington State Department of Fisheries

May 1992

Technical Report

Number: 118

TECHNICAL REPORTS

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State of Washington
DEPARTMENT OF FISHERIES

TECHNICAL REPORT NO. 118

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Final Report of Research

May 1992

Edited by

Steve Schroder
Kurt Fresh
WASHINGTON DEPARTMENT OF FISHERIES

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EXECUTIVE SUMMARY AND RECOMMENDATIONS

INTRODUCTION

Wild and hatchery coho smolts emigrating through the Chehalis Basin and its estuary have consistently survived at lower rates than coho originating from other coastal watersheds. This survival difference is important since 80 to 100 thousand coho plus additional chinook and steelhead could be added to annual coastal catches if it were corrected. In addition, the low abundance of wild coho from the Chehalis River has often put constraints on ocean fisheries.

From 1987 through 1990, scientists from the Washington Department of Fisheries, National Marine Fisheries Service, Oregon Cooperative Fishery Research Unit-Oregon State University, University of Washington, U.S. Fish and Wildlife Service, Washington Department of Ecology, and U.S. Environmental Protection Agency investigated where, geographically, fish from the Chehalis watershed were being impacted. A secondary objective was to identify the factors that were stressing or directly killing the fish. The approach used was to compare coho juveniles sampled throughout the Chehalis River and its estuary (the inner harbor) with fish that had been collected from the Humptulips River and North Bay where survival is considered normal.

Four possible explanations for the survival problem were investigated. These were: 1) that freshwater rearing areas in the Chehalis and Humptulips watersheds possess environmental characteristics that produce coho with dissimilar abilities to

survive in seawater, i.e. to complete smoltification; 2) that pathogens or parasites present in the Chehalis Basin or its estuary directly induce mortalities or interfere with smoltification; 3) that the eventual death of many smolting coho that emigrate through the Chehalis River estuary is caused by chronic physiological stress and reduced immunocompetence initiated by poor water quality in the inner harbor; and 4) that predators in the Chehalis Basin and estuary cause some or all of the observed losses of coho salmon.

RESULTS

The evaluations made on smolting coho in the Humptulips and Chehalis basins demonstrated that both drainages produce comparable wild smolts. Indeed, some wild fish originating from the Chehalis Basin were higher quality smolts than those coming from the Humptulips watershed. On the other hand, coho produced from the Humptulips Hatchery had consistently better indices of smoltification than fish from the Simpson Hatchery. This may have been caused by the disease episodes that occurred at the hatchery during our two year evaluation period. The results of the freshwater smolting assays clearly indicated that rearing conditions in the Chehalis Basin were not responsible for the coho survival problem during our study.

The disease and parasite surveys disclosed that coho in the upper watersheds of both rivers had low pathogen levels and similar parasite loading rates. Moreover, wild coho from both basins responded equivalently to a secondary stress test, indicating that

they had experienced similar levels of stress while rearing in freshwater. Immunocompetence assays made on wild fish showed that coho from the Chehalis watershed had a slightly greater capacity to resist disease than those collected from the Humptulips. Yet, the opposite trend occurred in hatchery fish, probably because coho raised at Simpson Hatchery had experienced a disease outbreak just before being sampled.

The most notable feature of the health evaluations was the occurrence of a digenetic fluke, *Nanophyetus salmincola*, in both watersheds. Typically, coho captured in the inner harbor had higher counts of metacercaria (a resting stage of the parasite) than those sampled in the Humptulips estuary. We feel Chehalis River coho acquire high loadings of *Nanophyetus* as they emigrate through the lower portions of the river. Fish held in live boxes placed in the lower river became heavily infested with this parasite. Heavy infestation obviously places physiological burdens on fish and makes them vulnerable to additional stressors. Live box trials showed that a combination of parasitism and additional stress can cause heavily infested fish to die at higher rates than those with lower *Nanophyetus* burdens. In summary, the health screenings indicated that parasitism by *Nanophyetus* could play a role in the coho survival problem in Grays Harbor if fish experience additional physiological stress.

Juvenile coho that had been beach seined or held in enclosures in the inner harbor commonly had elevated cortisol titers and reduced immunocompetencies when compared to fish collected in North

Bay. In addition, fish barged through the inner harbor and later challenged by a natural outbreak of *Vibrio* in seawater netpens had mortality rates that were four times those experienced by fish barged through North Bay. Mixed function oxidase tests conducted on coho held in live boxes revealed that xenobiotic compounds (biologically foreign chemicals) were widely dispersed throughout the inner harbor. Collectively these data demonstrate that the inner harbor contains environmental conditions that can stress coho at a very sensitive stage in their life-cycle. Such stressors by themselves or in combination with others may cause mortality.

Two continuous-flow bioassays were performed to examine the effects of specific effluents on smolting coho. Fish exposed to 30% Weyerhaeuser pulp-mill effluent for five days had higher mortality rates than other treatment populations. Necropsy data collected on these fish seven months after exposure showed several organ dysfunctions. However, fish exposed to lower concentrations of Weyerhaeuser and ITT Rayonier effluents were not different from control fish. Yet all coho used in the bioassays died at very high rates suggesting that factors beyond exposure to effluents had affected the assays. Mixed function oxidase tests revealed that EROD activities (liver enzymes involved in the metabolism of toxicants and other foreign compounds) had increased in fish exposed to Weyerhaeuser effluents. This response was both dose- and time-dependent and showed that the effluent contained xenobiotic compounds. Tests with "Y"-mazes demonstrated that coho would avoid low concentrations of pulp-mill effluent if given a choice. These

tests also illustrated that concentrations of ITT Rayonier effluent which the fish could not detect were capable of impairing olfactory acuity. Moreover, the stamina of smolting coho was reduced when they were forced to swim in waters laden with pulp-mill effluents.

A suite of water quality assessments were made to determine if chemical compounds injurious to aquatic life were present in Grays Harbor. Some of these chemicals were found, but none could be conclusively linked to the salmon survival problem. Nonetheless their presence has degraded the inner harbor environment.

The chemical constituents of several major effluent streams, i.e. wastewaters from two sewage treatment plants, and pulp effluent from ITT Rayonier and Weyerhaeuser were also examined. The wastewaters from the sewage treatment plants were typical of such facilities and did not elicit any unusual bioassay effects. Numerous potentially toxic chemicals were found in both the ITT and Weyerhaeuser effluents, but all were below concentrations previously shown to impair aquatic organisms. Nevertheless, bioassays performed to assess the toxicity of these effluents established they were toxic to some organisms. Unidentified constituents in the effluents appeared to be responsible, since no correlations between toxicity and concentrations of known chemicals were observed. This finding is not surprising since the individual compounds targeted for analysis represented less than one percent of the estimated 4,000 kg/day of chlorinated organics discharged into the inner harbor by the two pulp mills in 1989. Analytical methods to detect many of the remaining compounds have not been

developed and whether these chemicals by themselves or in combination are toxic to young salmon remains to be discovered.

Comparable water quality assessments were made on Chehalis River water. These tests showed only trace amounts of metals and organic compounds. Chemicals like pesticides and herbicides that can impair smolting coho were not detected.

Previously gathered information on predators known to exist in Grays Harbor was reviewed to determine whether these animals could significantly reduce coho survival. This review indicated the foraging habits of birds and mammals would preclude them from being responsible for the survival problem. Field sampling, however, revealed that a robust population of squawfish exists in the Chehalis River while none were detected in the Humptulips River. Thus, a two year study was conducted to estimate their impact. The results of this work established that squawfish could consume up to 7% of the hatchery fish released into the Chehalis drainage. However, only about 0.5% of the wild fish were eaten by this predator. Although responsible for some loss, squawfish predation could not account for the consistently large survival difference that exists between Chehalis and Humptulips fish.

CONCLUSIONS

First, whatever is preventing Chehalis coho from surviving at expected rates does not impact the fish while they are rearing in freshwater. Previous work by the Washington Department of Fisheries in the Chehalis watershed shows that when the basin is adequately seeded it produces smolts at rates comparable to other

watersheds in western Washington. The present study verified these fish were also well smolted and thus should survive at rates comparable to other coastal populations. Second, the predation evaluation indicated that piscivores in the river and estuary played only a small part in the survival differential. Third, we ascertained that heavy parasitism by *Nanophyetus* coupled with additional stressors can cause coho to die prematurely. The lower portion of the Chehalis River possesses ideal habitat for the first intermediate host of this parasite (a freshwater snail) and consequently some emigrating coho probably become heavily infested as they head toward the sea. Conditions in the inner harbor were shown to stress fish; thus, we hypothesize that degraded water quality coupled with high parasite loadings work in concert to cause exceptionally high mortalities to occur in Chehalis River coho.

RECOMMENDATIONS

One of the few constants in the history of the Chehalis coho survival problem is that the chemical nature of effluents entering the inner harbor continuously changes. Both mills, for instance, have recently gone to high substitution of chlorine in their bleaching processes to reduce the quantity of chlorinated organics discharged into the inner harbor. Additionally, both have instituted new measures to prevent or decrease the introduction of spills or chemicals into their waste streams. ITT Rayonier has improved its solids removal and stopped discharging activated sludge. Moreover, this mill has increased aeration of its large

treatment pond and thus will reduce the immediate oxygen demand adjacent to its outfall. The Weyerhaeuser mill has installed oxygen delignification, a process that requires less use of chemicals, surfactants and bleaching agents during the pulping and bleaching process. Furthermore, Weyerhaeuser has stopped using sulfuric acid for coliform control. This mill's effluent is now continuously discharged into the inner harbor with a pH $\geq$ 5 as opposed to a twice a day release of pH 3 effluent. Both mills just applied for new NPDES permits. A recent requirement of these permits is that pulp effluents must pass a sensitive bioassay which uses oyster larvae. This condition is currently under review but if it is upheld, and effluents are improved to meet it, appreciable gains in water quality will be realized. The Army Corps of Engineers has also agreed not to dredge the inner harbor during the coho migration period. Collectively these changes may substantially improve the water quality of the inner harbor. Recently, however, the inner harbor was deepened and widened. The new harbor configuration may retain fish, hold mill effluents and other waste waters for longer periods than previously existing conditions and therefore exacerbate water quality impacts.

Most of these modifications took place after our field work had been completed. Thus, our first recommendation is to ascertain the consequences of these changes. This can be accomplished by continuing the wild and hatchery coded-wire tagging program that has been conducted in Grays Harbor for the past decade. The marine harvest contribution rates of these fish should be appraised over

the next three brood years (i.e. 1990, 91 and 92) to determine if the survival problem has been alleviated. Until that assessment, studies designed to further investigate the mechanisms responsible for coho mortality in the Chehalis watershed should be curtailed. Our second recommendation is to expand the tagging program to include fall chinook. We feel that this species may be more impacted than coho because of its longer residency in Grays Harbor.

Third, if Chehalis coho continue to survive at unacceptable rates, then the combined effects of *Nanophyetus* infestation and exposure to various effluents on smolting coho should be investigated. In addition, *Ceratomyxa*, a myxosporidian parasite known to cause mortalities in salmonids, was just discovered in the lower Chehalis River. The effect of this organism on juvenile coho survival should be also investigated.

Fourth, resources should not be spent on characterizing rearing habitats in the Chehalis watershed. This basin's capacity to produce expected numbers of high quality smolts has been empirically assessed. Habitat should be maintained and improvements encouraged. However, the benefits of these efforts will be diluted if the survival block that exists in the lower river and estuary is not corrected.

Fifth, last spring the USF&WS performed a contaminants survey in Grays Harbor. Some preliminary data obtained from that study showed that *Corophium* (an epibenthic amphipod that feeds on particulate matter) collected in Bowerman's Basin had dioxin concentrations of around 24 to 25 parts/trillion. This finding was

interesting since previous evaluations of dioxins, furans and related compounds in Grays Harbor sediments, waters, and animal tissues have been low. *Corophium* is an important salmonid food item and is also consumed by many of the shorebirds that use Bowerman's Basin as a rest stop during their seasonal migrations. What physiological effects dioxins and furans have on the animals that feed on *Corophium* should be analyzed. Moreover, to determine if the levels observed in Bowerman's Basin were atypical, extensive sampling of this amphipod should occur throughout Grays Harbor. Such sampling should be concentrated in mud flat areas that have not been disturbed by navigational dredging. At a minimum, collections should occur in the mud flats associated with North and South Channels, since dioxin has been detected in these areas. Dioxins and furans in other marine crustaceans, like Dungeness crabs and burrowing shrimp should be also evaluated because of their feeding habits and importance in estuarine food webs.

Finally, the biological and water quality assessments made during this study showed that the inner harbor was degraded. Improving water quality in this area is recommended, not only to promote salmon survival but also to enhance the production of other important species. Some of the recent changes described above may have already improved inner harbor water quality. By continuing to tag and monitor coho and other Chehalis salmonids, the success of these and related efforts to improve environmental conditions in Grays Harbor will be cogently portrayed by the species we are trying to restore.

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ACKNOWLEDGEMENTS

An undertaking of this magnitude requires the assistance of a great many people, not only in the field but also in the planning, writing, and editing phases. Some of the individuals and organizations that helped, deserve special thanks for their outstanding contributions. Conrad Mahnken, Wally Zaugg, Walt Dickhoff, Lee Harrell, Brian Beckman, Bill Waknitz, Gary Winans, and Glenn Snell from the NMFS, were key participants in this study. Connie and his staff helped design the overall study and were responsible for evaluating the smolt status of coho collected in both freshwater and marine habitats. In addition, they performed all the pathogen screening work, helped setup and perform a variety of bioassays, assisted in fish collection, were major contributors to this report and acted as editors, figure generators, and reviewers. Other NMFS divisions also made significant contributions. Usha Varanasi, Doug Weber, John Stein, Tracy Collier, and Mark Nishimoto performed all the mixed function oxygenase evaluations and also helped during the continuous-flow bioassays. Their reports and careful reviews of parts of this document are greatly appreciated.

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The assistance of Tom Quinn, Mary Moser, and Alan Olson from the University of Washington is greatly appreciated. Tom helped develop portions of the overall study design, and he and Mary and Alan tracked coho juveniles through the inner harbor and the Chehalis River. Before conducting these studies they carefully evaluated the effects of various types of acoustic tags on fish behavior. Furthermore, Mary evaluated the range and suitability of several acoustic tags. Lowell Stuehrenberg of NMFS generously

provided all the radio tags that were used to follow coho in the Chehalis River. In addition Lowell supplied the tracking stations that were employed to monitor the movements of radio tagged smolts. Lynwood Smith of the University, performed all the swimming endurance tests and also helped plan the experimental approach used in this study.

Quite a number of people from the Washington Department of Fisheries helped conduct this study. Two of our staff, Mark Carr and Gene Sanborn, deserve special thanks for their exceptional contributions. Mark's superb operation of our sampling boats and fish collection skills were invaluable. Moreover, he was responsible for maintaining a host of sampling equipment and for getting us where we needed to be to beach seine fish, to run *in situ* assays, and to electroshock coho and squawfish in the lower Chehalis and Humptulips Rivers. Mark also produced a number of figures and carefully reviewed this document. Gene designed and built a large and complex continuous-flow bioassay tank farm which he and Mark helped run while the fish were being exposed to effluents. Furthermore, Gene coordinated and participated in the collection of thousands of gallons of effluents, a job that often lasted 12 or more hours/day which could not have been accomplished without the assistance of Dan Stuckey and his heavy equipment operators. Dave Seiler, Pat Hanratty, Steve Neuhauser, Mike Ackley and Pete Topping not only helped procure fish samples for us but also built and operated the barges used to transport fish in the inner harbor and North Bay. Dave was also a very important

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Several individuals from the Washington Department of Ecology made substantial contributions. Foremost among them is Art Johnson. Not only was Art largely responsible for most of the water quality work that Ecology performed during the course of this study but he was also an invaluable editor. His excellent comments on the water quality portion of this report were greatly appreciated. Lynn Singleton was involved in the early design stages of this project, and he made several insightful suggestions which strengthened the experimental approach. Other Ecology staff were also helpful, either in the field or in developing experimental protocols.

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should be used to hold fish in both the *in situ* and continuous-flow bioassays. Moreover, he made critical procedural suggestions on how a variety of chemical assays should be performed and on what types of blood chemistry parameters needed to be measured. Evan Hornig coordinated most of EPA's water quality work while Dave Terpening helped extensively in the field and also ensured that a number of Grays Harbor samples were incorporated into national programs. The EPA chemists at EPA's Manchester Lab deserve special thanks for the hundreds of complex analyses they were required to perform. Rob Pedersen, although not originally involved with this study, was responsible for analyzing and writing the results of EPA's field work. His willingness to coordinate this effort with us and Art Johnson was very helpful.

A number of institutions and individuals in the local community made important contributions as well. Gene Schermer and John Smith from Grays Harbor Community College gave us permission to set up a lab at the College and generously lent us several microscopes. The city of Aberdeen let us moor boats at several of its docks. Jim Barkman, the manager of the Aberdeen Sewage Treatment Plant and his staff were especially supportive. Jim not only allowed us to set up our tank farm at his plant, but he assisted us in numerous ways while we were constructing and operating the tank complex. Dean Grossman, president of Ocean Shores Harbor Inc. was also very generous. The Grays Harbor Regional Planning Commission coordinated a number of meetings and actively lobbied the State Legislature for funding support.

Monies to perform this study came from a variety of sources. A small pilot study performed in 1987 was funded by the Weyerhaeuser Co., ITT Rayonier, Washington Department of Ecology, Washington Department of Fisheries, and the Grays Harbor Soil Conservation District. Work performed from 1988-1990 was supported by a special legislative allocation of funds sponsored by Senator Brad Owen. Besides this direct funding, all the agency's and universities involved in performing this study made substantial in-kind contributions. Ecology and EPA for example, supported all of the water quality investigations that were performed.

Probably the most remarkable aspect of this multi-agency study was the dissolution of institutional and agency barriers. Personnel, equipment and supplies were shared by all the cooperating agencies to ensure that the study's objectives would be met. The formation of such a large and diverse scientific team is a credit to all of the individuals who participated in this investigation.

PART I: INTRODUCTION

GENERAL BACKGROUND

Grays Harbor is the northernmost of three large estuaries located on the Washington coast. Two major river systems empty into the harbor; one, the Humptulips, has a 245 square mile (628 km^2) watershed and discharges into North Bay, an undeveloped area of the estuary. The other river, the Chehalis, drains 2,200 square miles (5,640 km^2) and empties into a narrow region of Grays Harbor referred to as the inner harbor (Fig. 1). Unlike North Bay, this portion of the estuary is heavily industrialized; it currently receives 48 to 50 million gallons (181 to 189 x 10^6 Liters) of pulp mill effluent per day plus smaller amounts of leachates from a variety of sources such as landfills and log storage areas plus effluents from three sewage treatment plants. Besides these inputs, the navigation channel in the inner harbor is dredged regularly, so at certain times of the year, including the spring, suspended sediments are also present.

Over the past sixty years numerous water quality and fish survival studies have taken place in Grays Harbor (Beyer et al. 1979; Seiler 1989). For instance, the Washington Pollution Control Commission (now the Department of Ecology) investigated complaints of dead and distressed fish and shellfish in the inner harbor in the early 1930's. Similar complaints occurred again several years later and at this time water quality assessments were made by the State Health Department and the Pollution Control Commission (Beyer

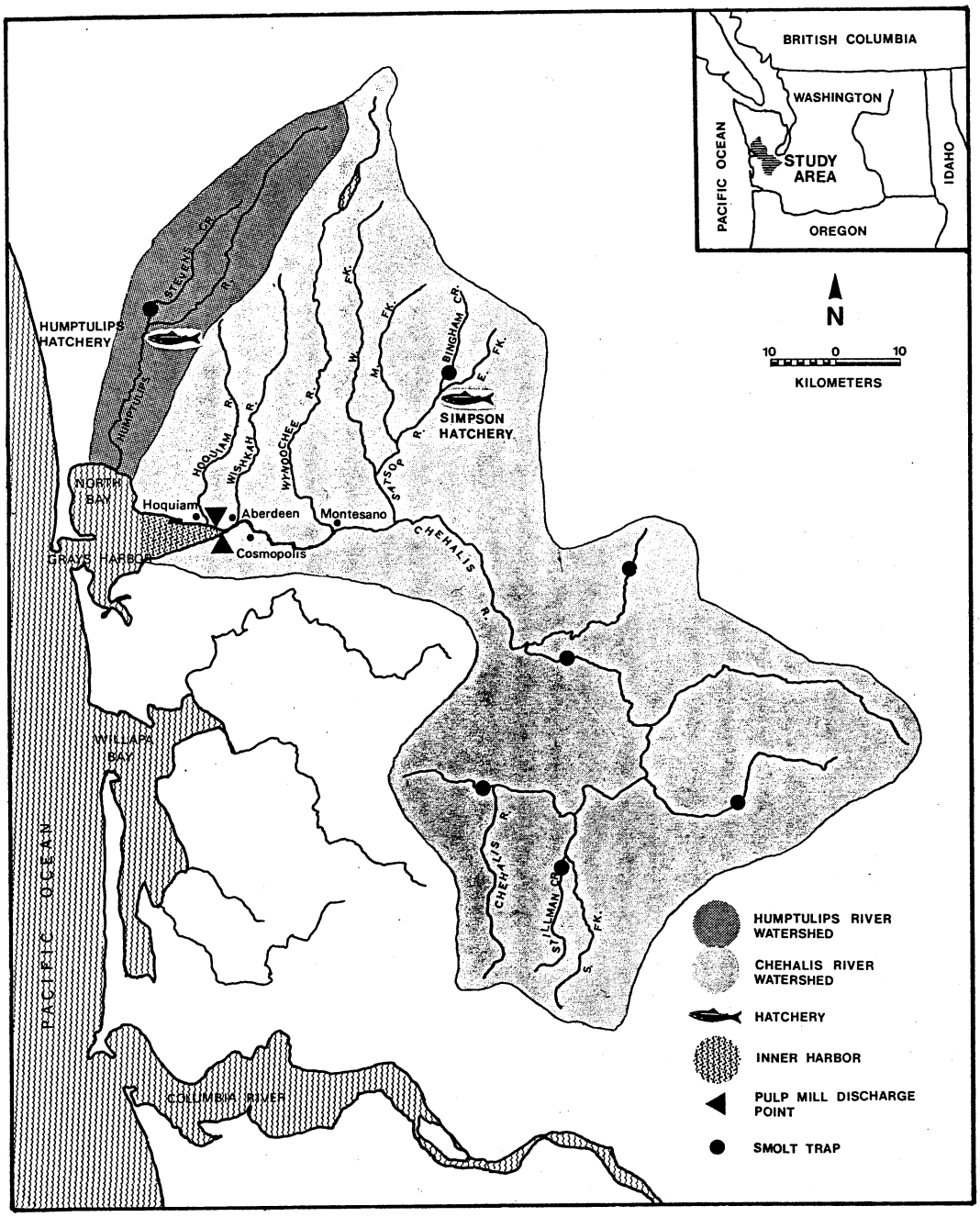

Fig. 1. Map of the Chehalis and Humptulips watersheds showing the locations of the inner harbor, North Bay, pulp mill outfalls, hatcheries, and freshwater fish collection sites.

et al. 1979). Eriksen and Townsend (1940) summarized these studies and performed a detailed hydrographic survey of the harbor. They concluded that waste sulfite liquor was causing low dissolved oxygen (DOs) levels (often near zero) in the inner harbor during low flows (May-October). They also investigated the possibility that adjacent mud flats were reducing dissolved oxygen levels but could find no evidence supporting this contention. Their hydrographic assessments of current patterns revealed that it took approximately 42 days to flush the inner harbor during low flows.

Other investigators subsequently calculated flushing rates in Grays Harbor and demonstrated that tidal patterns, Chehalis River flows, coastal upwelling, atmospheric conditions, and other environmental factors can strongly influence flushing rates. For example, Duxbury (1979) used a salt and freshwater budget approach and determined that within a year, volume exchange rates could range from 0.5 to five days. Pearson and Gotaas (1951) and Stein and Denison (1965) measured how rapidly spent sulfite liquor left the harbor. These studies took place when river flows were relatively low (1300 and 2700 cfs). Both showed that approximately 20% of the effluent was removed per day and that 99% of a given batch of effluent would be transported out of the harbor in approximately 20 days. Duxbury (1979) cites some of this work and states ..."the ability of the inner harbor volume exchange process to remove SSL [spent sulfite liquor] is at best only 65% efficient." Under the river flow conditions observed by Stein and Dension, Duxbury's model also predicted a 20% per day removal rate

for SSL. In a review of these studies, Loehr and Collias (1981) report that the interplay between Chehalis River flows and tidal currents cause particles of water to oscillate back forth in the lower Chehalis River and harbor. Rapid turnover occurs during high river flows while longer residency times take place during periods of low flows.

Because of the proclivity of wastes to remain in the inner harbor during spring and summer months, the ITT Rayonier pulp mill started to pump about 80% of its concentrated sulfite waste liquor into storage lagoons on Rennie Island. These materials were then released throughout the winter when freshwater flows were higher. This tactic, which began in 1946, improved inner harbor water quality (Orlob et al. 1951; Neale 1955) but still left portions of the harbor with substandard dissolved oxygen levels. The pending construction of a sulfite pulp mill by the Weyerhaeuser Company at Cosmopolis prompted further water quality measurements by the Pollution Control Commission (Peterson et al. 1957). Measurements were made before and after the mill began discharging waste liquor. As in previous studies, low dissolved oxygen concentrations were observed during summer months but once flows exceeded 2500 cfs (70.8 m^3) they were generally adequate. Peterson et al. also found that the Weyerhaeuser plant added an additional 60,000 lbs (27,180 kg) of BOD/day (Biochemical Oxygen Demand) to the inner harbor and decreased DO levels by 1 part/million in the South Channel, where the mill outfall is located.

Subsequent sampling by the Commission in 1958 disclosed that

during the late spring and summer, DOs in the inner harbor (from Cosmopolis to the west end of Rennie Island) were inadequate until the ITT Rayonier mill had shut down for routine maintenance. After the mill resumed operation, low dissolved oxygen concentrations again occurred over a 9 mile stretch of the river and estuary (Beyer et al. 1979). It was hypothesized that cold, upwelling ocean water may have been partially responsible for the depressed DOs observed. However, Peterson et al. (1957) and Pearson and Holt (1960) found that upwelling only occurred sporadically, and Pearson and Holt (1960) concluded that the likelihood of this water having a pronounced affect on DOs was low since its ability to flush the inner harbor was substantially less than a comparable volume of freshwater entering at the head of the estuary (Beyer et al. 1979).

A cooperative study involving the Washington State Departments of Fisheries, Ecology, and Game, U.S. Geological Survey and Weyerhaeuser Company was performed during 1964-1966 to further delineate conditions in the inner harbor. This work disclosed that the inner harbor was receiving approximately 500,000 lbs (226,500 kg) of BOD/day and that about 94% of this demand was originating from pulp mill effluents (McCall 1971). In addition, the consumption of oxygen by inner harbor mud flats was examined by using standard bell jar methods. These experiments confirmed the earlier observations made by Eriksen and Townsend (1940) that the mud flat areas produce slightly more oxygen than they consume (Herrmann 1971). In summarizing the entire study, Pine and Tracy (1971) state that improvements in water quality must be made if the

aquatic resources in the harbor are to be protected or enhanced.

At about the time these investigations were occurring, the Washington Department of Fisheries (WDF) observed that a disproportionate percentage of coho (*Oncorhynchus kisutch*) and chinook (*O. tshawytscha*) salmon harvested in Grays Harbor originated from the Humptulips watershed (Deschamps and Johnson 1957). To refine this observation, two mark and recapture experiments were conducted in the mid 1960's. In each instance, 200,000 coho from the Simpson Hatchery (located in the Chehalis watershed--see Fig. 1) were fin clipped, trucked to the Humptulips River and released. At the same time, another 200,000 fin-clipped coho were released several miles above the Simpson Hatchery and allowed to emigrate through the lower Chehalis and its estuary. The contribution rates of these fish to ocean fisheries were different; those that had been trucked and released into the Humptulips survived at about twice the rate as fish that had been released at the Simpson Hatchery (Deschamps and Senn 1969; Wright and Bernhardt 1969; Wright 1970; Seiler 1989).

These results prompted WDF to carry out another fish survival study in 1969 and 1970. In this instance, fall chinook juveniles seined from the inner harbor were placed in live boxes located throughout the inner harbor. The fish were periodically monitored and environmental parameters, including the Pearl-Benson Index which is an indirect measure of spent sulfite liquor, were simultaneously recorded. In both years, high mortalities rapidly occurred, particularly in the live boxes that were placed closest

to mill outfall areas (Deschamps and Phinney 1971). These results occurred, despite generally favorable DO, pH, BOD, and PBI (Pearl-Benson Index) values, suggesting that other factors besides these parameters were creating a toxic environment for juvenile salmonids. Deschamps and Phinney conclude their report with a strong recommendation that detailed laboratory bioassays be performed to more fully evaluate the affects of pulp mill effluents on smolting salmon.

While these studies were being conducted, the ITT Rayonier mill changed its pulping process from a calcium to a sodium based one. Additionally, the Washington Department of Ecology (Ecology) directed ITT to use primary treatment to improve effluent characteristics and both pulp mills were required to reduce their BOD loads to a total of 40,000 lbs/day (18,120 kg) during the low-flow period. The ITT mill also installed a clarifier in 1972 to help remove suspended solids (Deschamps and Phinney 1971; Beyer et al. 1979).

The Weyerhaeuser Company proposed a joint industry-regulatory agency study in 1974 to evaluate the effect of these changes, which were largely mandated by the Federal Water Pollution Control Act Amendments of 1972. During this investigation, ITT Rayonier, Ecology, WDF, and Weyerhaeuser performed live box studies, continuous-flow bioassays, fish stamina tests and ancillary laboratory experiments (DOE 1975). The results of these studies were inconclusive; fish mortalities occurred during some of the bioassays but they could be attributed to a variety of causes (DOE

1975).

In 1973, just prior to this cooperative effort, WDF placed coded-wire tags (CWTs) into two groups of 100,000 fall chinook that were being reared at the Simpson Hatchery. One of these groups was released into the Humptulips while the other was released into the Satsop River near the Simpson Hatchery and allowed to emigrate through the Chehalis system. The chinook that were trucked and liberated into the Humptulips River contributed 18 times as many fish to ocean fisheries as those released from the Simpson Hatchery (Seiler 1989).

These results, which became available in 1979, received little attention because the Clean Water Act of 1977 directed the pulp industry to make major improvements in their wastewater treatment (Seiler 1989). Because of these changes, BOD and suspended solids were significantly reduced in the inner harbor and dissolved oxygen levels were increased. After these improvements, many felt that the salmon survival problem in Grays Harbor had been resolved (Mathews 1981).

In 1980, WDF began a comprehensive, long-term program to evaluate the production and survival of wild and hatchery coho in the Grays Harbor Basin. The goal of this ongoing effort is to quantify Grays Harbor coho salmon production and thereby improve harvest management. Annual coded-wire tagging at the Simpson and Humptulips hatcheries began in 1982. In addition, wild fish have also been tagged at various locations throughout both the Humptulips and Chehalis basins. Results of the first six years of

tagging (Figs. 2a and 2b) have consistently shown that hatchery and wild coho emigrating down the Chehalis River and through the inner harbor contribute to ocean fisheries at one half the rate of comparable fish descending the Humptulips (Seiler 1989; Seiler pers. comm.). A comprehensive review of the ocean catch patterns of these fish revealed that coho from both river systems have comparable oceanic distributions (Seiler pers. comm.). Thus, the observed contribution rates were not caused by unequal fishing pressure but rather by some factor that was causing Chehalis coho to die before they reached harvestable size.

Where this mortality might be occurring was partially examined in 1985. In this study, WDF tagged three separate lots of Humptulips Hatchery coho with CWTs. Two of the three tagged groups were placed in fish planting trucks. One of the trucked groups was transported and released into tributaries of the lower Chehalis, while the other group was driven around the Humptulips Hatchery for 90 minutes and then released into the Humptulips River; this second group was used to evaluate the effects of trucking. The third group was released from the Humptulips Hatchery without being trucked and was therefore a control. In 1986, these fish were harvested in the ocean. The tag recovery data collected on the trucked fish showed that smolts which emigrated down the Humptulips and through North Bay contributed more than 20 times as many fish to ocean sport and commercial catches as those that emigrated through the lower Chehalis (Fig. 3). Since both groups of fish had been trucked for an equivalent period and used the same ocean

CONTRIBUTION TO OCEAN FISHERIES
GRAYS HARBOR COHO SALMON

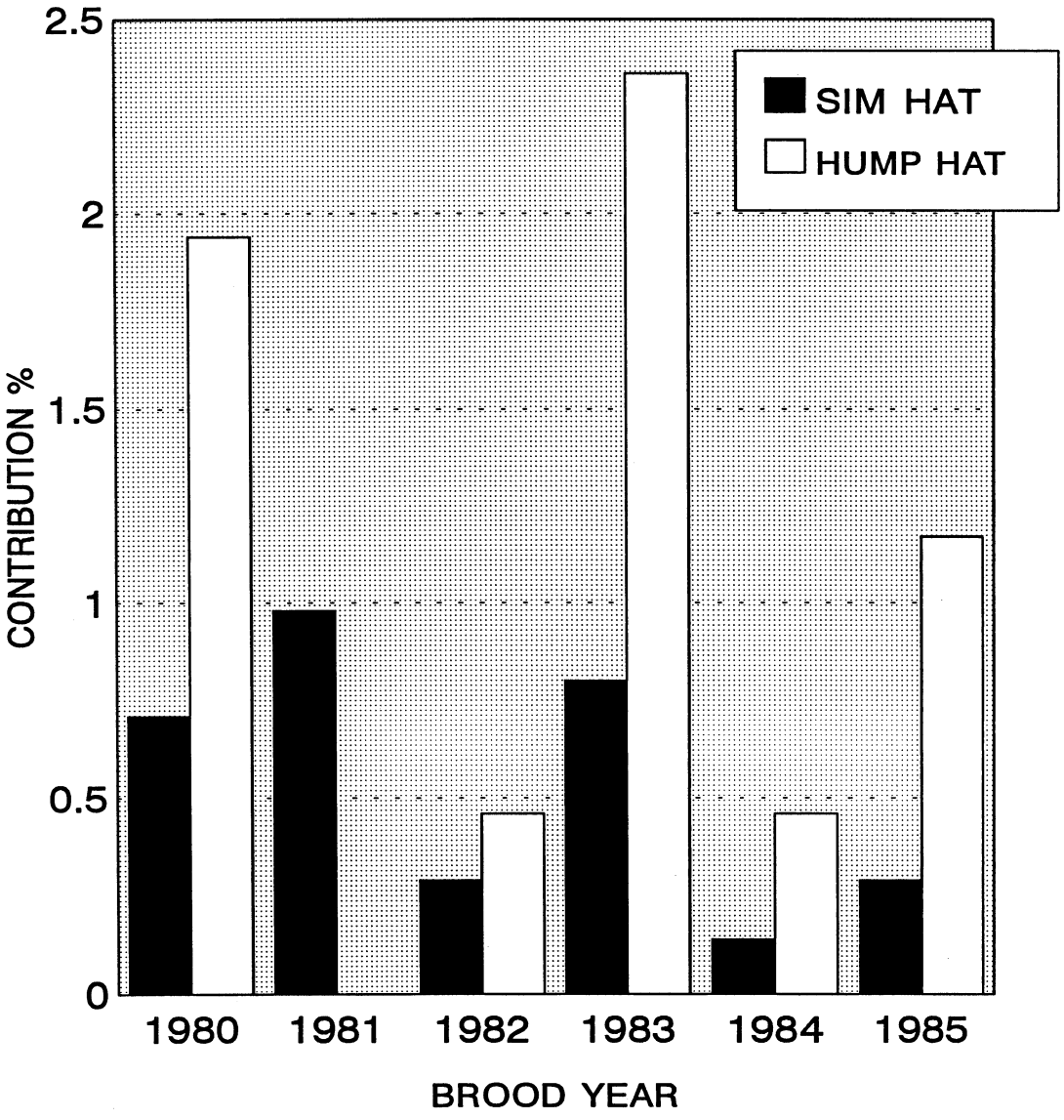

Fig. 2a. A comparsion of the contribution rates of Simpson (Chehalis watershed) and Humptulips hatchery coho to ocean fisheries. In 1981, no Humptulips fish were tagged because of a disease outbreak at the hatchery.

CONTRIBUTION TO OCEAN FISHERIES
GRAYS HARBOR COHO SALMON

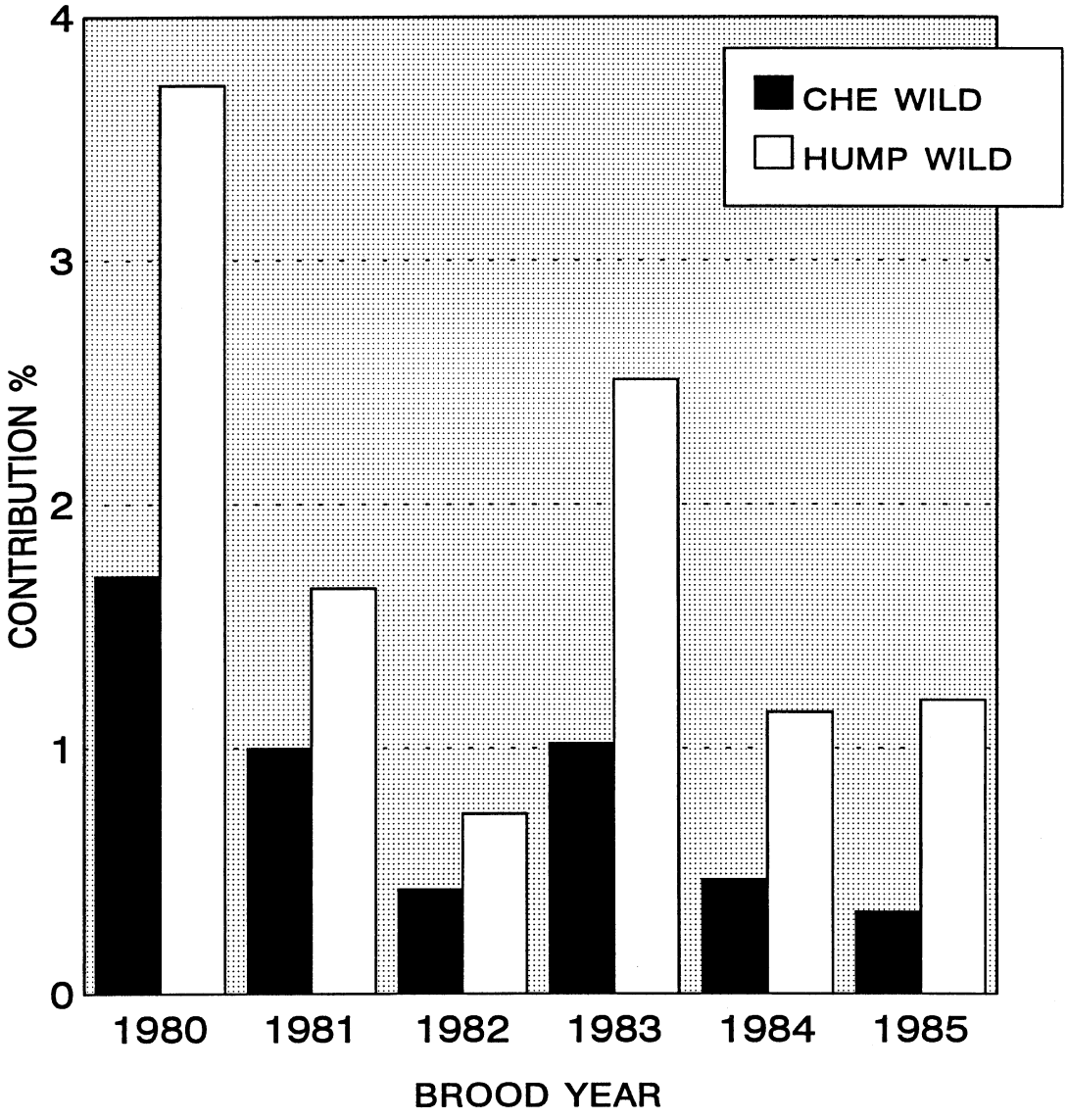

Fig. 2b. A comparison of the contribution rates of Chehalis and Humptulips wild coho to ocean fisheries.

CONTRIBUTION TO OCEAN FISHERIES
HUMPTULIPS COHO SALMON

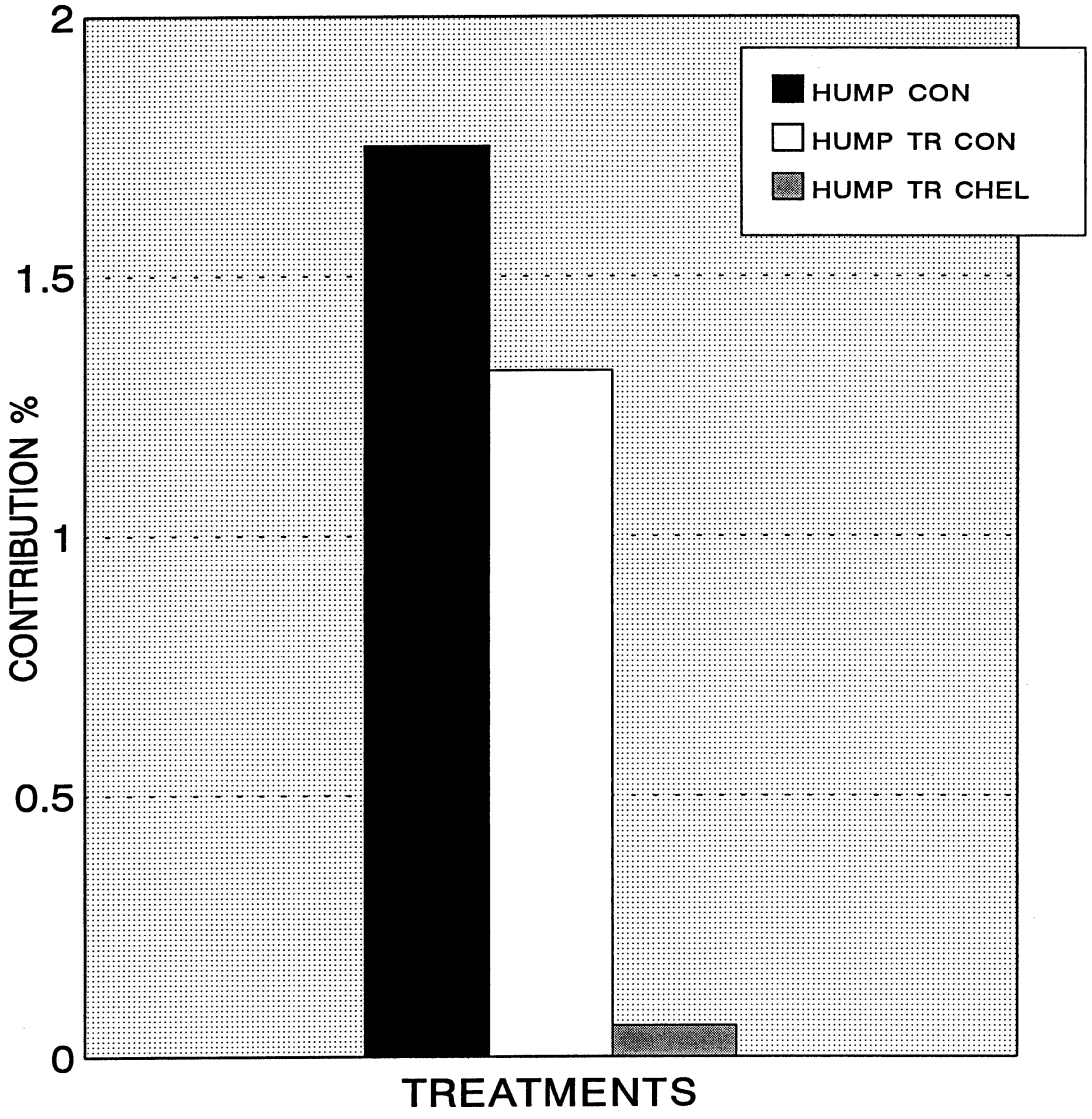

Fig. 3. The results of an experiment that looked at the effects of trucking and release sites on the contribution rates of Humptulips coho salmon to ocean fisheries. The bars, left to right, show the contribution rates of fish that were a) released at the Humptulips Hatchery, b) released at the Humptulips Hatchery after being trucked for 90 minutes, and c) liberated into the lower Chehalis watershed after 90 minutes or less of trucking.

pastures, their differential performance appears to be linked to the migration routes the fish used to reach the outer portions of Grays Harbor. These results are similar to those shown in Figs. 2a and 2b which also suggest that coho emigrating from the Chehalis are being impacted in the lower Chehalis or its estuary, the inner harbor.

DEVELOPMENT OF A STUDY PLAN

Early in 1986, data from most of the post 1982 CWT studies had been analyzed and shared with Ecology and the United States Environmental Protection Agency (EPA). This information was also used to help develop a Chehalis Basin fish survival paper (Seiler unpubl. rep.). This report, a by-product of the Landmark Watershed Planning process, generated considerable interest in the Aberdeen-Hoquiam area. Because of local concerns and the desire of federal and state agencies to resolve the Chehalis fish survival problem, a Policy Committee comprised of industry, federal, state, and local representatives was established. The Policy Committee first met in the fall of 1986. It authorized the formation of a Technical Committee which was given the responsibility of producing a plan that could be used to determine what factors were causing the poor survival of Chehalis coho.

The problem that the Technical Committee addressed was not trivial. Seiler (pers. comm.) estimates that the Chehalis basin is capable of producing 1 to 3 million wild coho smolts per year when the river system is adequately seeded. If these fish survived at rates comparable to other coastal streams they would add an

additional 80 to 100 thousand coho adults to the annual coastal harvest. Additional losses of fall and spring chinook salmon and steelhead trout (*Oncorhynchus mykiss*) further augment the economic importance of the Chehalis fish survival problem.

When the Technical Committee began to develop a research plan, it became obvious three questions had to be investigated before the survival problem had any hope of being resolved. First, where within the watershed and its estuary were the fish being impacted; next, how could such impacts be measured; and finally, what linkages, if any, exist between fish survival and environmental conditions the fish experience while they reside in or migrate out of the Chehalis drainage and its estuary. These questions were packaged into the following four general hypotheses:

1) The upper watersheds of the Humptulips and Chehalis possess characteristics that produce coho with dissimilar abilities to successfully complete smoltification, i.e. to adjust to seawater.

2) Pathogens present in the Chehalis basin or its estuary induce delayed mortalities by interfering with the smoltification process.

3) Poor water quality in the inner harbor caused by point and nonpoint sources induce chronic physiological stresses that prevent successful smoltification or reduce the competency of the immune system to such an extent that the fish eventually die.

4) The Humptulips and Chehalis watersheds and their estuaries possess different kinds or abundances of natural predators and these disparities are great enough to account for some or all of

the observed reduction in coho production from the Chehalis basin.

As these hypotheses indicate, our approach was a comparative one that relied upon letting the fish provide us with information about their well being. Data collected on coho obtained from the Humptulips River and its estuary made it possible to contrast a typical coastal coho population with one that was suffering from abnormal mortality. Humptulips fish could be used as controls because previous data (Seiler 1989) indicated that coho from this system contribute to ocean fisheries at rates comparable to other coastal watersheds or at about twice the rate as fish emigrating from the Chehalis River.

Our hypotheses were tested by following a research plan that laid out a series of tasks that were begun in the spring of 1987 and completed in the winter of 1990. The components of the plan are shown in Tables 1 through 4, which chronologically list the tasks performed to test each hypothesis. In each table, the tasks have been split into two major categories, those that were linked to the biological performance of juvenile coho and those that examined the water characteristics of the habitats they were living in. As the tables show, many of the tasks performed in 1987 were directed toward establishing or developing procedures that could be used in the last two years of the study. Results of this pilot work have been described (WDF/NMFS 1988) but for completeness, portions of our 1988 report will also be presented here.

Tables 1, 2, and 4 are largely self explanatory, however, Table 3 which presents the steps taken to investigate inner harbor

Table 1. Chronological listing of the tasks used to determine whether the freshwater rearing and upper migratory areas in the Chehalis watershed were interfering with smoltification.

TASK #	DESCRIPTION OF TASK
	Biological Assessments Made In 1987
1	The smolt status of hatchery and wild coho juveniles collected throughout the Chehalis and Humptulips watersheds was evaluated. Fish were periodically collected from March through June and temporal changes in Na-K adenosine triphosphatase (ATPase), triiodothyronine (T_3), thyroxine (T_4), hematocrits, fish length, weight and condition factor, body shape and coloration were monitored.
	Water Quality Assessments Made in 1987
2	Water samples collected throughout the Chehalis and Humptulips watersheds were used in a series of 7 day chronic toxicity tests that measured the survival and reproductive capacity of the freshwater cladoceran, *Ceriodaphnia dubia*. Samples were collected in Feb., June and Sept.
	Biological Assessments Made In 1988
3	The smoltification assays that tracked temporal shifts in ATPase, T_3, T_4, cortisol, hematocrits, growth, condition, body shape and coloration were repeated. Hatchery and wild fish from the Chehalis and Humptulips watersheds were sampled from early February through June.
4	The smolt status of coho collected at the mouths of both rivers was also monitored by examining fish captured with an electroshocking boat.
	Water Quality Assessments Made In 1988
5	Additional *Ceriodaphnia* chronic toxicity tests were performed on water samples collected in February 1988 from the Chehalis and Humptulips rivers.

Table 2. Chronological listing of the tasks used to determine if pathogens or stressors present in the Chehalis basin induced delayed mortalities by interfering with the smoltification process.

TASK #	DESCRIPTION OF TASK
	Biological Assessments Made In 1987
1	Disease and parasite screenings were performed on coho salmon collected at the Humptulips and Simpson hatcheries and on wild fish obtained in both watersheds. Histological examinations of liver, kidney, gill and heart tissues were performed and bacterial surveys were made. A review of the incidences of disease at both hatcheries from 1979 through 1987 was also conducted.
	Biological Assessments Made In 1988
2	Disease and parasite screenings comparable to those made in 1987 were performed on fish collected from the Humptulips and Simpson hatcheries and on wild fish obtained throughout both watersheds.
3	The stress levels in hatchery and wild fish collected in the Humptulips and Chehalis basins were ascertained by 1) assessing temporal changes in cortisol titers, 2) exposing fish to a standardized secondary stress test, and 3) assaying their immunocompetence.

Table 3. Chronological listing of the tasks used to determine whether conditions in the inner harbor are responsible for the poor survival of coho emigrating from the Chehalis River.

TASK #	DESCRIPTION OF TASK
	Biological Assessments Made In 1987
1	The smoltification and disease status of coho salmon captured by beach seine in the inner harbor and in North Bay were examined.
2	The migration routes of coho leaving the Chehalis and Humptulips rivers were determined by beach seining in the estuaries of both rivers.
3	The range and signal strength of various radio and acoustic tags that could be used to track coho migrants as they moved through the lower Chehalis and the inner harbor were appraised.
4	The effects of acoustic tags on the behavior and swimming performance of tagged coho juveniles were determined.
5	Barges that could move smolting coho through the inner harbor and North Bay were built and tested.
6	How various levels of *Nanophyetus* (a digenetic trematode) infestation may affect smoltification was explored. Wild coho from both watersheds and cultured fish from the Humptulips Hatchery were placed into seawater net pens and held for 6 months. The relationship between seawater mortality and infestation rate, i.e., the number of metacercarial cysts present in the posterior one-third of the kidney was examined.
	Water Quality Assessments Made In 1987
7	Waste waters and effluents from the Weyerhaeuser and ITT Rayonier pulp mills, Aberdeen sewage treatment plant and dechlorinated water from Lake Aberdeen were screened for toxicants. These assessments were done to collect preliminary chemical and bioassay data on the effects of these materials on smolting coho.
8	An inventory that identified the locations of all urban storm drain outlets in the inner harbor was conducted.

9 Smolting salmon, starry flounders, soft-shelled clams, pulp mill effluents, and suspended and bottom sediments from the inner harbor were collected to help determine if dioxins and related compounds were present. Much of this work was carried out in 1987 by the U.S. Environmental Protection Agency as part of its National Bioaccumulation Study.

Biological Assessments Made In 1988

10 The smoltification and disease status of coho migrants moving through the inner harbor and North Bay was evaluated.

11 The immunocompetence of coho smolts captured by beach seine in the inner harbor and North Bay was assessed.

12 Coho smolts with acoustic tags were tracked through the inner harbor to ascertain their migration speed and routes.

13 Coho from the Humptulips Hatchery were barged through the inner harbor and North Bay in a way that mimicked their normal out-migration patterns. The ATPase, cortisol, and immunocompetence values of these fish plus their disease and parasite status were determined.

14 A continuous-flow bioassay tank farm complex was built at the Aberdeen Sewage Plant. Smolting coho from the Humptulips Hatchery were placed in the complex and exposed to Weyerhaeuser and ITT Rayonier pulp mill effluents, Aberdeen and Hoquiam STP waste waters, Chehalis River water and to positive (12ppb Cu^{++}) and negative control waters for five days. The fish were then transported to the U.S. Fish and Wildlife's Marrowstone Island Laboratory where they were reared in seawater for 8 months. A broad range of blood chemistry parameters as well as growth and survival were routinely monitored.

15 Mixed-function oxidase tests were performed on coho collected from the barge and the bioassay tank farm. In these assays, the activity of liver cytochrome, P-450, is determined. Elevated P-450 levels provide evidence of exposure to biologically significant concentrations of certain chemical contaminants, several of which are toxic to aquatic species.

16 Preliminary studies that examined how pulp mill effluent affected the swimming performance of smolting coho were performed. In addition, how these materials influenced immediate changes in body weight was examined.

17 Two-choice mazes were used to ascertain whether coho juveniles would avoid various concentrations of pulp mill effluents if given a choice.

Water Quality Assessments Made In 1988

18 Three standard bioassay protocols, the *Selenastrum capricornutum* test which measures algae growth, the purple sea urchin fertility test and the seven-day *Ceriodaphnia* test were performed to assess the toxicity of ITT Rayonier and Weyerhaeuser pulp mill effluents. Two of these tests, the *Selenastrum* and *Ceriodaphnia*, were also used to evaluate waste waters from the Aberdeen and Hoquiam Sewage Treatment Plants.

19 Bottom sediments collected in the inner harbor, North Bay and at the western end of the South Channel were examined for EPA priority pollutants, resin acids, guaiacols, fatty acids, herbicides, dioxins and furans.

20 Class II inspections were made on both pulp mills. During these inspections effluents were examined to identify pollutants of concern and characterized to see if they met NPDES permit requirements. Three bioassay procedures, rainbow trout, *Daphnia pulex* and mysid shrimp tests, were used to measure acute toxicity; another assay using oyster larvae was performed to assess chronic effects. Besides these effluent assays, an assay that looked at the survival and reburial rate of *Rhepoxynius* (an amphipod) placed into sediments collected near the mill outfalls was conducted. The Ames test was also used to detect whether the effluents were mutagenic. Finally, the laboratory procedures used by the mills to monitor their effluents were reviewed.

21 Standard assessments of water quality (DO, pH, and temperature plus heavy metal concentations) were made while fish were being run through the barging and continuous-flow bioassays.

22 A continuous-flow centrifuge and XAD resin columns were used to monitor the concentration of materials (e.g., dioxins, furans, etc.) present in the waters and suspended particulate matter of the inner harbor and in the effluents used in the continuous-flow bioassay.

23 The acute toxicity of the pulp mill effluents and waste waters from the Aberdeen and Hoquiam sewage treatment plants used in the continuous-flow bioassay were assessed by conducting bioassays with fathead minnows, water fleas and smolting coho. These tests were run in a portable continuous-flow bioassay trailer and took place while the larger bioassay tank farm was in operation.

Biological Assessments Made In 1989

24 Coho from the Humptulips Hatchery were introduced into seven live boxes, one was located in the lower Chehalis River, five were placed in the inner harbor, and the last two were situated in North Bay. Coho from the Humptulips Hatchery were transferred into the live boxes at two different times and held for 5, 9 or 14 days. When the fish were removed from these structures, some had their ATPase levels, cortisol titers, and immuno-competencies immediately assessed. The remaining fish were transferred into seawater net pens and held for an additional six months to evaluate their survival. Moreover, coho from net-pens at Westport were placed into the live boxes for 8 days to see how the ATPase values of these seawater adapted fish would respond to the environmental conditions surrounding their live boxes. A part of this appraisal examined the association between *Nanophyetus* infestation and marine survival.

25 Continuous-flow bioassays were used to expose smolting coho salmon from the Humptulips Hatchery to 0 (dechlorinated Wishkah River water), 5, 10 or 30% pulp mill effluents for 5 days. In some cases, coho were exposed to mixtures of these effluents, i.e. they were made up of equal amounts of ITT Rayonier and Weyco effluent that gave a final effluent concentration of either 5, 10 or 30%; other groups were exposed to a single effluent. To evaluate how longer exposures to these materials may affect the fish, some groups were held for 14 days in either pure or mixed solutions of 5% pulp mill effluent. After being exposed to effluents, cortisol titers, immunocompetence, ATPase and blood sodium values were obtained on fish from each treatment. Additionally, fish from each treatment were split into two groups, one was trucked to the Manchester Field Station (NMFS) and placed into saltwater net pens to see how the fish would respond to a natural disease challenge. The remaining fish from each treatment, were transferred to the Marrowstone Island Field Station (USFWS) and held in seawater for 9 months to evaluate growth and survival.

26 Mixed-function oxidase tests were performed on coho that had been used in the live box and continuous flow bioassay experiments.

27 Smolting coho were placed into two-choice mazes to determine the concentrations of pulp mill effluents they could detect. Additionally, to see if these substances interfered with a smolt's ability to recognize a known noxious substance (L-serine), coho were placed into mazes that possessed concentrations of pulp effluent that had not been avoided in the first series of tests but that were now laden with L-serine. In this situation, a lack of avoidance would indicate that the effluent was masking or otherwise interfering with a fish's ability to detect other important biological odorants.

28 Smolting coho were tracked through the inner harbor by using acoustic tags. This work was done to gather more information on the migration patterns of coho in the inner harbor and to see if yearly variation in these patterns exist.

Water Quality Assessments Made In 1989

29 Unannounced samples of pulp mill effluents were taken from March through June. The objectives of this sampling program were to: 1) further define the chemical character and toxicity (via bioassays) of these effluents, 2) test for relationships between toxicity and concentrations of effluents, and 3) assess effluent variability.

30 A continuous-flow centrifuge (with XAD resin columns) was used to sample Chehalis River, inner harbor and North Bay waters. This same sampling equipment was also used to characterize the effluents used during the continuous-flow bioassays.

Table 4. A chronological list of the tasks performed to determine whether differences in the kinds or abundance of predators in the Chehalis and Humptulips watersheds can account for some or all of the coho survival problem in Grays Harbor.

TASK #	DESCRIPTION OF TASK
	Biological Assessments Made In 1987
1	A literature review on squawfish, harbor seal and bird predation on salmonid fishes was conducted.
	Biological Assessments Made In 1988
2	The Humptulips and Chehalis Rivers were sampled to determine if both watersheds possessed squawfish.
3	The stomach contents of 159 squawfish collected in the Chehalis River prior to, during, and after smolt migration were examined.
	Biological Assessments Made In 1989
4	The abundance of squawfish $\geq$ 300 mm in the lower 80 km of the Chehalis River was estimated with a mark-recapture procedure.
5	The food habits of 508 squawfish $\geq$ 300 mm collected in two portions of the Chehalis River were examined prior to, during and immediately after the smolt migration period. Since one of the sampled areas possessed only wild coho while the other had a mixture of wild and hatchery fish, it was possible to determine if both types of coho were equally susceptible to squawfish predation.
6	The migration speed and routes of wild coho emigrating down the lower 50 miles of the Chehalis River were determined by tracking fish equipped with radio tags.
7	An estimate of the number of wild and hatchery coho consumed by squawfish in the lower 80 km of the Chehalis River was made.

water quality needs additional explanation. Three different approaches were used to assess the effects of the inner harbor environment on migrating coho. In the first one, fish were simply beach seined throughout the Chehalis and Humptulips estuaries and their health and physiological status were compared. This type of *in situ* sampling can indicate that differences exist among fish captured in various places but since it is impossible to know how long a fish has been in an area such effects are not easily attributed to a particular site. To explicitly assess environmental impacts, it was necessary to carry out a number of controlled bioassays. Two types were used, those that took place in the inner harbor (or *in situ* bioassays) and those that were conducted in a laboratory setting that allowed the effects of particular effluents to be tested either singly or in combination. To determine an appropriate exposure duration for these tests, acoustic tags were used to discern how long wild coho smolts usually stayed in the inner harbor.

The results obtained from performing the tasks listed in Tables 1 through 4 are presented Part II and Part III of this document. Those that examined how the fish responded to the habitats they were living in, are included in Part II, whereas all the water quality assessments are integrated into Part III.

The water quality assessments briefly described in Tables 1 through 4 were performed to characterize: 1) the receiving environment of the inner harbor, including bottom sediments, the water column, and animal tissues, 2) the major effluents released

into the inner harbor, and 3) the Chehalis River water. The results of this work have been placed into Part III by topic, e.g. the findings of all studies that examined Chehalis River water were placed in the same section. The other three water quality topics have been treated similarly.

Many of the ideas, language, and data presented in the following pages originated from contract reports. In many cases, each cooperator was responsible for investigating numerous, but not necessarily linked, parts of the Grays Harbor investigation. Hence, figuratively, each research group manufactured one or more pieces of a puzzle. To present our findings on a hypothesis or topic basis required that each report be dismantled and merged with related parts that had originated from other sources.

A few readers may wish to examine all results produced from a particular research team. Some of this information has been released as reports or is in the peer reviewed literature; in other cases, technical reports or papers that cover this work will be released in the future. The purpose of Table 5 is to identify which parties performed various segments of the work presented here, so that those who desire more information will know whom to contact.

In the Executive Summary, all findings generated by this work have been summarized and recommendations designed to help further resolve the coho survival problem in Grays Harbor are presented.

Table 5. Sources for further details on information collected during the Grays Harbor Salmon Survival Study that has been presented in this report.

PART I: INTRODUCTION

1. Steve Schroder
   Washington State Department of Fisheries
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2. David Seiler
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PART II: BIOLOGICAL INVESTIGATIONS

Hypothesis One: Smoltification

General Information on Smoltification

3. Walton Dickhoff
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4. Conrad Mahnken
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ATPase

5. Waldo Zaugg
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   (509-538-2626)

Thyroid Hormones

6. Walton Dickhoff
   See #3

Cortisol

7. Carl Schreck
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   Oregon State University
   Corvallis, OR 97331
   (503-754-4531)

Morphometrics

8. Gary Winans
   National Marine Fisheries Service
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   Seattle, WA 98112
   (206-553-2737)

Color Patterns and Smolt Appearance

9. Bill Waknitz
   National Marine Fisheries Service
   P.O. Box 38
   Manchester, WA 98353
   (206-553-6479)

Hematocrits

10. Lee Harrell
    National Marine Fisheries Service
    P.O. Box 38
    Manchester, WA 98353
    (206-553-0633)

11. Bill Waknitz
    See #9

Hypothesis Two: Fish Health

12. Lee Harrell
    See #10

Hypothesis Three: Inner Harbor Conditions

General

13. Steve Schroder
    See #1

14. Conrad Mahnken
    See #4

Beach Seine Studies

15. Kurt L. Fresh
    Washington Department of Fisheries
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    Olympia, WA 98504
    (206-586-2116)

Fish Tracking

16. Tom Quinn
    Fisheries Research Institute, WH-10
    University of Washington
    Seattle, WA 98504
    (206-543-7532)

17. Moser, M., A. Olson, and T. Quinn. 1990. Effects of ultrasonic dummy transmitters on juvenile coho salmon, pp. 353-357. In N.C.Parker, A.E. Giorgi, R.C. Heidinger, D. J. Jester, E. Prince, and G.A. Winans (eds). Fish-marking techniques. American Fisheries Society Symposium No. 7.

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Fish Barging

19. Bill Waknitz
    See #9

20. Waldo Zaugg
    See #5

21. Carl Schreck
    See #7

Live Box Studies

22. Bill Waknitz
    See #9

23. Steve Schroder
    See #1

Continuous-Flow Coho Smolt Bioassay

24. Aldo Palmisano
    Gary Wedemeyer
    U.S. Fish and Wildlife Service
    National Fishery Research Center
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    Naval Station
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Swimming Performance and Avoidance

25. Lynwood Smith
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    University of Washington
    Seattle, WA 98195
    (206-543-4650)

26. Carl Schreck
    See #7

Hypothesis Four: Predation

27. Kurt L. Fresh
    See #14

PART III: GRAYS HARBOR WATER QUALITY ASSESSMENTS

General

28. Art Johnson
    Department of Ecology
    Environmental Investigations and Laboratory Services
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29. Gretchen Hayslip
    Rob Peterson
    Rick Albright
    Environmental Protection Agency
    Region 10
    1200 6th Ave.
    Seattle, WA 98101
    (206-553-1868)

Analyses of Effluents

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Chehalis River Water Quality

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37. See #29

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Receiving Environment

39. Johnson, A. and R. Coots. 1989. Survey of chemical contaminants in the bottom sediments of Grays Harbor estuary. Washington Department of Ecology, Olympia.

40. See #29

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PART II: BIOLOGICAL INVESTIGATIONS

HYPOTHESIS ONE: SMOLT STATUS OF COHO SALMON

Introduction and Background

Smoltification, the parr-to-smolt transformation of juvenile salmon, is the developmental process associated with seaward migration and adaptation to ocean residence. Several extensive reviews describing smoltification have been published (Wedemeyer et al. 1980; Folmar and Dickhoff 1980; Hoar 1988). In healthy, unstressed and growing coho salmon, smoltification typically occurs during the second spring of residence in fresh water. The completion of smoltification in fresh water is essential for successful survival and growth in the ocean.

The onset and duration of smoltification is regulated primarily by increases in daylight and water temperature during the spring, although other environmental factors may have a role as well. Many physiological, behavioral, and morphological changes in coho salmon undergoing smoltification have been characterized. Thus, it is possible to measure the progress of smoltification in juvenile fish in fresh water and evaluate their potential for seawater survival.

In this section, we assess whether smolts produced by the Chehalis and Humptulips river basins in 1987 and 1988 were comparable by evaluating the timing and magnitude of changes in various smolt-related characteristics. This comparison was designed to reveal whether noticeable differences in smolt status

existed between coho originating from the two watersheds and whether these differences, if any, could account for the persistently lower survival of Chehalis River fish.

Monitoring Smoltification in Salmonids

To assess smolt status we used the following five criteria: 1) changes in growth, body shape, coloration, and condition factor, 2) gill Na^+-K^+-ATPase activity (hereafter referred to as ATPase activity), 3) thyroid hormone concentration in blood plasma, 4) cortisol concentration in blood plasma, and 5) volume of red blood cells in whole blood (hematocrits). These parameters are all established indices of smoltification and are thus directly linked to seawater survival. For example, Zaugg (1989) demonstrated a relationship between adult recoveries of fall chinook salmon and the development of gill ATPase activity in smolts. Additionally, progressive changes in thyroid hormone levels that typically occur in smolting coho while in fresh water have been correlated with long-term survival and growth in salt water net pens (Folmar and Dickhoff 1981). Moreover, the elevation of blood cortisol during smoltification appears to promote the acquisition of seawater tolerance in smolts (Specker and Schreck 1982). A brief description of each of these indices is provided below.

Growth, Body Shape, Condition Factor, and Coloration

Previous work has shown that significant changes in external appearance occur during smoltification (Salo and Noble 1954; Vanstone and Markert 1968; Hoar 1976; Prentice et al. 1981; Gorbman et al. 1982; Winans 1984; McMahon and Hartman 1988). For one, the

animals shift from color patterns characteristic of cryptically-colored parr to silvery smolts. A decrease in condition factor accompanies the change in color (Vanstone and Markert 1968; Hoar 1976; Clarke and Nagahama 1977; Rodgers et al. 1987). Specific changes also occur in body proportions, particularly in the caudal peduncle. The peduncle tends to grow more quickly than other body areas early in smoltification resulting in a thinner, more streamlined tail region (Winans 1984; Winans and Nishioka 1987). Streamlining of the body is transitory and other body regions "catch up" through differential growth later in development. The streamlining process, specifically the differential growth of the caudal peduncle, is linked to increasing thyroid hormone concentrations and elevated ATPase values (Winans and Nishioka 1987; Zaugg and Beckman unpublished data).

ATPase Activity

One of the most important events that occurs during smoltification is development within the gill of a "salt pumping" system to excrete excess salt. Fish accumulate salts internally in seawater environments by osmosis and by drinking seawater. If fish were unable to excrete salt, physiological functions would be impaired and result in death. Excess salt is "pumped" out of the body into the surrounding seawater environment by a process involving the energy-requiring ion transport enzyme called adenosine triphosphatase (ATPase). Since operation of this enzyme system requires the presence of sodium and potassium ions and takes place in the gills, it is referred to as gill sodium, potassium-

stimulated ATPase (or ATPase). Elevated levels of ATPase are essential to smolt survival in seawater. Present evidence indicates that all anadromous salmonids must increase their ATPase levels while they reside in fresh water. This must also occur to a certain extent for artificially-produced salmon while they reside in the hatchery, but more completely as they migrate seaward.

Thyroid Hormones

Thyroid hormones play important roles in almost every phase of development in salmonid fishes, including growth, coloration, migratory restlessness, and acquisition of seawater readiness (Grau et al. 1981; Dickhoff and Sullivan 1987). Involvement of the thyroid in the smoltification process was first suggested by Hoar (1939) who observed that thyroid tissues of Atlantic salmon (*Salmo salar*) were active during the parr-smolt transformation. Since that time, endocrinologists have discovered that two thyroid hormones, triiodothyronine (T3) and thyroxine (T4), apparently influence the rate of the smoltification process (Grau et al. 1981). Studies of coho salmon have shown that there is a noticeable peak and decline in T4 levels just prior to outmigration (Dickhoff et al. 1978). In fact, Folmar and Dickhoff (1981) found that survival of hatchery coho appeared to be linked to the portion of this surge completed prior to seawater entry.

Cortisol

Cortisol is a corticosteroid produced by interrenal tissue that has a number of functions. Increased cortisol production is one of the primary responses of fish to physiological stress

(Wedemeyer et al. 1990). Elevated levels of cortisol enable a fish to rapidly use energy reserves. Cortisol not only helps a fish cope with stress but it also plays a role in smoltification. Elevations in resting plasma cortisol levels have been observed during smoltification in coho salmon (Specker and Schreck 1982). Cortisol appears to affect Na^+ transport and activity of ATPase in the gill. Additionally, Richman and Zaugg (1987) suggested that cortisol specifically stimulated chloride cell proliferation or differentiation.

Hematocrit Counts

Hematocrits increase during smoltification (Koch 1982; Sullivan et al. 1985; Hoar 1988) and also serve as an indicator of health and stress (Wedemeyer and Yasutake 1977). The normal range of hematocrits is between 30 and 50% (Wedemeyer and Chatterdon 1971).

General Model of Smoltification

Published data on changes in body shape, gill ATPase activity, and thyroxine (T4) and cortisol concentrations can be summarized in the form of a general model (Fig. 1.1) that shows how these indices are expected to change during smoltification in fish held in fresh water. Smoltification in this model begins in February (parr stage) and, when fish are retained in fresh water, reaches completion (smolt stage) by mid-May. Fish that migrate seaward undergo further physical and physiological changes.

Multi-character changes in body shape can be monitored by principal component analysis. In smolting coho, the second

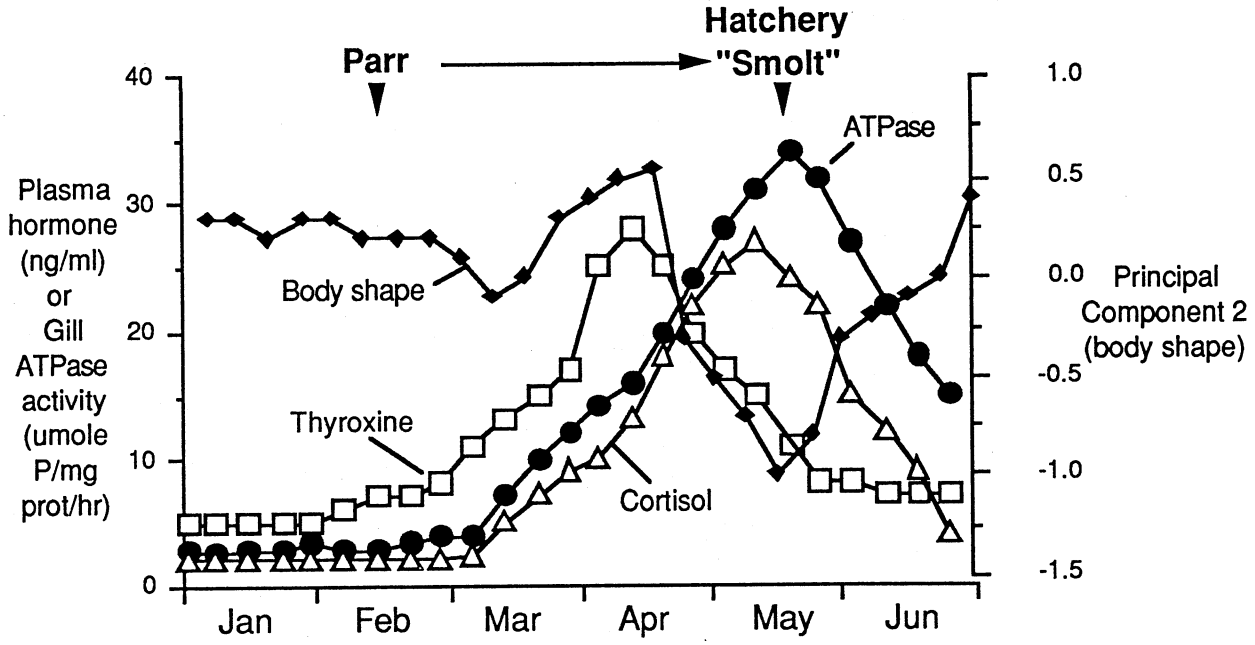

Figure 1.1. Changes in body shape (principal component 2), gill Na^+-K^+ATPase activity, and blood plasma levels of thyroxine and cortisol during the parr-to-smolt transformation of coho salmon held in fresh water (Modified from Dickhoff et al. 1990).

principal component (PC2) tracks temporal changes in the shape of the caudal peduncle and tail. Typically, PC2 values remain relatively constant until one or two months before completion of smoltification. At this time a rapid rise in PC2 value occurs for about one month. Then, the value for PC2 swiftly declines to below zero and increases to above zero as the fish adjusts to seawater. Blood plasma T4 concentration increases, reaches a maximum, and then declines during smoltification, so that it has returned to near its initial value during the later stages of development. Both blood plasma cortisol concentration and gill ATPase activity increase during smoltification, and reach their maximal values by mid-May. This association of blood cortisol concentration with gill ATPase activity is expected, since cortisol has been shown to stimulate gill ATPase activity (Richman and Zaugg 1987).

Deviations from expected **changes** in smolt parameters as shown in Figure 1.1 would suggest that smoltification is suboptimal or not synchronous in the particular population of juvenile salmon studied. Variation in the timing of smolt development and in the absolute value of smolt parameters are expected and thus are not good indicators of suboptimal smoltification. Different populations of juvenile salmon, either in hatcheries or in the wild, experience differences in biotic and abiotic factors such as food supply and water temperature, which influence growth, development, and smoltification. Thus, the emphasis of our analysis was on determining whether fish were going through expected changes.

Materials and Methods

Fish Sampling Procedures

In this section the study sites and methods used to collect coho for the various smoltification assays are described. As explained above, our sampling design was structured around the need to sequentially remove fish from a variety of locations through time. We sought to capture yearling coho salmon throughout the late winter and spring months in each watershed to determine if normal physiological preadaption for seawater entrance took place. Fortuitously, the Chehalis and Humptulips watersheds share many common features; for example, both have WDF hatcheries that release large quantities of yearling coho salmon and both have areas where natural coho production occurs.

Whenever possible, multiple pieces of information were collected on the same fish. Not only did this include smolt parameters such as ATPase, thyroxine, and cortisol but measurements of stress levels and health as well. Thus, in order to avoid repeating descriptions for how samples were obtained for each assay (and for the fish stress and health analyses), the collection methods are all presented here.

Collection of Hatchery Fish. Beginning in mid-winter and continuing through mid spring, coho were regularly collected from the Humptulips and Simpson hatcheries (Fig. 1.2) to assess their smolt status and health. In 1987, collections occurred from 3/12 to 6/8 while in 1988, sampling occurred from 2/16 to 5/28. In both

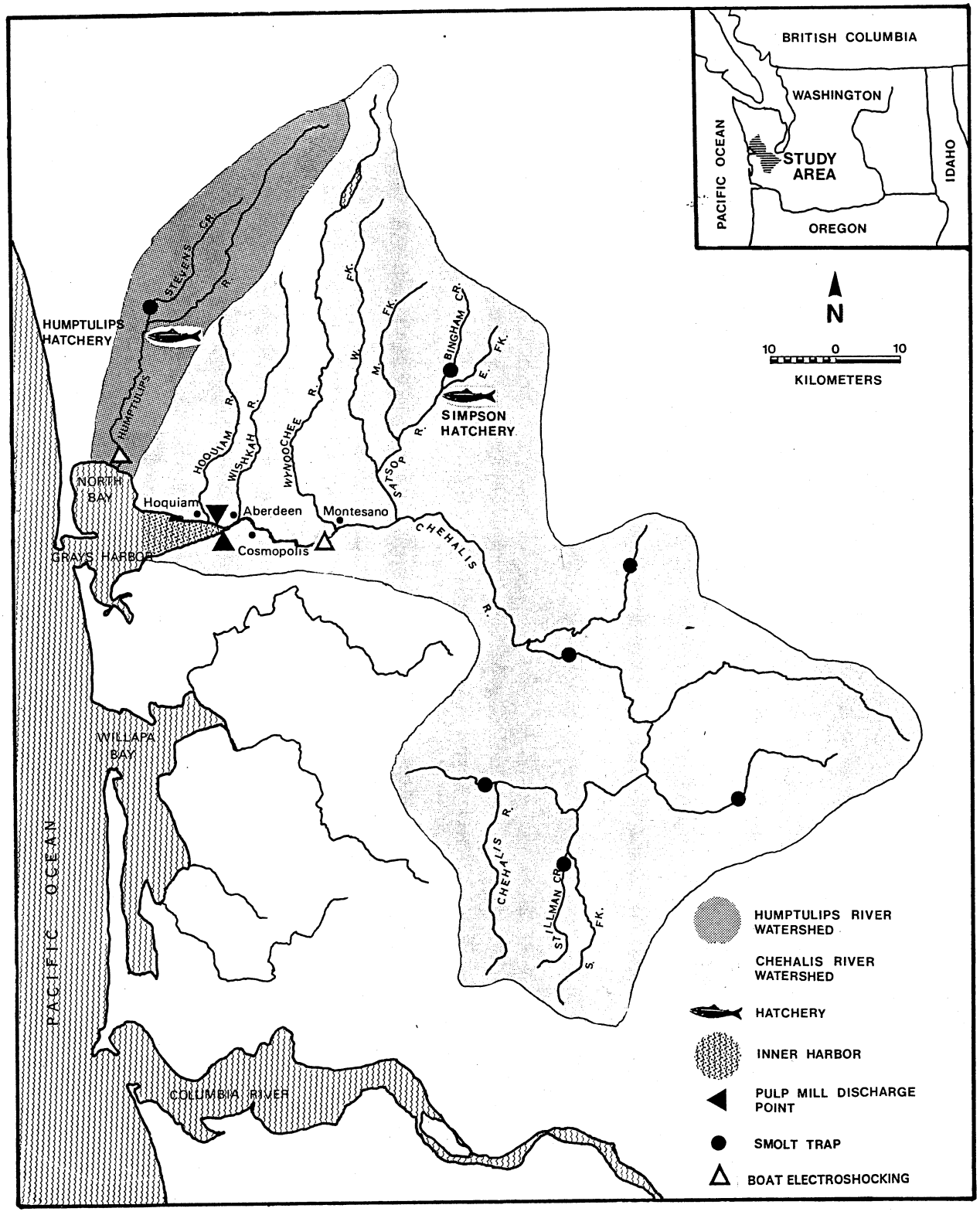

Fig. 1.2. Map of the Chehalis and Humptulips watersheds showing the sites where hatchery, wild resident, and migrant coho were collected for smolt, health, and stress evaluations.

years, samples were obtained approximately once every two weeks.

In 1987, two populations of coho were sampled at the Humptulips Hatchery. The group in pond #18 was reared under typical conditions, i.e. the fish were fed once per day by broadcasting food into the pond. The other population (pond #17) was placed in a pond equipped with feeders that the fish could activate and consequently their feeding frequencies and the amount of food they received per day were controlled by the fish themselves. In early May, just prior to the release of the production groups, several thousand fish from each population were placed in sequestered rearing areas. Sequestered groups were sampled periodically until early June to develop a more complete picture of their smolting cycle and to discern if they would revert back to parr if they were prevented from migrating.

Two populations were once again sampled at Humptulips Hatchery in 1988. The first was the regular production group held in pond #18 (referred to as the Humptulips production group) until release in late April. The second group consisted of several thousand fish from pond #18 that had been placed into a separate rearing area in early March. As in 1987, the sequestered fish were used to develop a complete picture of the smolting cycle of the Humptulips fish.

At the Simpson Hatchery, coho are raised in one large rearing pond. As at Humptulips Hatchery, prior to the release of the production group in late April and early May, 1987, several thousand fish were collected and sequestered in their own rearing area. A similar sequestered group was established in early March,

1988.

All samples of hatchery fish were collected with large pond or block seines and the fish held in well aerated containers until they were processed. On days when cortisol or immunocompetence assays were performed, fish were collected for these assays with a long handled dipnet prior to using the seines. During each sampling episode, about 100 fish from each hatchery population were measured, weighed, and visually evaluated for smolt status. Most fish that were weighed and measured were liberated below their respective hatchery while fish that had been collected but not measured (i.e., extras) were returned to their rearing areas. Some fish (<30) were sacrificed during each sampling period for smolt, stress, and health assays. The same assays and evaluations were generally performed each year with the exception of cortisol analyses and immunocompetence assays which were done only in 1988.

Collection of Wild Coho. Like the hatchery fish, collections of wild fish in the Humptulips and Chehalis basins were made periodically from mid March through early June of 1987 and 1988. Four locations were sampled in the Chehalis River watershed and two in the Humptulips River basin (Fig. 1.2). In the Chehalis basin, sites were located throughout the watershed to provide a profile of smoltification as the fish migrated downstream and to assess whether there were particular areas of the basin where fish were not smolting properly.

Chehalis Basin. The Black Hills and the Coastal Mountain Range split the Chehalis basin into two approximately equal parts

(in Fig. 1.2, this boundary occurs at the "scoop" trap location). The upper watershed is more agricultural and has a slightly different microclimate and soil composition than the lower Chehalis and Humptulips watersheds which are very similar to one another. Consequently, wild fish were collected in both parts of the Chehalis River watershed. Stillman Creek (Fig. 1.2) was chosen to represent a typical upper watershed stream while Bingham Creek (Fig. 1.2) was selected to represent a lower Chehalis basin stream. These particular sites were used because an on-going WDF study evaluating coho production in the Chehalis River drainage maintains smolt traps on each of these streams (see Seiler 1989).

The Stillman Creek trap consisted of a series of screen panels placed in the stream bed. The panels sieved the entire stream except for two small openings which lead into holding boxes. Samples of migrants were removed from these boxes. At Bingham Creek, fan traps were placed across the entire breadth of the stream. These traps, which are made of perforated aluminum screening and bent into a fan-like shape, sieve water and pass fish to a holding box where they can be easily retrieved.

Besides the traps on tributaries, fish were also collected from a scoop trap located in the middle of the mainstem Chehalis River at the boundary between the upper and lower portions of the watershed. The trap sat on two floating steel pontoons and is analogous to an inclined plane trap since perforated screening, placed at an angle, sieves fish from the river and passes them into a holding box. Fish were periodically collected from the trap to

obtain representative samples of migrants. The trap sampled about 5 to 7% of the fish emigrating down the Chehalis River from the upper 900 miles^2 (2,250 km^2) of the watershed.

Coho yearlings collected at the aforementioned traps were migrants. That is, they had progressed far enough through smoltification to migrate seaward. On some occasions, we also collected resident coho, or fish that had yet to begin their seaward migration, from areas upstream of these traps by using a Smith-Root Mark VII backpack electroshocker. During each sampling episode, at least 15 coho were collected and used in various smolt and health assays. Actual sample sizes are given in Appendix 1.

In 1988, coho were also collected from the lower river near Montesano (R.K. 9-15) using a Smith-Root boat electroshocker. Our objective in sampling this portion of the river was to obtain samples of migrants shortly before they encountered estuarine conditions. The fish captured in this area, however, were of uncertain origin. They could be either wild or hatchery smolts since this area was downstream of where hatchery fish were released. In addition, wild fish could have been either migrants or residents since some fish were captured in rearing habitats along the river-bank while other fish were captured in more open water areas. The type of fish captured is important because smoltification measurements can be different in each type of fish. For example, active wild migrants from the upper river should have higher ATPase values then fish rearing in the lower river.

Humptulips Watershed. In the Humptulips River watershed, wild

fish were collected from Stevens Creek, the main tributary to the Humptulips River, in 1987 and 1988. Electroshocking gear was again used to collect resident fish and a trap, very similar to the one used at Stillman Creek, was employed to obtain migrants from a point on Stevens Creek near its confluence with the Humptulips River. In 1988, a boat electroshocker was used to collect coho from the lower Humptulips River, about 2-3 miles (3.7-5.5 km) from the river's mouth. The smoltification data collected from these fish, like that obtained from fish in the lower Chehalis River, was ambiguous because wild, hatchery, resident, and migrant fish were all present.

Methods used to Assess Smoltification

Growth, Coloration, Condition Factor, and Body Shape. To assess the external appearance of hatchery fish, the criteria of Prentice et al. (1981) was used (Table 1.1). Because the color patterns of wild fish are more intense than those of hatchery fish, more conservative criteria were used. Parr were assigned a value of one, transitional fish (in the intermediate stages of smoltification) a value of two, and smolts a value of three. Length and weight data were obtained on each fish sampled and used to calculate the condition factor ($K= W/L^3$ where W equals weight in gr and L equals length in cm) of every fish.

The methods used to measure changes in body shape have been described in detail by Winans (1984) and Winans and Nishioka (1987) and are only briefly summarized here. Fish were photographed alongside a metric ruler on a white background, either fresh in the

Table 1.1. Visual criteria used to identify the parr, transitional, and smolt stages in coho salmon (after Prentice et al. 1981).

Physical Attribute	Parr	Transitional	Smolt
1. Parr Marks	Distinct, extend well below lateral line.	Evident but faded. Confined mostly to the area above the lateral line.	Absent
2. External Color on Dorsal Surface	Generally brown to brownish yellow	Somewhat greenish, changing to blue	Blue to green
3. External Color on Ventral Surface	Generally yellow	Belly is white but yellow towards lateral line.	Belly is white but turns silver towards the lateral line.
4. Fin Color[1]			
-Caudal Fin	Yellow to orange red	Dark but translucent, dark margin becoming evident on	Translucent with some dark pigment, definite dark band on posterior edge
-Anal Fin	Yellow to orange-red, often with white posterior margin.	Clearing, becoming white and translucent, white tip on posterior margin.	Clear and translucent with small white tip on posterior.
-Ventral Fins	Yellow to orange-red	Slightly yellow or very light orange, becoming translucent.	Clear, translucent.
-Pectoral Fins	Yellow to orange-red	Moderately yellow or orange	Clear and translucent, sometimes with dark pigment next to the pectoral girdle.
5. Opercle	Shiny golden to yellow	Light golden color, not yet entirely silver	Definitely silver.

[1] During the transitional stage the fins progressively lose color and become translucent in the following order: caudal-anal-ventral-pectoral. Where a fish is, in this sequence can be used to determine how far a transitional fish has progressed towards the completion of smoltification.

field or after they had been frozen for several months; effects of freezing on morphometric measurements are minimal (Winans unpublished data). Twelve morphometric features or landmarks were identified around the outline of the fish form (Fig. 1.3). The relative positions of landmarks in X-Y coordinate space were recorded with a digitizing board and the Euclidean or morphometric distance between pairs of landmarks was calculated using the Pythagorean Theorem.

Using the 12 landmarks, 26 morphometric distances were calculated in a truss-network pattern as illustrated in Fig. 1.3. These types of measurements produce a systematic, geometric characterization of fish shape that is very sensitive to changes in body form. Changes in body shape were assessed with principal components analysis (Manly 1986) using S.A.S. (Statistical Analysis System) version 6.02 for microcomputers. Components describe specific relationships among variables and are considered "shape" descriptors. The second principal component or PC2 describes tail shape. Because values for PC2 change significantly during smoltification, this was used as an index of smolt development.

ATPase Activity. The method used to determine ATPase activity in this study is described by Zaugg (1982). A portion of gill tissue is homogenized in a buffered solution and partially purified membrane fragments containing the desired enzyme are isolated by centrifugation. The enzyme is activated in the presence of Mg^{++}, Na^{+}, K^{+}, and ATP from which inorganic phosphate is liberated and measured chemically. The rate at which inorganic phosphate is

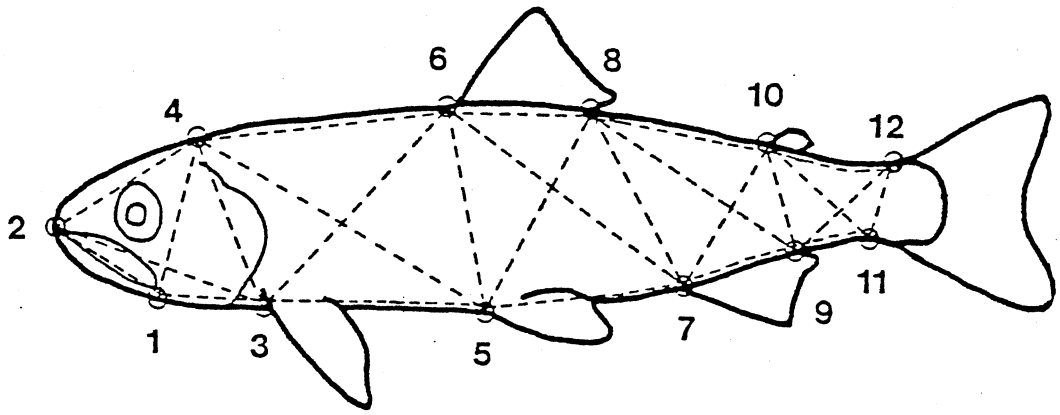

Fig. 1.3. The truss-network used to ascertain temporal shifts in coho morphology. Landmark points are indicated by open circles and the 26 morphometric distances measured are shown as dashed lines.

liberated from ATP is determined (μmoles P_i mg protein^{-1} hr^{-1}) and used as an indicator of smolt development. Typical rates of activity are 5-10 for coho salmon parr, 12-30 for developing hatchery smolts, 15-35 for active seaward migrants, and 30-50 for fish adapting to seawater.

Thyroid Hormones. Levels of T3 and T4 in blood plasma were measured by obtaining blood from the caudal vein of anesthetized fish after the tail had been severed. Blood plasma samples were then placed on dry ice and transported to NMFS's Montlake Laboratory in Seattle, where the radioimmunoassay method of Dickhoff et al. (1978, 1982) was employed to determine T3 and T4 concentrations.

Cortisol. To measure plasma cortisol, fish were netted and immediately killed with a lethal dose of MS-222 buffered with $NaHCO_3$, a method that does not affect levels of resting cortisol. Fish were bled by severing the caudal peduncle and collecting blood in ammonium-heparinized capillary tubes. Plasma was separated by centri-fugation and stored at -20 C until assayed. Plasma cortisol was measured in 10 μL of plasma following a radioimmunoassay (RIA) procedure (Foster and Dunn 1974) modified for use with coho salmon plasma by Redding et al. (1984).

Hematocrit Counts. At the time that blood plasma samples were collected to evaluate T3 and T4 concentrations, blood hematocrit counts were also determined by collecting a sample of blood in microhematocrit tubes, centrifuging the sample and measuring percent red blood cells.

Results and Discussion

Growth, Coloration, Condition Factor, and Body Shape

Hatchery Fish. The proportion of fish that entered smoltification based upon color patterns was determined at each hatchery in 1987 and 1988 (Fig. 1.4a and 1.4b). In 1987, smolt status showed a normal progression from parr-to-smolt in all production groups, regardless of hatchery location. At Humptulips Hatchery, about 22% of the fish in mid-March were parr and by early May, parr were no longer in evidence. Comparable changes were observed in production fish at Simpson Hatchery, although a greater proportion of Simpson fish were still in the transitional stage at release.

At both hatcheries in 1987, the percentage of smolts in the sequestered populations declined (Fig. 1.4a). There appear to be several reasons why this occurred. First, the ability of the Simpson fish to volitionally exit their rearing pond meant that the fish used to create the sequestered population were probably transitional fish just beginning to enter the final stages of smoltification. Second, in both hatcheries, fish were moved from large rearing ponds to the smaller sequestered rearing areas. The stress associated with adjusting to a new rearing area may have been enough to delay or inhibit smoltification. Finally, since these fish were prevented from migrating and entering seawater, some of them began to revert back to the parr stage.

On the first sampling date in 1988 (February), 20% of the production fish at Humptulips Hatchery had begun smolting, whereas

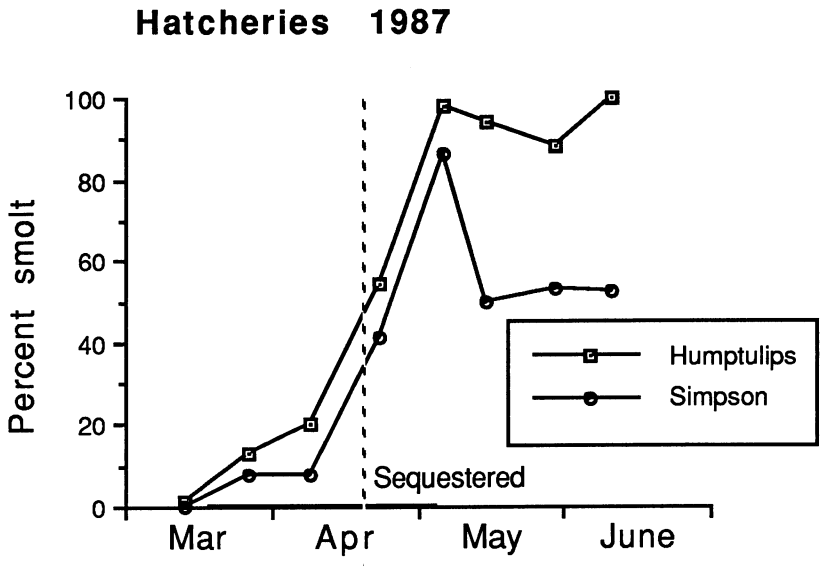

Figure 1.4a. Proportion of production fish entering smoltification (percent smolt) at Humptulips and Simpson Hatcheries during 1987 determined by visual examination of external appearance according to Prentice et al. (1981).

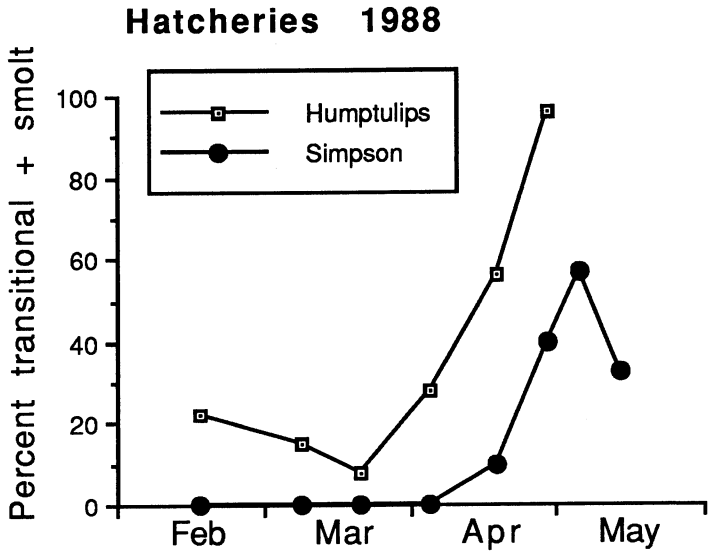

Figure 1.4b. Proportion of production fish entering smoltification (percent transitional + smolt) at Humptulips and Simpson Hatcheries during 1988 determined by visual examination of external appearance according to Prentice et al. (1981).

all fish at Simpson Hatchery were still parr (Fig. 1.4b). During April there was a marked increase in the proportion of transitional and smolt appearing fish at both hatcheries. This increase occurred earlier and to a greater extent in fish at Humptulips compared to Simpson Hatchery. The color patterns of sequestered fish were generally not as clear as those of production fish. However, sequestered fish at Humptulips Hatchery were more advanced in smolt development compared to sequestered fish at Simpson Hatchery.

Changes in length and weight of hatchery fish are presented in Figures 1.5 and 1.6. In 1987, all three production groups grew at similar rates but the Humptulips fish were consistently larger than the Simpson fish. At release, the mean fork length (FL) of fish at Humptulips was 154 mm while it was 148 mm at Simpson; the average weight of Humptulips fish was 37.0 gr while Simpson fish averaged 34.5 gr.

Fish size in 1988 was generally smaller than in 1987. In February 1988, production fish at Humptulips Hatchery were smaller than those at Simpson Hatchery (Fig. 1.6). However, by March, fish at Humptulips Hatchery were larger than those at Simpson Hatchery. The growth rate of fish at Humptulips was sustained throughout sampling and was higher than fish at Simpson Hatchery. The growth rate of the sequestered fish was lower than production fish in both hatcheries. At release, the average size of Humptulips fish was 145 mm FL and 33.0 gr. while fish at Simpson averaged 131 mm FL and 23.0 gr.

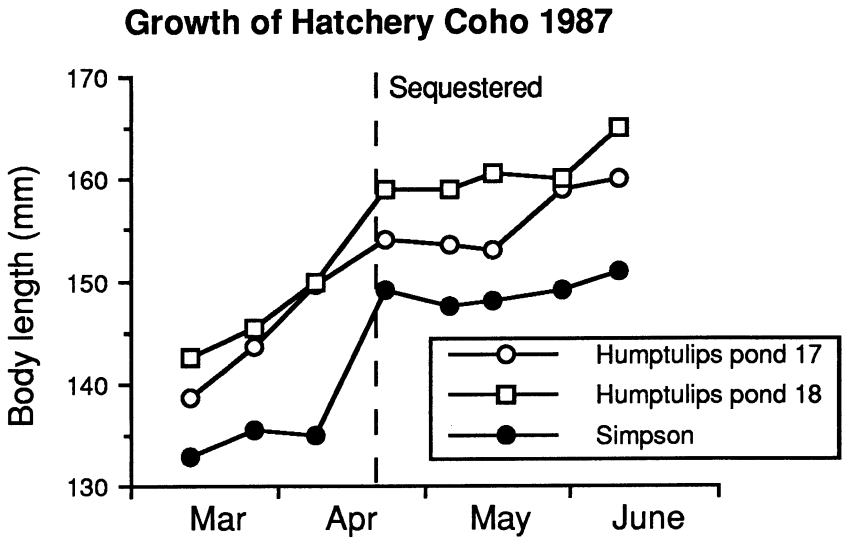

Figure 1.5. Temporal changes in fork length (mm) of coho salmon from Simpson Hatchery and from pond 17 (demand feeding) and pond 18 (broadcast feeding) at Humptulips Hatchery. The sequestered groups at Simpson and Humptulips hatcheries were established on April 28 and April 22, respectively.

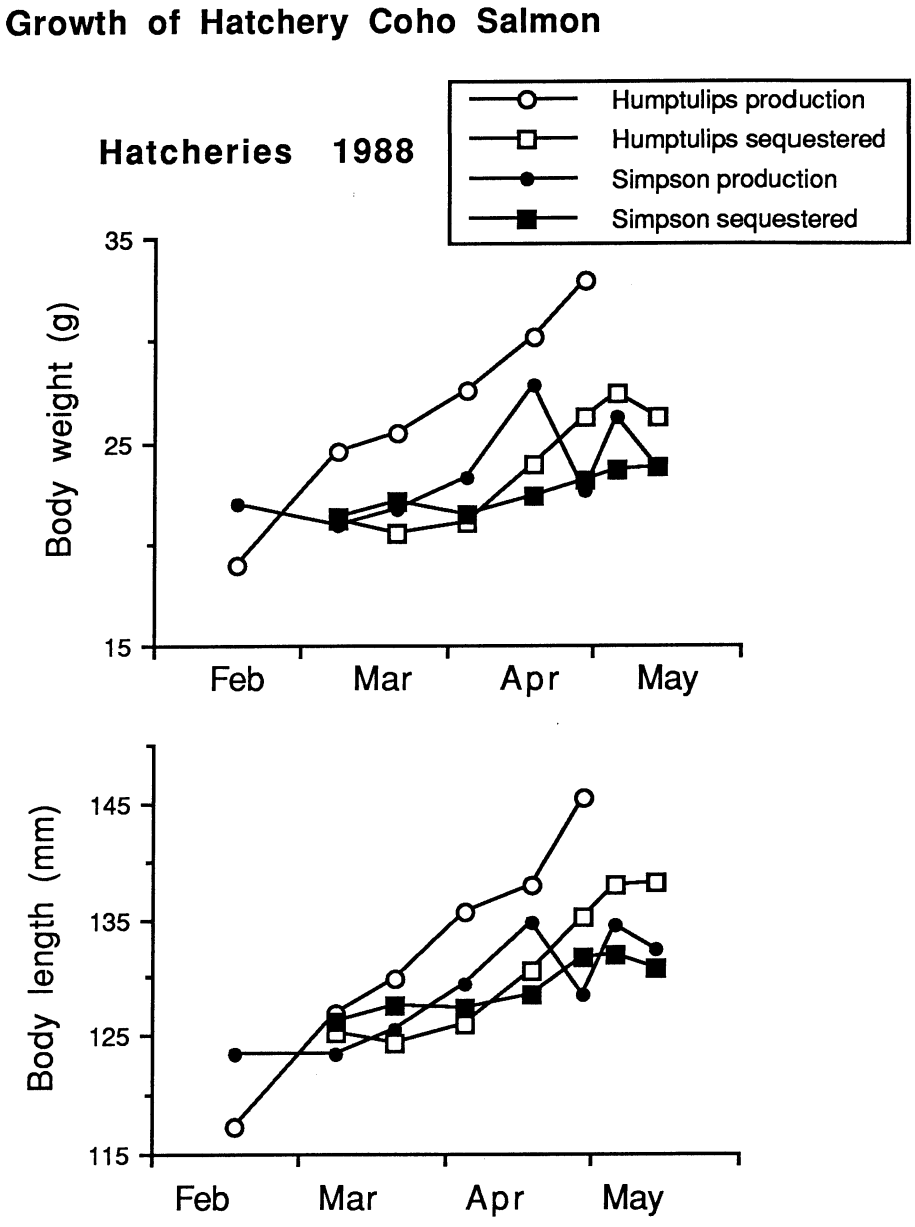

Figure 1.6. Temporal changes in fork length (mm) and body weight (g) from production and sequestered groups at the Simpson and Humptulips Hatcheries during 1988.

In general, the condition index of smolting salmon is expected to decline as smolting progresses (e.g., Hoar 1988). In 1987, the condition factor declined as expected in all hatchery groups up to the time they were released (Fig. 1.7). The condition factor of the two sequestered groups dropped soon after they were separated from the production groups; this was probably due to the stress of moving the fish.

In 1988, the condition factor for all hatchery groups generally declined (Fig. 1.8). The condition factors of production fish at both hatcheries were initially higher and showed a more marked decline over time when compared to sequestered fish. No consistent differences in condition factors were observed comparing between hatcheries.

In 1987, the pattern of smolt development as determined by principal component analysis was similar in Simpson and Humptulips hatcheries, but the timing of the PC2 drop differed by about one week (Fig. 1.9). At Simpson Hatchery, there was a penultimate rise in PC2 near late April, followed by a drop to a negative mean value that was statistically different from zero at the beginning of May. At Humptulips Hatchery, the penultimate increase in PC2 occurred at the beginning of May and the PC2 value became significantly negative in mid-May. These results suggest that smolt development was slightly more advanced at Simpson Hatchery compared to Humptulips Hatchery in 1987.

In 1988, the values for PC2 at the hatcheries became significantly negative at the end of April for fish at Humptulips

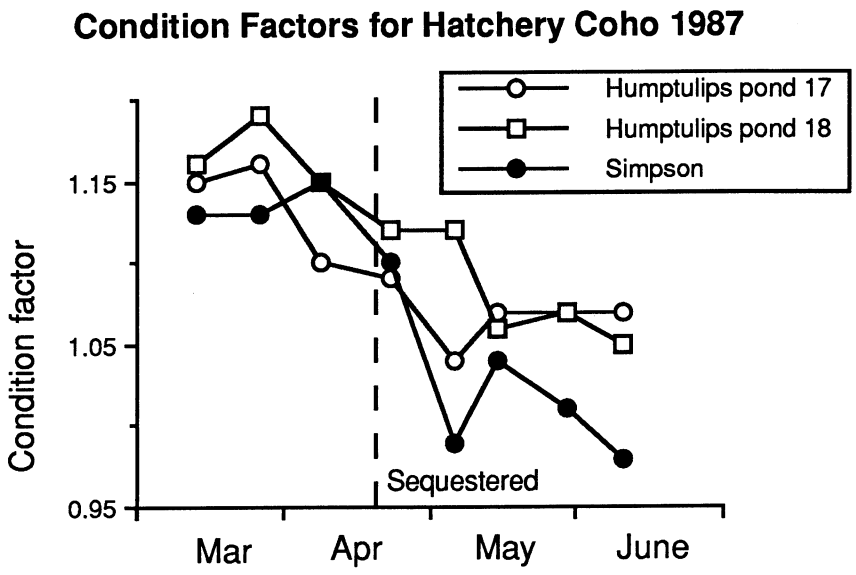

Figure 1.7. Condition factor (weight in grams x 100 divided by length in cm^3) of coho salmon from Simpson Hatchery and from pond 17 (demand feeding) and pond 18 (broadcast feeding) at Humptulips Hatchery. The sequestered groups at Simpson and Humptulips hatcheries were established on April 28 and April 22, respectively.

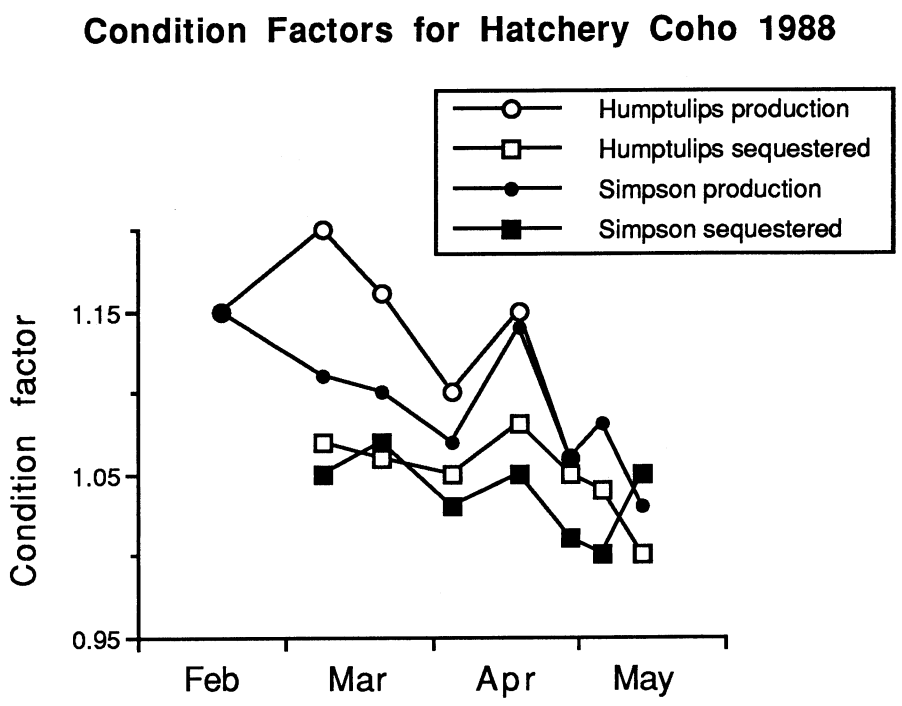

Figure 1.8. Condition factor (weight in grams x 100 divided by length in cm^3) of coho salmon sampled at Humptulips and Simpson Hatcheries during 1988.

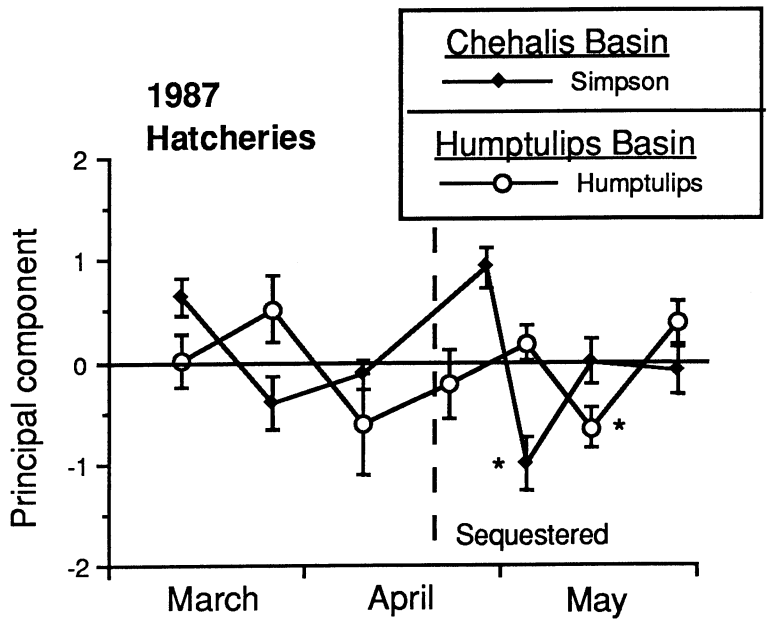

Figure 1.9. Changes in body shape (principal component) of coho salmon sampled at the Simpson and Humptulips hatcheries in 1987. Symbols represent means; brackets indicate ± one standard error; asterisks indicate values significantly less than 0 ($P \leq 0.05$).

Hatchery, and at the beginning of May for fish at Simpson Hatchery (Fig. 1.10). These data suggest that morphometric changes occurred slightly earlier in fish at Humptulips Hatchery compared to Simpson Hatchery, the opposite of that observed in 1987. In summary, comparison of fish from the two river basins revealed similar patterns of body shape change for the two hatcheries that differed only in the timing of peak morphometric change by about one week.

Wild Fish. The smolt color index for wild fish collected with electroshocking gear (residents) and traps (migrants) is given in Fig. 1.11 and 1.12. In both years, the number of resident fish identified as parr decreased throughout the spring. Resident fish tended to be more advanced in 1987 than in 1988 over the same time period. For example, the mean color index for resident fish collected in March 1988 indicated that all fish had a parr appearance whereas in March 1987 there were more transitional fish. During April and May, the mean color value increased to intermediate between parr and transitional in residents collected from Bingham Creek (Chehalis Basin) and Stevens Creek (Humptulips Basin). However, the index tended to be higher in 1987, indicating these fish had more of a transitional appearance.

Migrants were consistently more advanced in their color patterns than residents. In both years, the mean color values of migrant fish were greater than 2.0 on the first sampling dates in Bingham, Stillman, and Stevens creeks (Fig. 1.11 and 1.12). Thus, most of the fish that were actively migrating were either transitional fish or smolts which is what would be expected. Some

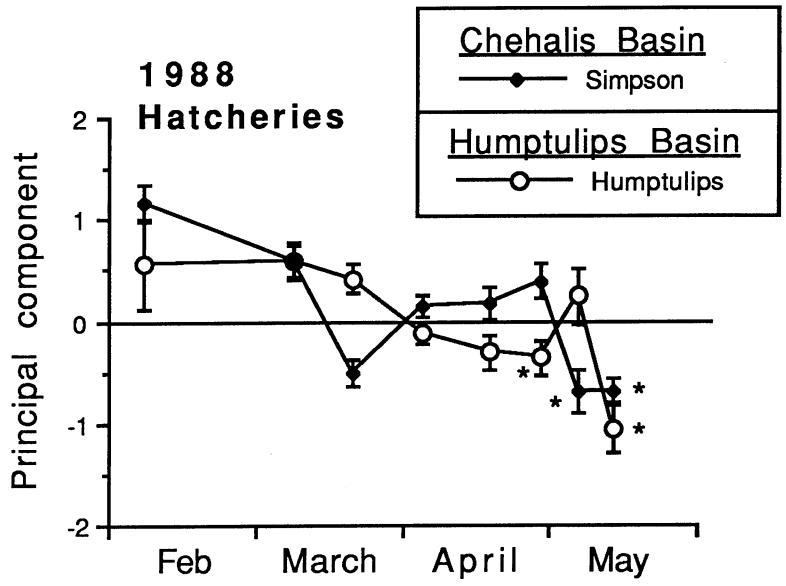

Figure 1.10. Changes in body shape (principal component) of coho salmon sampled at the Simpson and Humptulips hatcheries in 1988. Symbols represent means; brackets indicate ± one standard error; asterisks indicate values significantly less than 0 ($P \leq 0.05$).

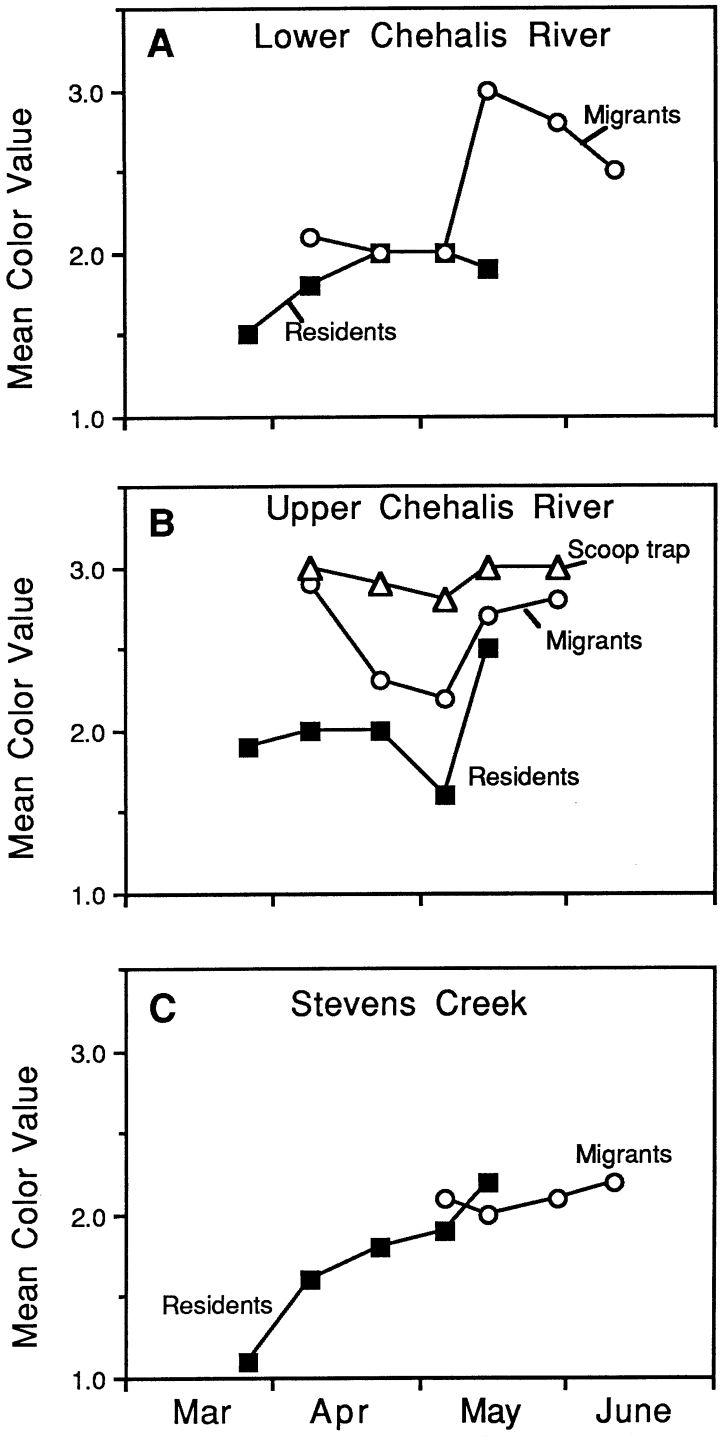

Figure 1.11. Mean color index of resident (collected by electroshocking) and migrant (obtained from smolt traps) coho salmon from Bingham Creek (A), Stillman Creek (B), and Stevens Creek (C), 1987. Values range from 1 (= parr) to 3 (= smolt).

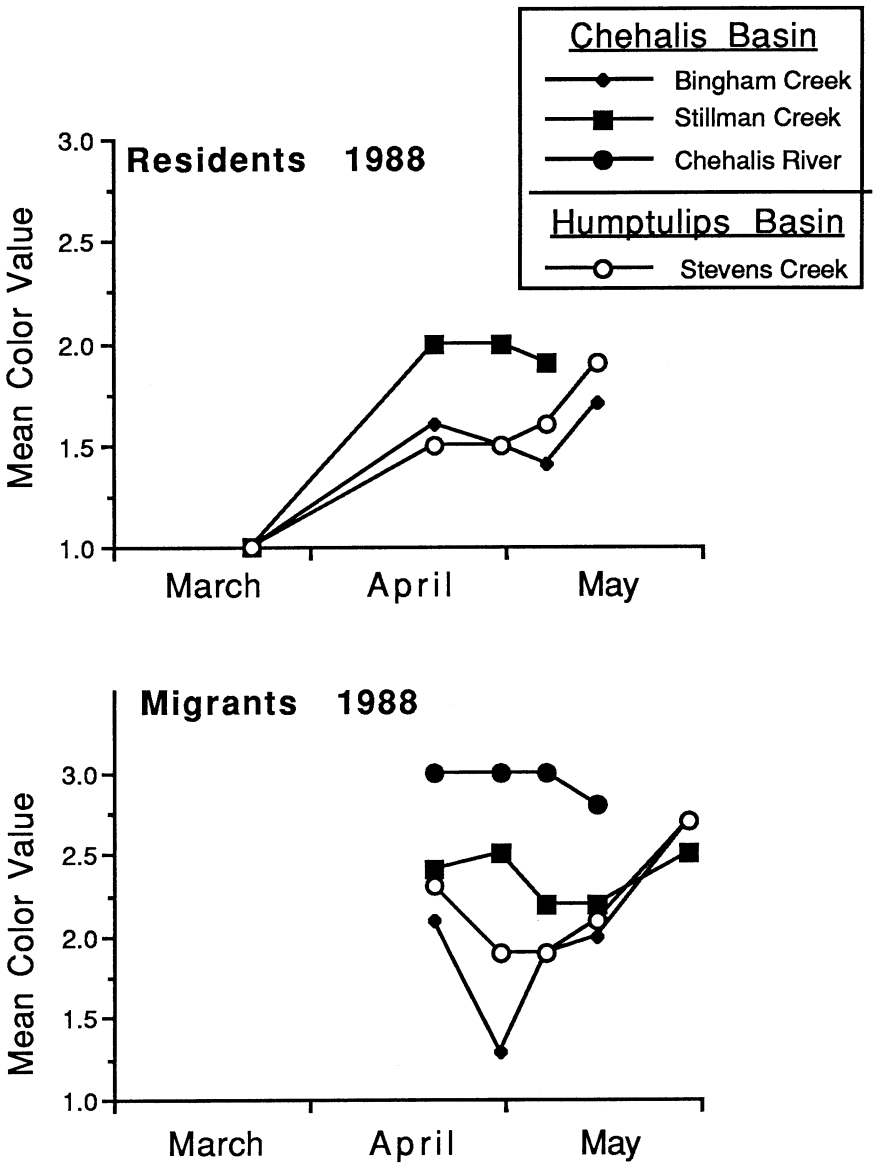

Figure 1.12. Mean color index (Prentice et al. 1981) of wild coho salmon sampled during 1988. Fish were collected by electroshocking (residents) or by trapping (migrants). Values range from 1 (= parr) to 3 (= smolt).

differences were observed between years at the same location. In 1988, mean color values rose slightly in May for migrants in Bingham and Stevens creeks, but remained relatively constant for migrants in Stillman Creek. The Stevens Creek fish possessed color patterns in 1988 that were either intermediate between Stillman and Bingham creeks or nearly identical. In 1987, the color index declined in Stillman Creek until mid-May when it increased. In Bingham Creek, the index increased sharply in early May and then declined slightly thereafter. There was little change in Stevens Creek fish in 1987 with most fish predominately transitionals.

In both years, the color index of migrants in the Chehalis River at the scoop trap location (Chehalis Basin) was at or near its maximum (3.0) throughout April and May (Fig. 1.11 and 1.12). At the time of their capture in the scoop trap, fish were well into their seaward migration and thus well smolted. In summary, consistent differences were not indicated in the color patterns of wild juvenile coho in the Chehalis and Humptulips river basins.

Although absolute values were different, changes in fork length over time for resident groups were very similar in both 1987 and 1988 with fish size generally increasing over time (Fig. 1.13 and 1.14). For migrants, fork length in 1987 generally declined over time. This pattern of large fish migrating first has been observed in other coho populations (e.g., Salo and Bayliff 1958; Dawley et al. 1986). In 1988, lengths of migrants at all sites increased between the first and second sampling periods after which it either declined or remained largely unchanged. Migrants in

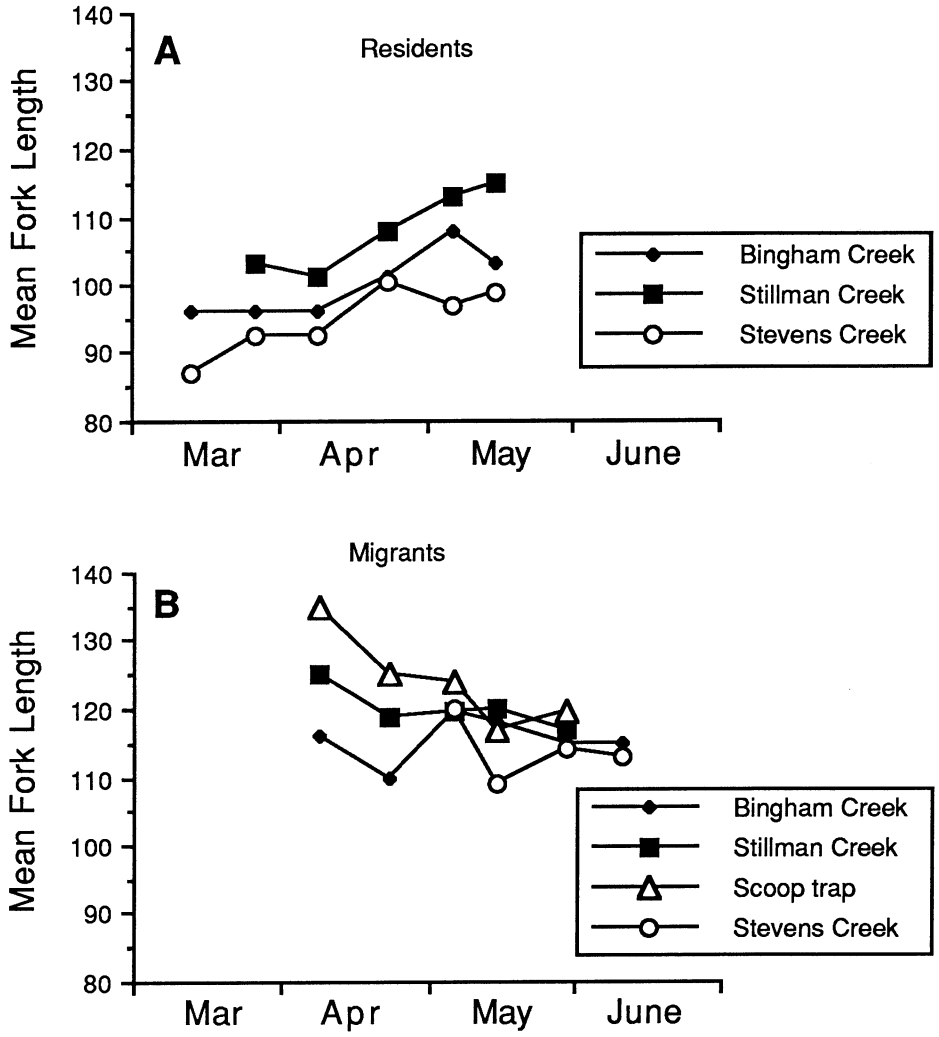

Figure 1.13. Temporal changes in fork length (mm) of resident (A) and migrant (B) coho salmon from the Humptulips and Chehalis river watersheds during 1987. Residents were obtained by electroshocking and migrants were obtained from smolt traps.

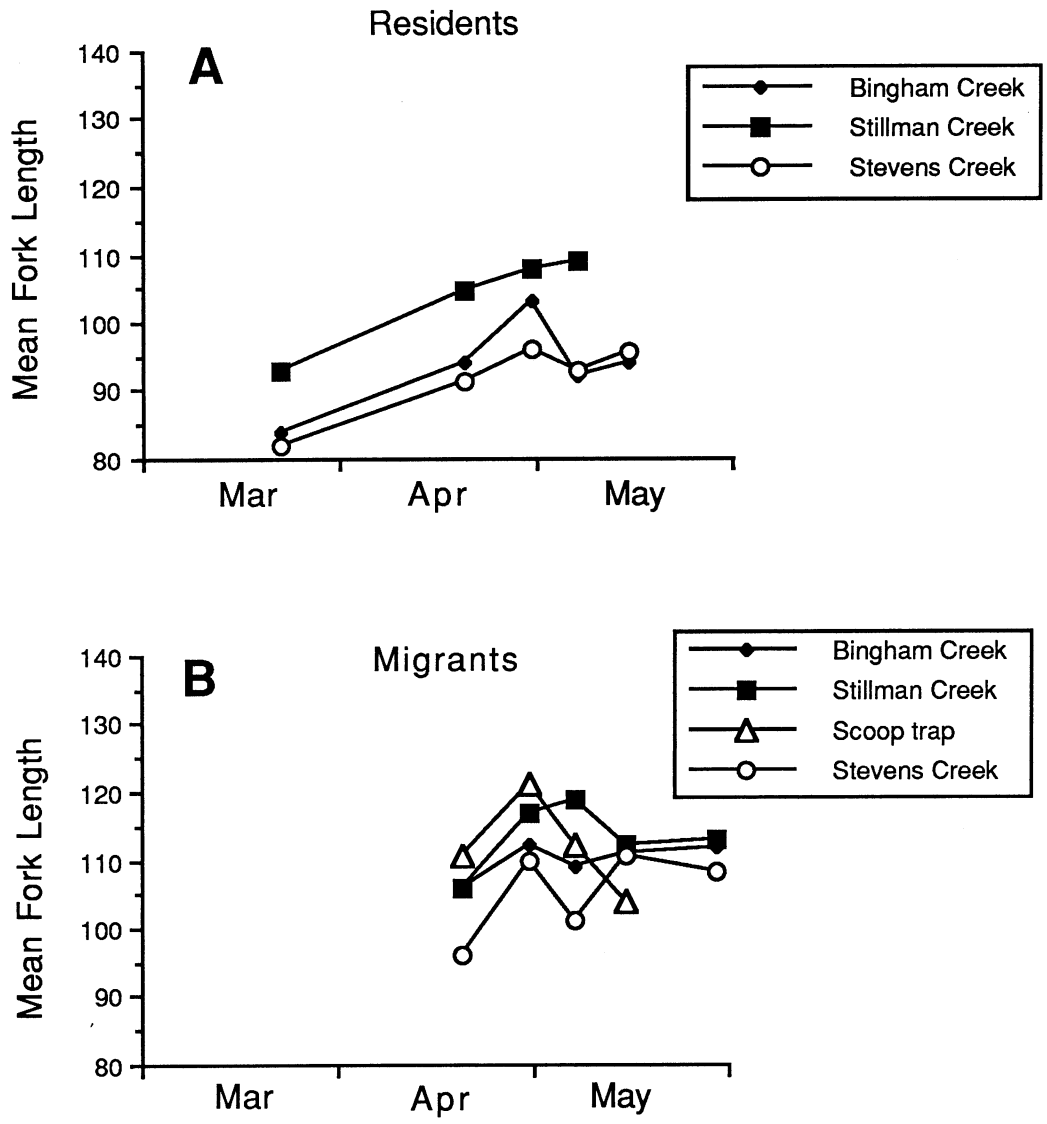

Figure 1.14. Temporal changes in fork length (mm) of resident (A) and migrant (B) coho salmon from the Humptulips and Chehalis river watersheds during 1988. Residents were obtained by electroshocking and migrants were obtained from smolt traps.

Stevens Creek tended to be smaller than those in the Chehalis watershed in both years. In general, the size of the migrants in both river systems were well within the ranges considered normal for Pacific Northwest coho populations.

In all cases, the condition factor of migrating wild fish was lower than resident coho in the same stream (Fig. 1.15 and 1.16). The condition factor of resident fish typically declined over time while that of migrants was relatively more constant; these are the same types of changes that have been observed in other populations of coho (e.g., Folmar and Dickhoff 1980). In summary, wild fish in the Chehalis and Humptulips basins undergo comparable changes in size and condition.

The principal components analysis performed on wild fish in 1987 showed that changes in PC2 were comparable in both river basins (Fig. 1.17). The mean value for PC2 became significantly negative in mid-May for resident fish in both Bingham and Stevens creeks. For migrant fish, PC2 values were significantly negative only for fish in Stevens Creek.

The PC2 values did not become significantly negative in resident coho from either river basin in 1988 (Fig. 1.18). They were significantly negative in migrant coho in Bingham Creek during late April, but not at other times, and were also significantly negative the only time samples of migrants were analyzed in Stevens Creek (mid-May). Significant differences were not obvious in the morphology of fish sampled in the two river basins. In 1988, wild fish had negative PC2 values at about the same time as their

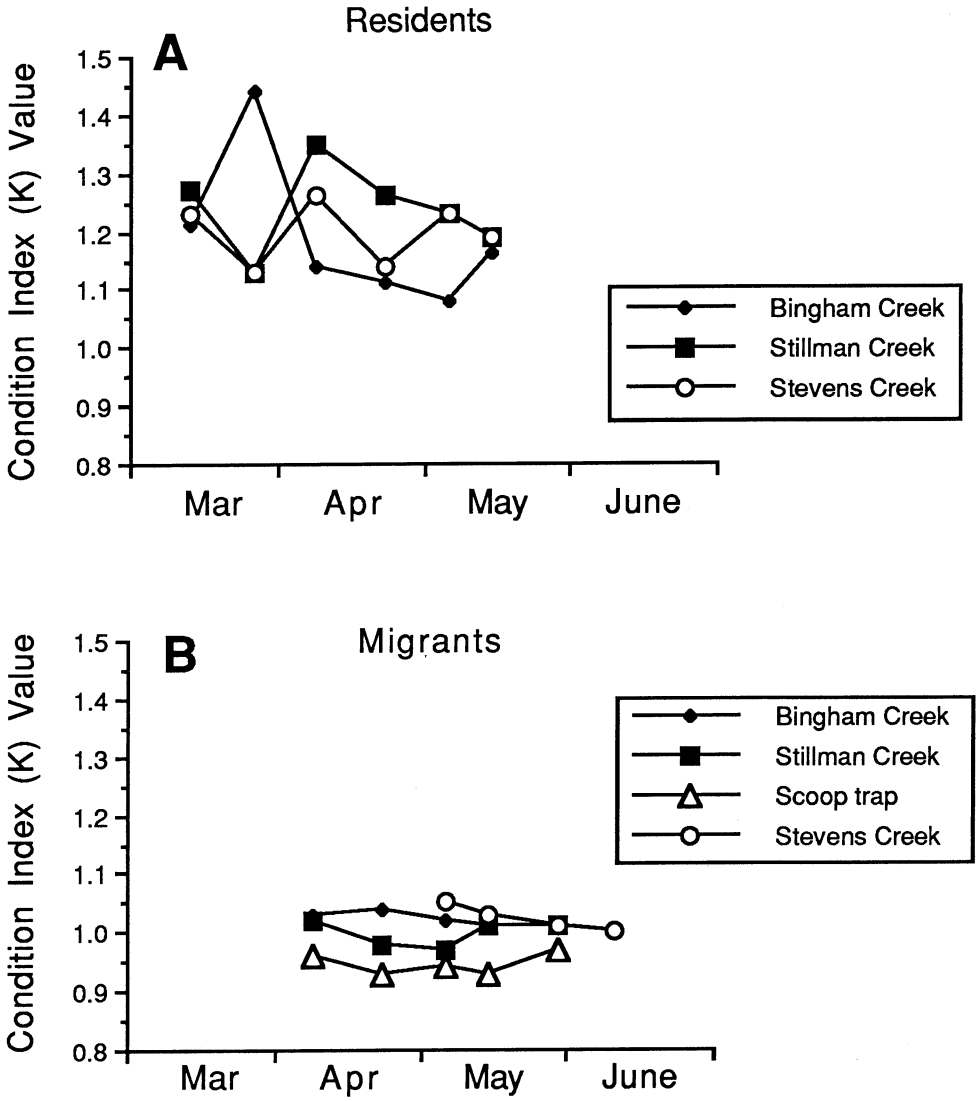

Figure 1.15. Temporal changes in the condition index of resident (A) and migrant (B) coho salmon from the Humptulips and Chehalis river watersheds during 1987. Residents were obtained by electroshocking and migrants were obtained from smolt traps.

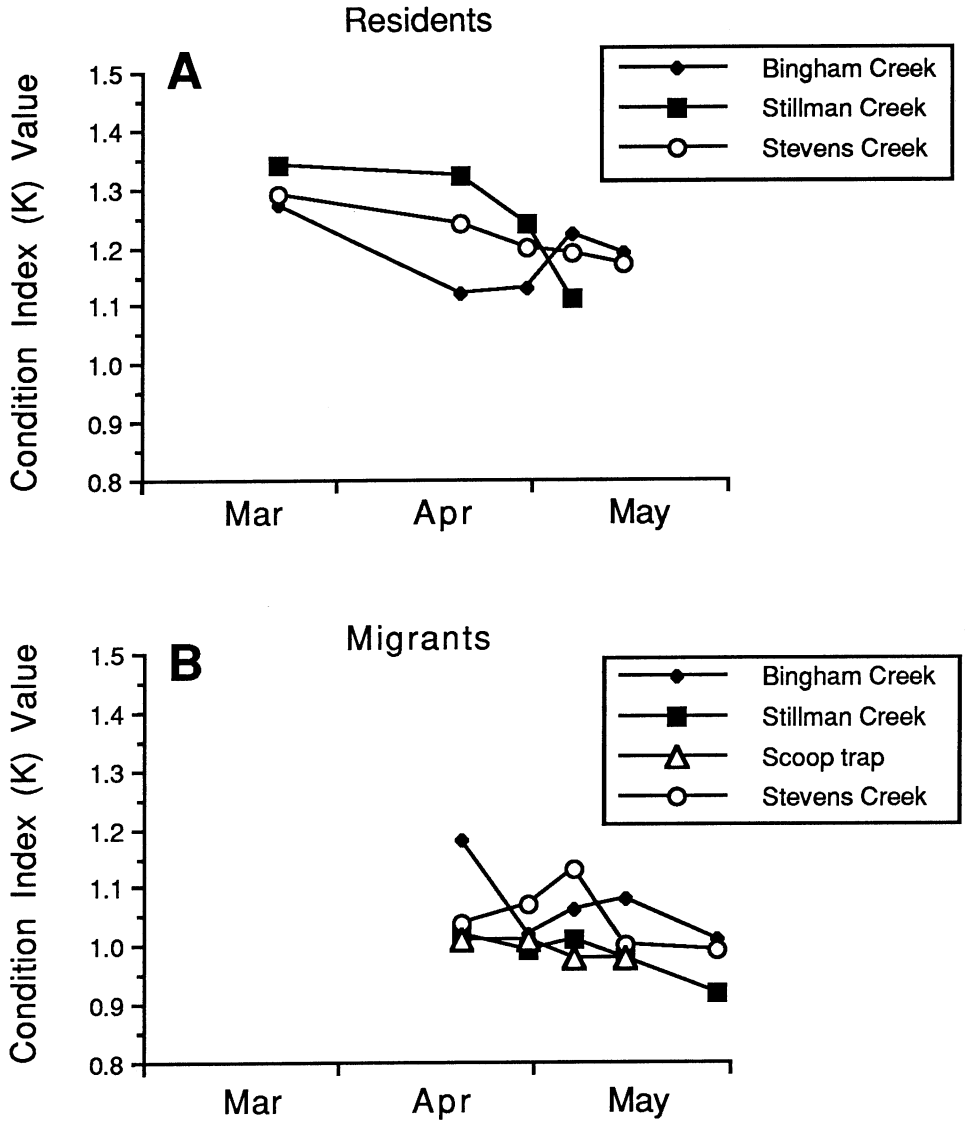

Figure 1.16. Temporal changes in the condition index of resident (A) and migrant (B) coho salmon from the Humptulips and Chehalis river watersheds during 1988. Residents were obtained by electroshocking and migrants were obtained from smolt traps.

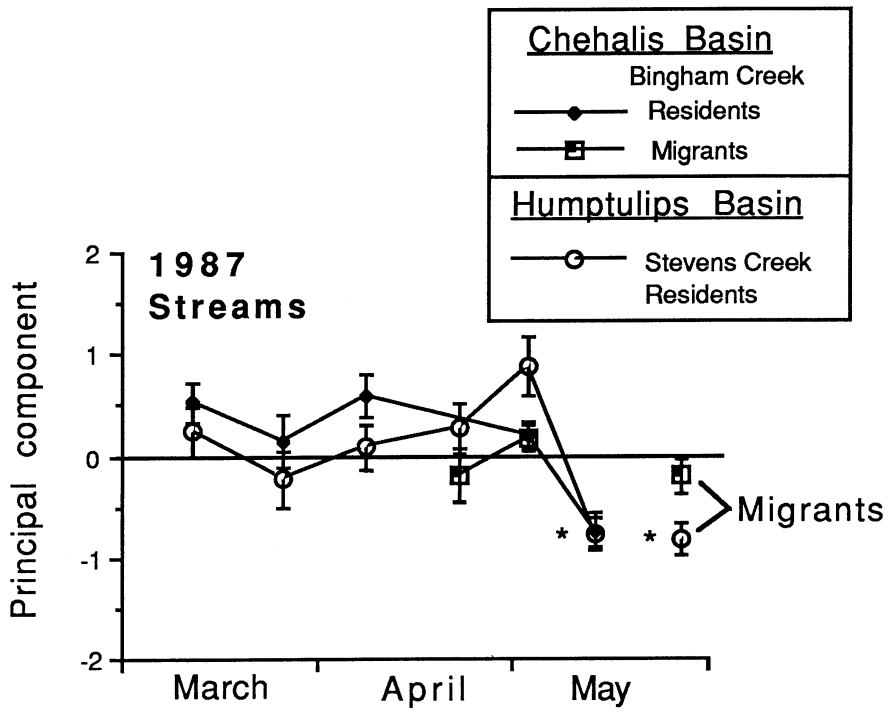

Figure 1.17. Changes in body shape (principal component) of wild coho salmon sampled from streams in the Chehalis and Humptulips basins in 1987. Fish were collected by electroshocking (residents) and from traps (migrants). Symbols represent means; brackets indicate ± one standard error; asterisks indicate values significantly less than 0 ($P \leq 0.05$).

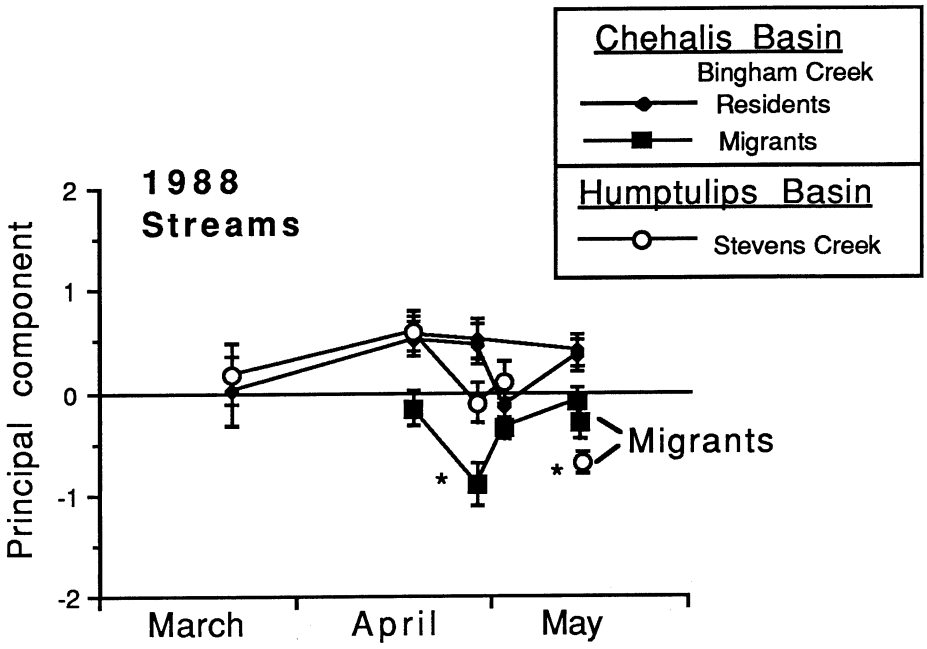

Figure 1.18. Changes in body shape (principal component) of wild coho salmon sampled from streams in the Chehalis and Humptulips basins in 1988. Fish were collected by electroshocking (residents) and from traps (migrants). Symbols represent means; brackets indicate ± one standard error; asterisks indicate values significantly less than 0 ($P \leq 0.05$).

respective hatchery stocks- 5/2 in the Chehalis Basin and 5/13 for the Humptulips Basin. Where data were available, it thus appears that wild fish, particularly migrants, followed the same pattern of morphometric changes.

Gill APTase

Gill ATPase activity increased markedly in fish at Humptulips Hatchery during 1987 (Fig. 1.19), with maximal levels occurring at the end of April. In contrast, a significant increase in ATPase did not occur in fish at Simpson Hatchery until the end of May (Fig. 1.19). These results suggest that, based upon ATPase levels, smoltification was more prominent and occurred earlier in fish at Humptulips Hatchery compared to Simpson Hatchery in 1987.

In 1988, gill ATPase activity in production and sequestered coho at both Humptulips and Simpson hatcheries increased in April with peak levels occurring in late April and early May (Fig. 1.20). The increase was slightly earlier and higher at Humptulips. The small elevation in Simpson fish is hardly typical of what is normally observed during smoltification. Whether the difference in ATPase development at the two hatcheries represents a significant difference in smolt development that would affect survival is unknown.

In all stream resident fish examined during 1987, gill ATPase increased significantly by the end of April (Fig. 1.21) and reached levels characteristic of smolts by May. There were no major differences between gill ATPase activities in wild resident fish from either the Chehalis or Humptulips basins. In 1987, migrant

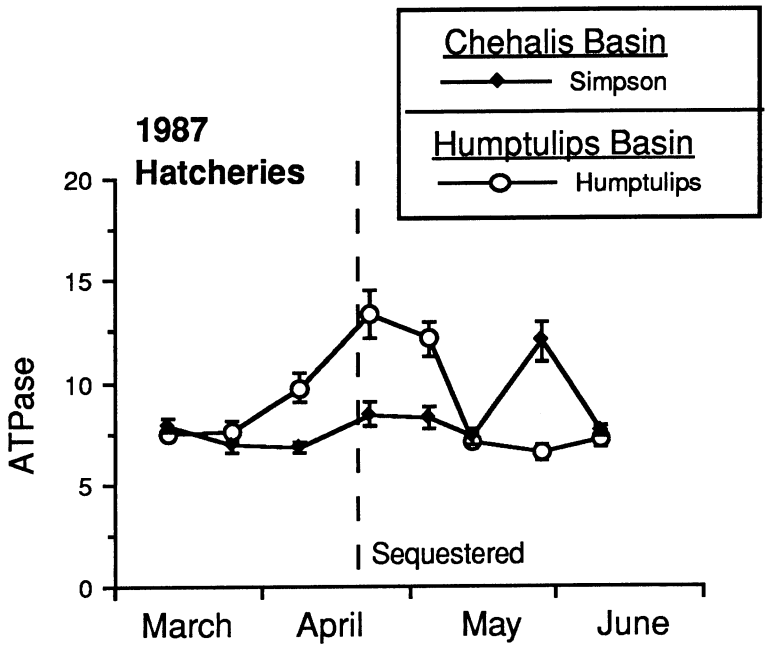

Figure 1.19. Changes in gill Na^+-K^+ATPase activity in coho salmon sampled at the Simpson and Humptulips hatcheries in 1987. Symbols represent means; brackets indicate ± one standard error.

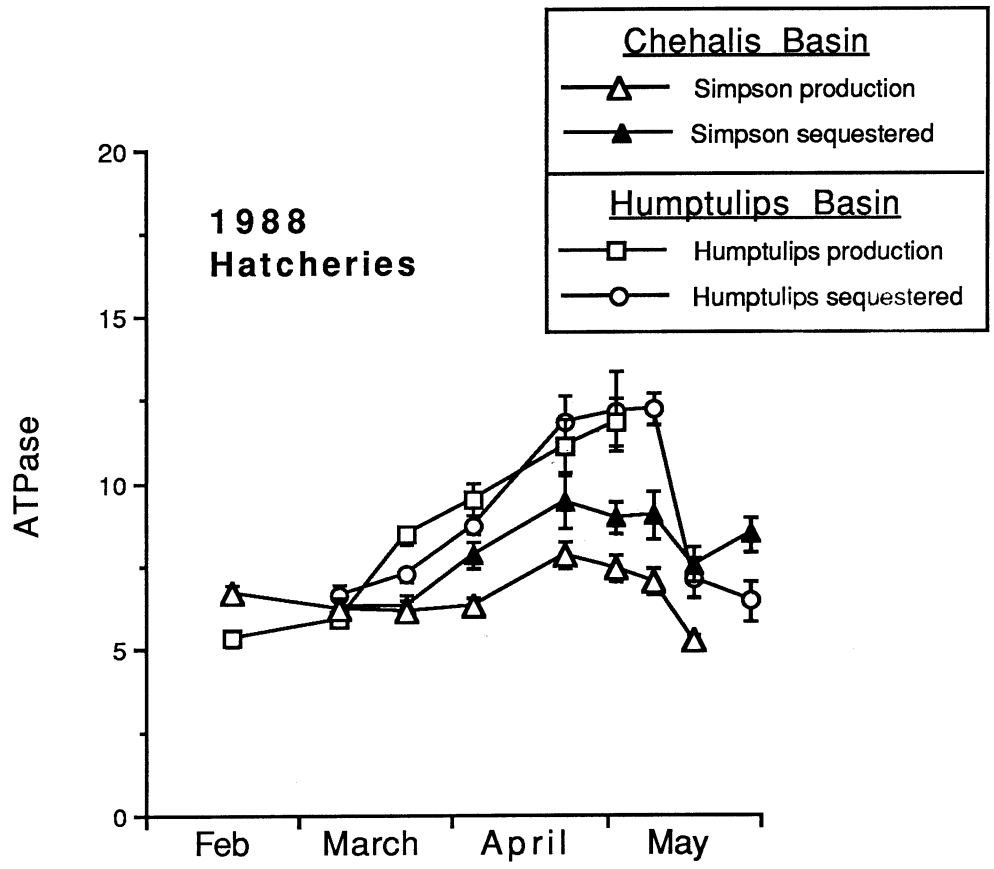

Figure 1.20. Changes in gill Na^+-K^+ATPase activity in coho salmon sampled at the Simpson and Humptulips hatcheries in 1988. Symbols represent means; brackets indicate ± one standard error.

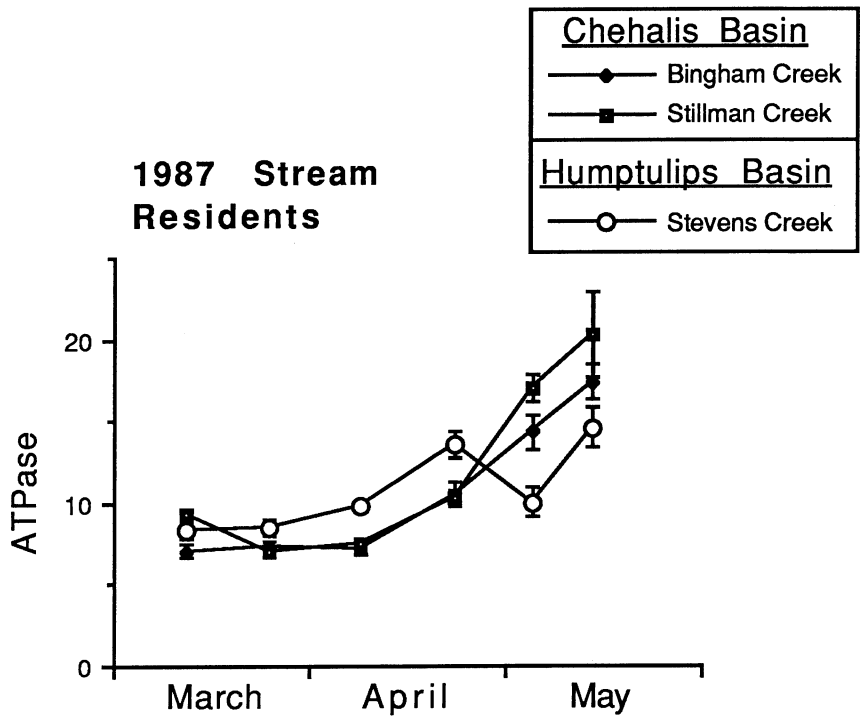

Figure 1.21. Changes in gill Na^+-K^+ATPase activity of wild coho salmon sampled from streams in the Chehalis and Humptulips basins in 1987. Fish were collected by electroshocking. Symbols represent means; brackets indicate ± one standard error.

fish had increasing ATPase values from April to May (Fig 1.22). By the end of May and continuing into June, these values were markedly elevated which is characteristic of migrating smolts (Zaugg 1982). Migrants caught at the scoop trap had higher levels of ATPase than any other sampling location. Fish in Stillman and Stevens creeks had slightly higher ATPase activities than those in Bingham Creek, suggesting that smoltification was about one week later in Bingham Creek (Fig. 1.22). These results indicate that smoltification was comparable in wild coho in the Chehalis and Humptulips basins during 1987.

Gill ATPase activity increased in May in all resident wild coho collected in 1988 (Fig. 1.23) and was greatest in fish from Stillman Creek. In May, ATPase returned to initial values (comparable to March) in fish from Bingham Creek, but remained elevated in fish from Stillman and Stevens creeks.

Migrant fish showed variable patterns of gill ATPase activity in 1988 (Fig. 1.24). ATPase activity in migrant populations in the tributaries increased from April to early May while at the scoop trap it was largely unchanged over this time period. ATPase declined sharply in early May in fish from Stevens and Bingham creeks and in mid-May for fish at the scoop trap. ATPase levels in fish from Stevens and Bingham creeks increased at the end of May. In Stillman Creek, levels declined steadily subsequent to the peak that occurred in early May. These data suggest that there were no consistent differences in gill ATPase activities in resident and migrant fish from the two river basins during 1988. Therefore

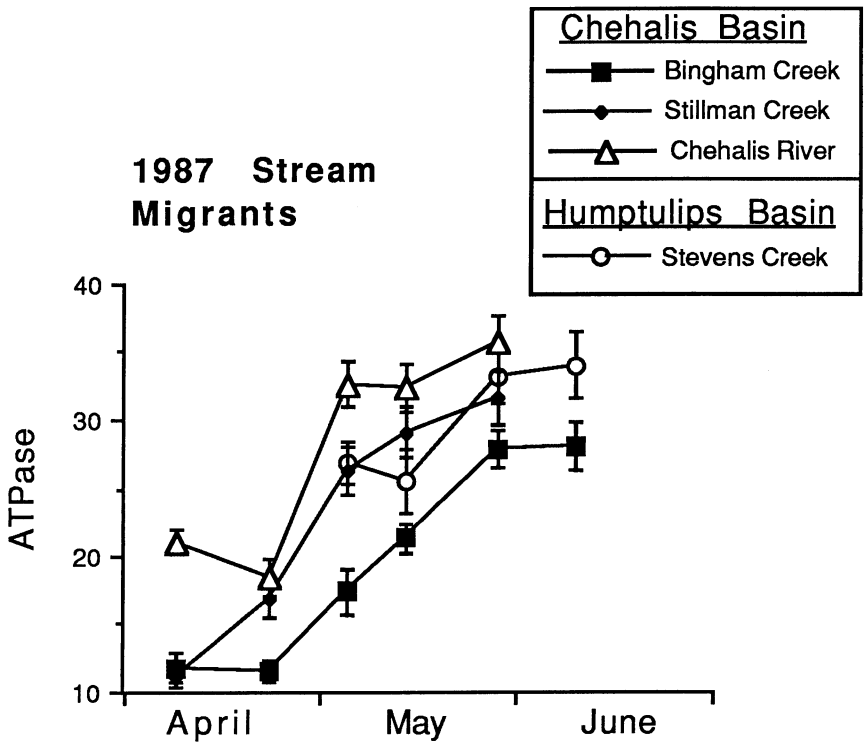

Figure 1.22. Changes in gill Na^+-K^+ATPase activity of wild coho salmon sampled from streams in the Chehalis and Humptulips basins in 1987. Fish were collected by trap. Symbols represent means; brackets indicate ± one standard error.

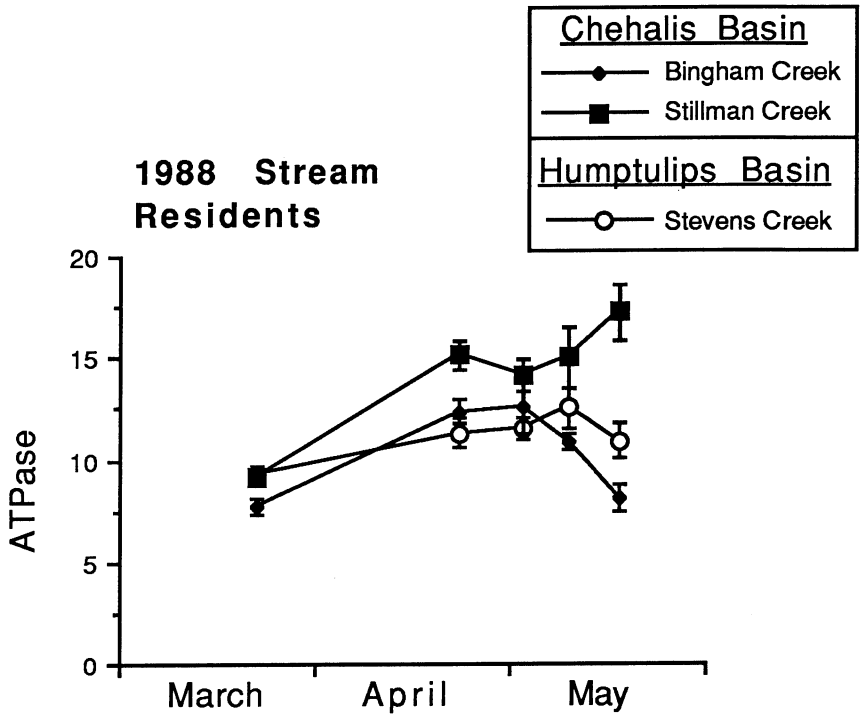

Figure 1.23. Changes in gill Na^+-K^+ATPase activity of wild coho salmon sampled from streams in the Chehalis and Humptulips basins in 1988. Fish were collected by electroshocking. Symbols represent means; brackets indicate ± one standard error.

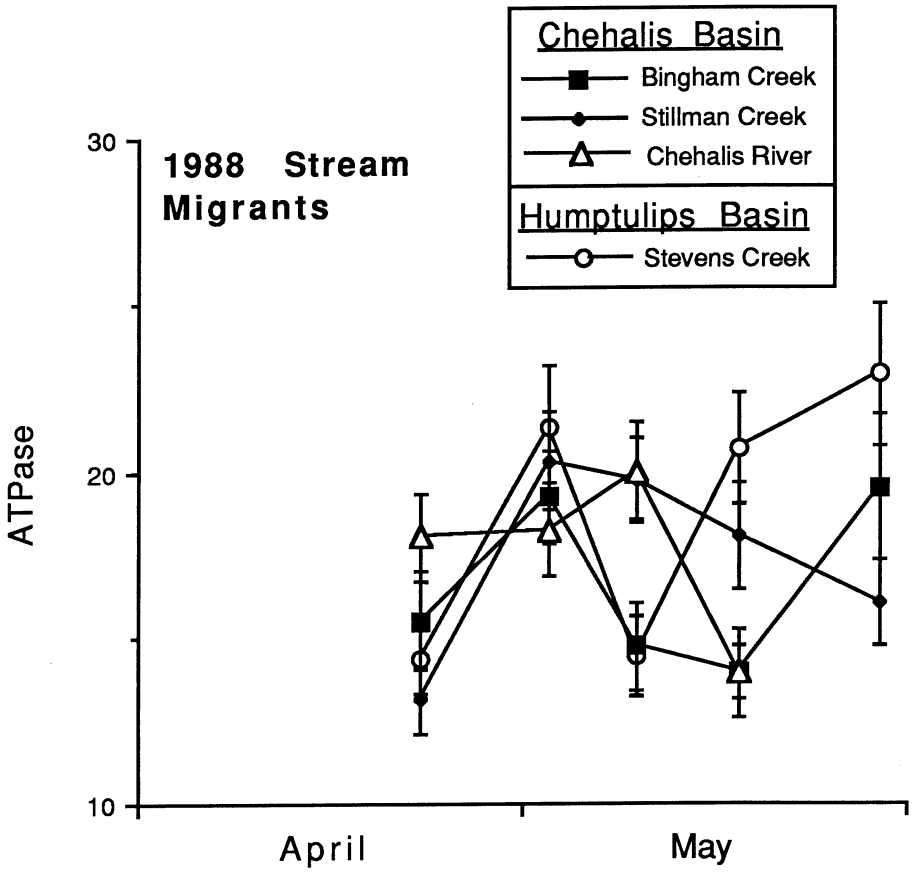

Figure 1.24. Changes in gill Na^+-K^+ATPase activity of wild coho salmon sampled from streams in the Chehalis and Humptulips basins in 1988. Fish were collected from traps. Symbols represent means; brackets indicate ± one standard error.

smoltification of wild fish was not markedly different in the two river basins in 1988.

Thyroid Hormones

In 1987, fish at Humptulips Hatchery showed typical changes in plasma concentration of T4 (Fig. 1.25) which peaked in early April and then decreased to near-basal levels by mid-May. In contrast, there was only a slight elevation in T4 levels in fish at Simpson Hatchery in May. These results suggest that higher quality smolts were produced at Humptulips Hatchery compared to Simpson Hatchery in 1987.

In 1988, fish from both hatcheries elevated their T4 during March and April (Fig. 1.26), although T4 levels were higher in fish collected from Humptulips compared to Simpson Hatchery. At both facilities, T4 declined to near-basal levels by mid-May. These results suggest that there were no major differences in smoltification at the two hatcheries in 1988.

Plasma T4 increased in fish obtained from streams in both river basins in 1987 (Fig. 1.27). In May, T4 was highest in resident fish in Bingham Creek and was equivalent in resident fish from Stillman and Stevens creeks. Data for migrant fish in 1987 were available only for Bingham Creek; T4 was elevated in migrants.

During 1988, plasma T4 increased in stream-resident coho in May (Fig. 1.28) with no differences apparent between fish from the two basins. Migrant fish sampled in 1988 from the mainstem Chehalis River and Bingham and Stevens creeks, increased their T4 levels during April and May (Fig. 1.29). T4 levels in migrant fish

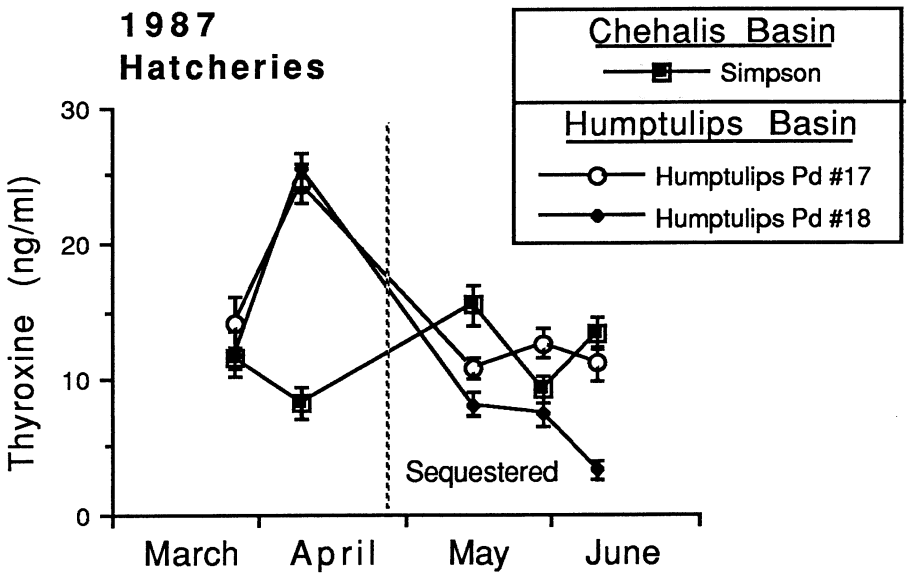

Figure 1.25. Changes in blood plasma concentration of thyroxine in coho salmon sampled at the Simpson and Humptulips hatcheries in 1987. Symbols represent means; brackets indicate ± one standard error.

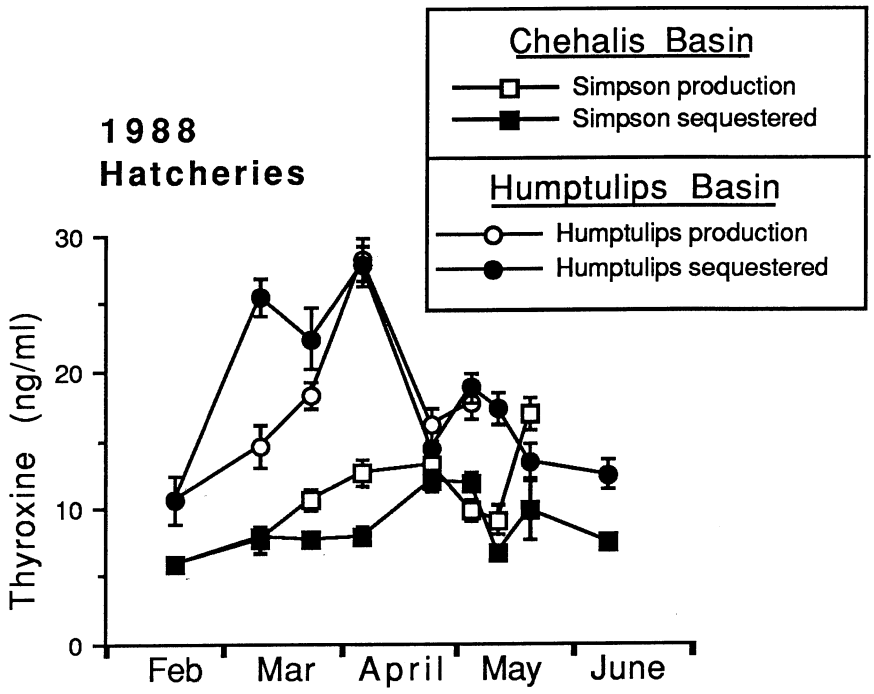

Figure 1.26. Changes in blood plasma concentration of thyroxine in coho salmon sampled at the Simpson and Humptulips hatcheries in 1988. Symbols represent means; brackets indicate ± one standard error.

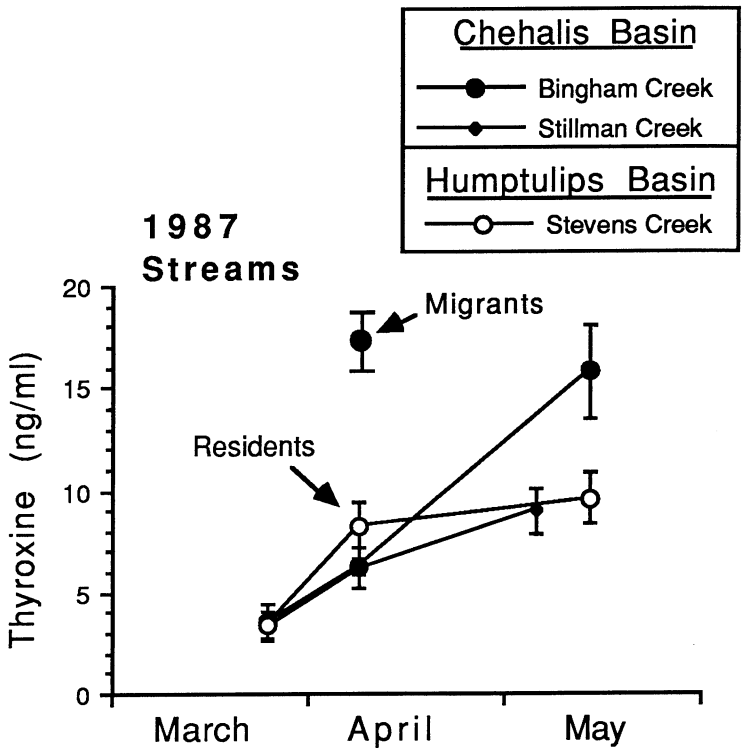

Figure 1.27. Changes in blood plasma concentration of thyroxine in wild coho salmon sampled from streams in the Chehalis and Humptulips basins in 1987. Fish were collected by electroshocking (residents) and from traps (migrants). Symbols represent means; brackets indicate ± one standard error.

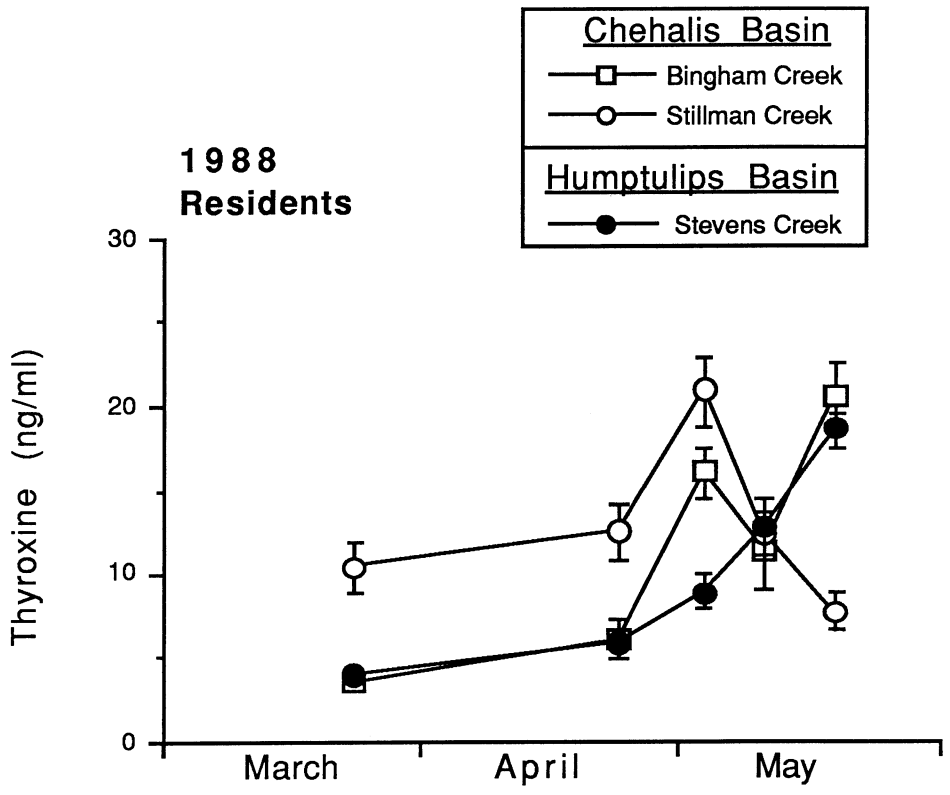

Figure 1.28. Changes in blood plasma concentration of thyroxine in wild coho salmon sampled from streams in the Chehalis and Humptulips basins in 1988. Fish were collected by electroshocking. Symbols represent means; brackets indicate ± one standard error.

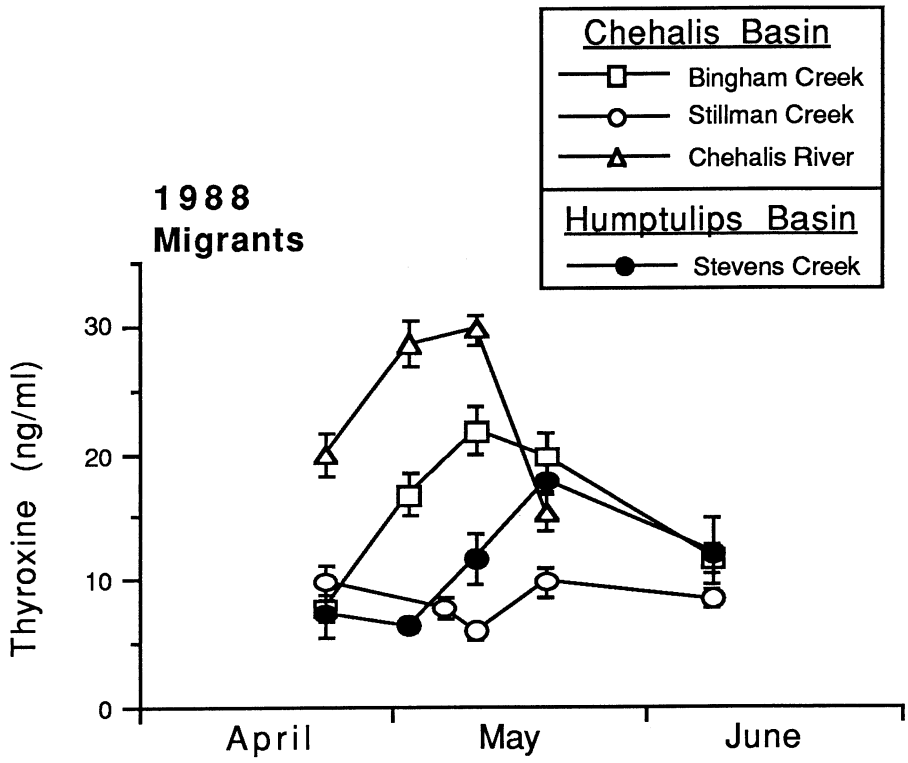

Figure 1.29. Changes in blood plasma concentration of thyroxine in wild coho salmon sampled from streams in the Chehalis and Humptulips basins in 1988. Fish were collected from traps. Symbols represent means; brackets indicate ± one standard error.

from Stillman Creek, however, remained relatively constant. In aggregate, these results indicate that T4 levels of migrant fish from the two basins were comparable during 1988.

Cortisol

Cortisol was evaluated in wild and hatchery fish only in 1988. Blood concentrations of cortisol increased at the end of April in both production and sequestered groups at Humptulips Hatchery (Fig. 1.30). In contrast, blood cortisol showed a clear increase only in the sequestered fish at Simpson Hatchery (Fig. 1.31). The highest cortisol level in production fish at Simpson was only approximately 15 ng/ml, which is markedly lower than peak levels of 25 to 40 ng/ml observed in sequestered fish at both hatcheries and in production fish at Humptulips. These results suggest that smolt development of production fish at Simpson Hatchery was lower than the other hatchery groups in 1988.

Plasma cortisol levels in wild coho collected from trap sites during 1988 were higher than those of hatchery fish (Fig. 1.32). Fish collected at the Stevens Creek trap (Humptulips Basin) showed little change in cortisol levels. Cortisol titers of fish collected from the scoop trap on the Chehalis River were the most variable. Fish from this site exhibited a declining trend in their cortisol levels from late April to mid-May. Conversely, at Bingham Creek, plasma cortisol levels obtained from trapped fish showed an increasing trend. In general, cortisol titers in migrants from all streams overlapped. Thus, there was no indication of significant differences in the quality of wild smolts from the Chehalis and

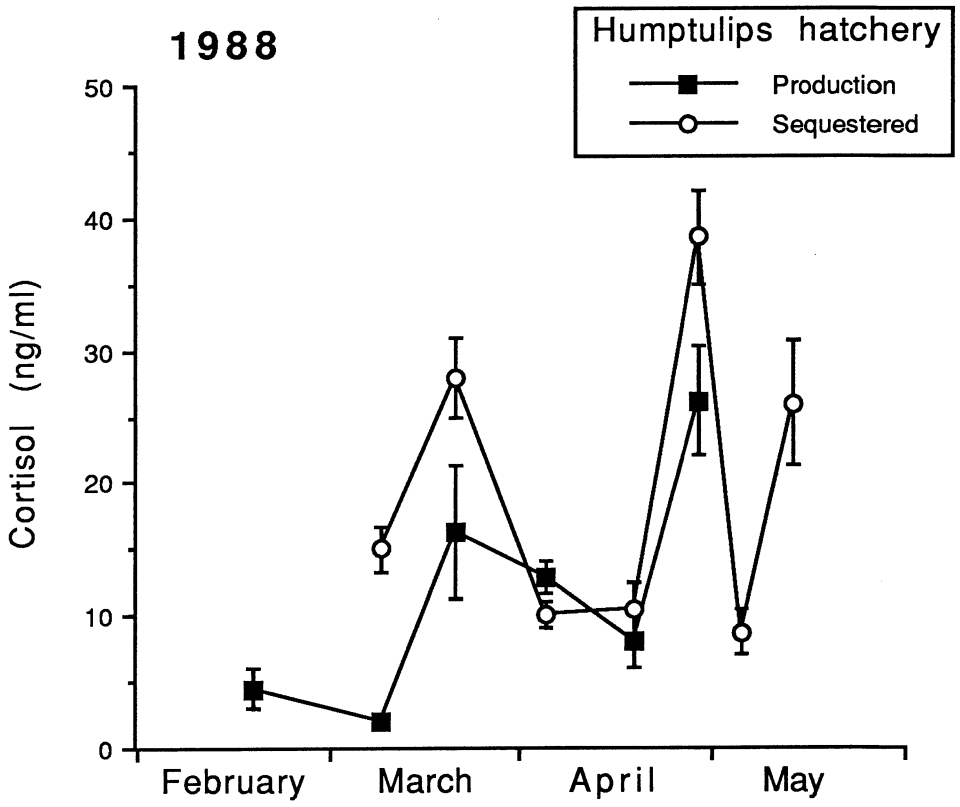

Figure 1.30. Changes in blood plasma concentration of cortisol in coho salmon sampled from the Humptulips hatchery in 1988. Symbols represent means; brackets indicate ± one standard error.

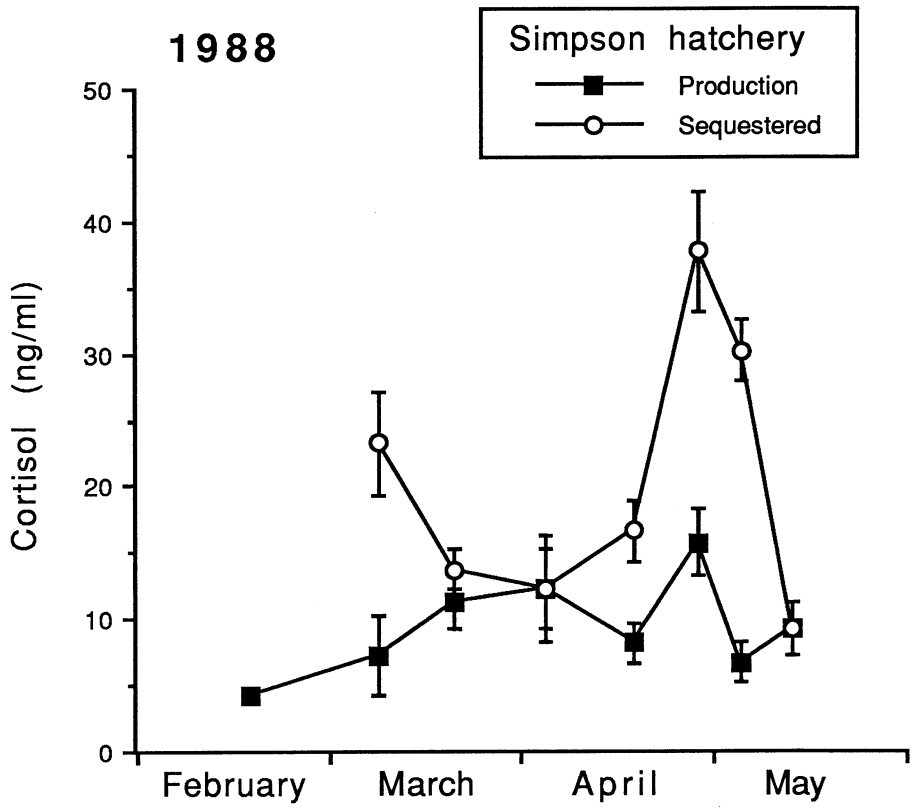

Figure 1.31. Changes in blood plasma concentration of cortisol in coho salmon sampled from the Simpson hatchery in 1988. Symbols represent means; brackets indicate ± one standard error.

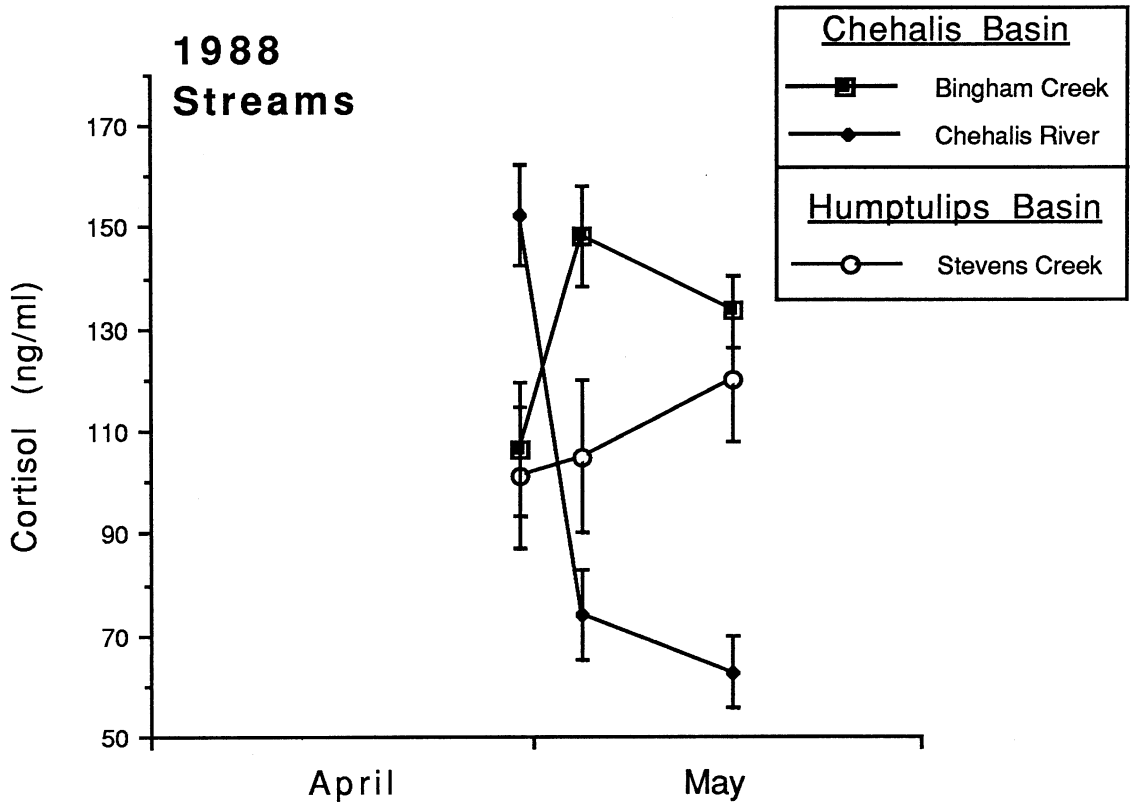

Figure 1.32. Changes in blood plasma concentration of cortisol in coho salmon sampled from streams in the Chehalis and Humptulips Basins in 1988. Fish were collected by trap. Symbols represent means; brackets indicate ± one standard error.

Humptulips basins based upon this parameter.

Hematocrit Counts

In 1987, hematocrit counts of hatchery fish (Fig. 1.33) ranged from 30 to 55% which is considered normal (Wedemeyer and Chatterdon 1971). Values obtained on Humptulips fish steadily increased over time, as expected, but the Simpson fish exhibited a more complex pattern. In late March and early April, these fish appeared to be stressed and their hematocrits declined, possibly as a result of a disease outbreak. By late April, however, the hematocrit counts began to rise as the fish recovered. The drop in hematocrits in mid-May was perhaps caused by sequestering the fish in a new rearing area.

Hematocrit counts obtained from coho salmon at the Humptulips and Simpson hatcheries in 1988 ranged, with two exceptions, from 33% to 47% (Figs. 1.34 and 1.35). Fish in the Simpson Hatchery production pond had hematocrit values of 28% and 25% on 18 April and 5 May, respectively. The low hematocrit counts in late April and early May at Simpson coincided with antibiotic therapy for bacterial kidney disease (BKD). Thus, at the time of release, production fish at Simpson Hatchery had lower hematocrit counts (25%) than production fish at Humptulips (47%). It is hypothesized that the lower hematocrit values in Simpson fish were due to BKD-induced destruction of hematopoietic tissue in the kidney.

Hematocrit values showed an increasing trend in wild resident and migrant fish sampled during both 1987 (Fig. 1.36) and 1988 (Figs. 1.37 and 1.38). Migrating fish had consistently higher

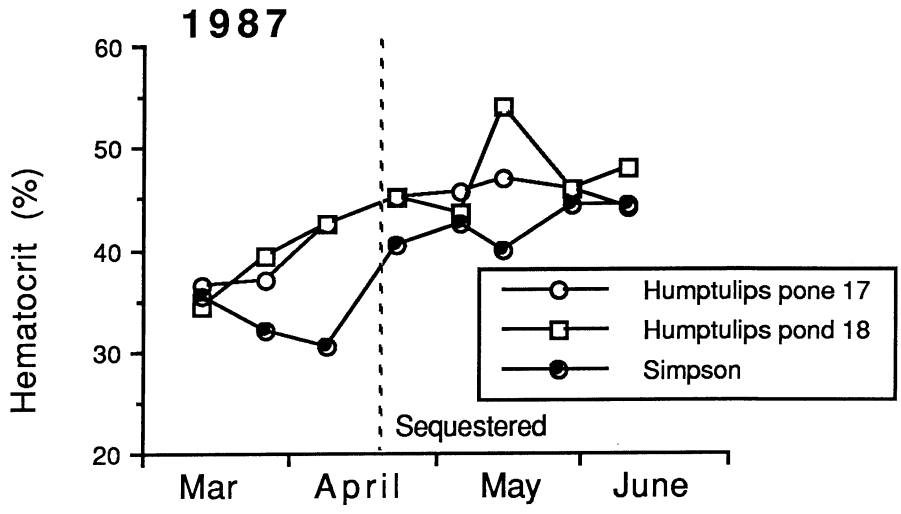

Figure 1.33. Changes in blood hematocrit values (%) of coho salmon from Simpson Hatchery and from pond 17 (demand feeders) and pond 18 (broadcast feeding) at Humptulips hatchery in 1988. Symbols represent means; brackets indicate ± one standard error.

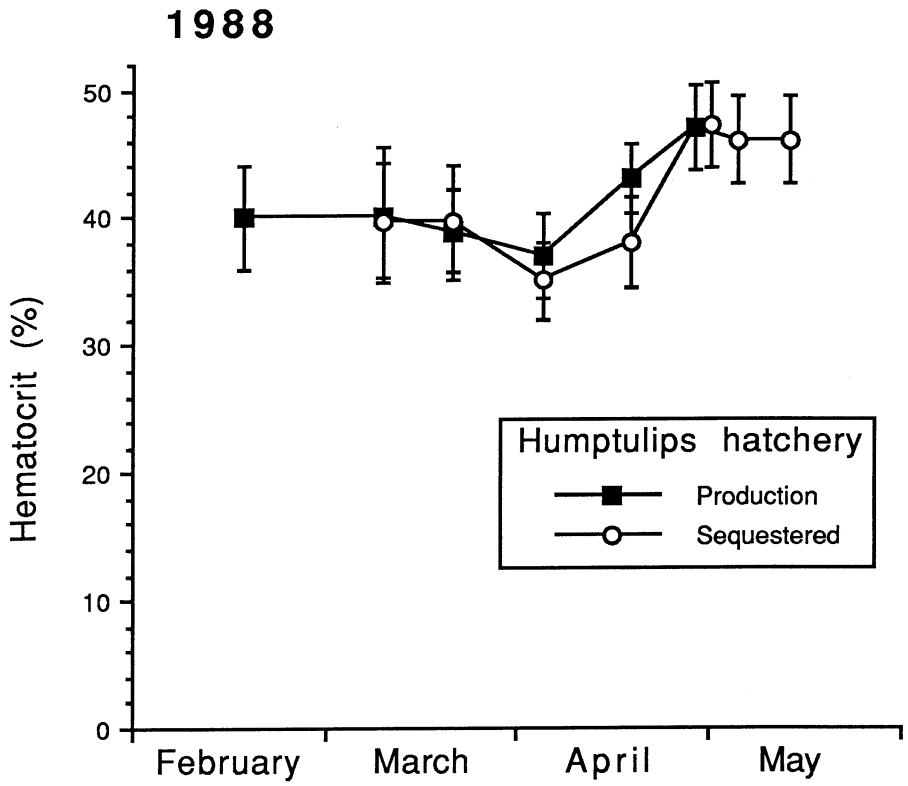

Figure 1.34. Changes in blood hematocrit of coho salmon sampled from the Humptulips Hatchery in 1988. Symbols represent means; brackets indicate ± one standard error.

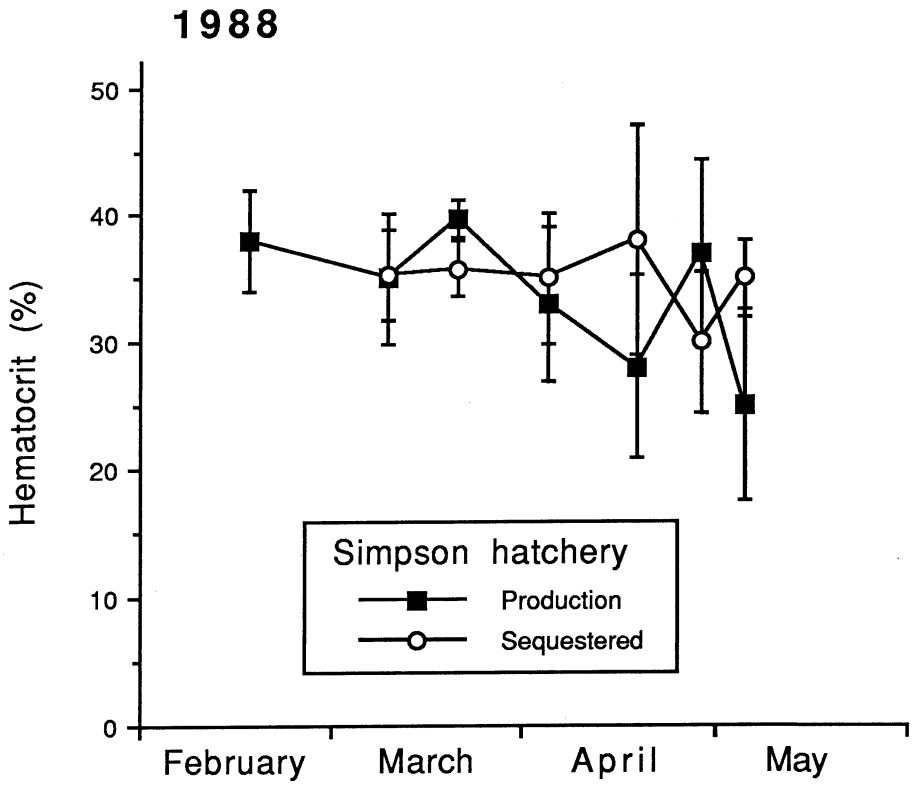

Figure 1.35. Changes in blood hematocrit of coho salmon sampled from the Simpson Hatchery in 1988. Symbols represent means; brackets indicate ± one standard error.

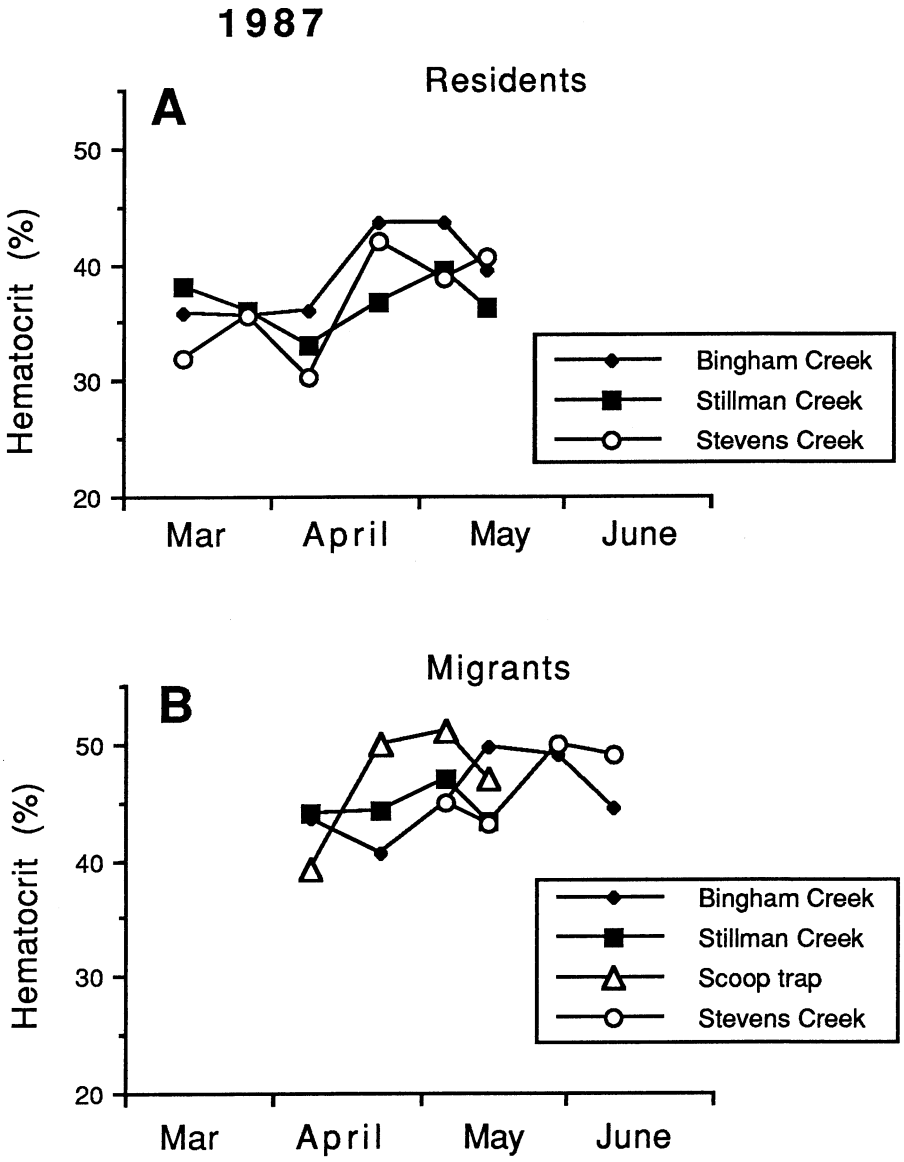

Figure 1.36. Temporal changes in blood hematocrit values (%) of resident (A) and migrant (B) coho salmon from the Humptulips and Chehalis watersheds. Residents were collected by electroshocking and migrants were obtained from smolt traps.

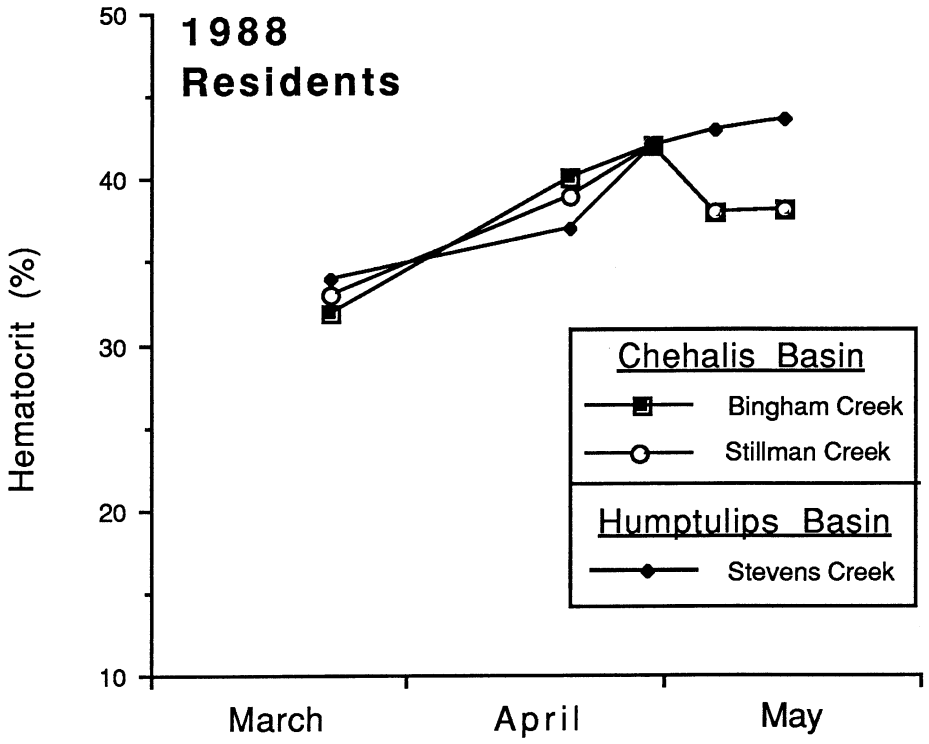

Figure 1.37. Blood hematocrit of wild coho salmon sampled from streams in the Chehalis and Humptulips Basins in 1988. Fish were collected by trap. Symbols represent means; brackets indicate ± one standard error.

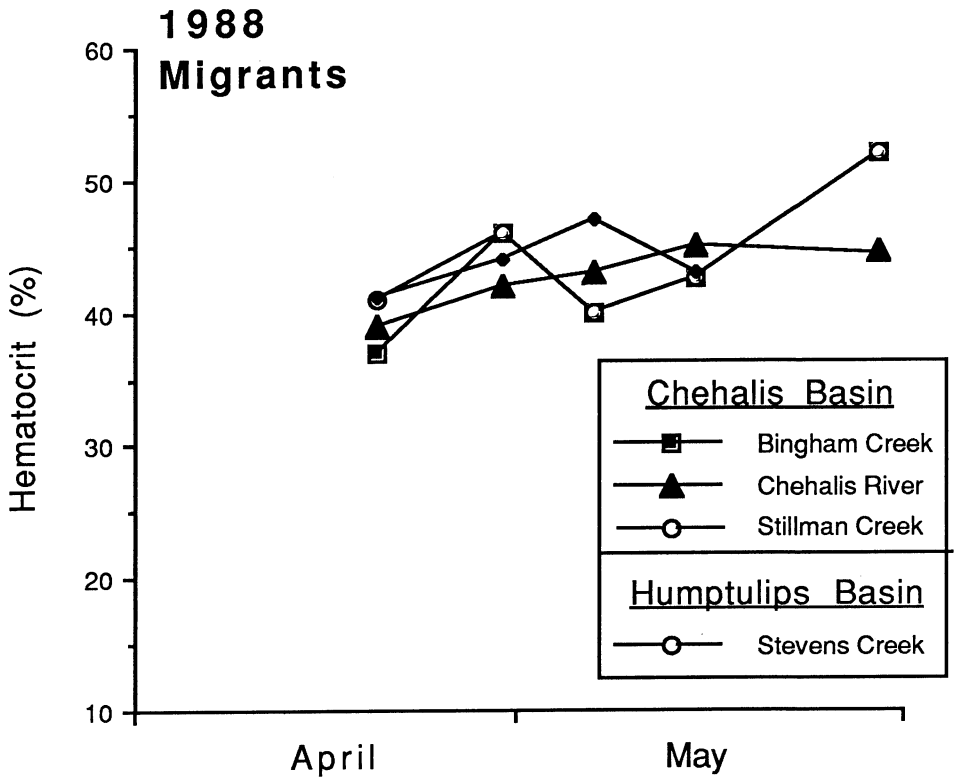

Figure 1.38. Blood hematocrit of wild coho salmon sampled from streams in the Chehalis and Humptulips basins in 1988. Fish were collected by trap. Symbols represent means; brackets indicate ± one standard error.

hematocrit counts compared to resident fish in both the Humptulips and Chehalis basins in 1988. While this was also generally true in 1987, hematocrit counts declined somewhat in migrants toward the end of spring. No significant differences could be discerned in hematocrit counts of wild fish in the two basins.

Summary

The results of assessments of gill ATPase activity and plasma thyroid hormone levels of hatchery-reared fish in 1987 suggested that smolts produced at Humptulips Hatchery were of higher quality than those at Simpson Hatchery. In contrast, morphological assessments conducted in 1987 using principal component 2 (PC2) indicated that coho produced at Simpson Hatchery were morphologically more smolted than fish at Humptulips Hatchery. In 1988, data on body shape, body weight, body coloration, plasma cortisol and blood hematocrit counts indicated that smolts at Humptulips Hatchery were of higher quality compared to those produced at Simpson Hatchery. We could detect no significant physiological difference in wild resident and migrant coho yearlings collected in the two river basins in 1987 or 1988.

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HYPOTHESIS TWO: ASSESSMENTS OF FISH HEALTH IN FRESHWATER

Introduction and Background

As described in the previous section, a major focus of the Coho Salmon Survival Study was to assess whether coho salmon juveniles produced within the upper watersheds of the Chehalis and Humptulips river basins were comparable. Results of these investigations demonstrated that wild smolts produced by the two watersheds were of comparable quality. Another factor that could have a significant influence on survival is the health of the fish. Clearly, parasites or pathogens could significantly impact the survival rate of fish produced in the Chehalis River system by inducing stress or interfering with smoltification.

WDF regularly monitors health of fish produced at the Humptulips and Simpson hatcheries. Diseases that can seriously affect survival of salmonids are usually obvious in a hatchery environment, resulting in abnormal morbidity and mortality during freshwater residence. WDF pathology reports indicate that minor problems with fish health occur periodically at the Simpson Hatchery, and some health problems also have been reported at the Humptulips Hatchery. For example, in one year (1981 brood), coho at Humptulips Hatchery were reportedly too sick to receive coded-wire tags.

In this section, the hypothesis that coho smolts exiting the rearing environments of the Chehalis River basin are less healthy than those exiting the Humptulips River is examined. The approach taken was to compare pathogen levels, parasite loadings,

histological parameters, hematology, and stress levels of coho produced from the two watersheds. Three major tasks were performed. First, disease and parasite screenings were performed on coho collected at Simpson and Humptulips hatcheries and on wild fish obtained from both watersheds in 1987. Second, similar screenings were performed on fish collected in 1988. Third, the immunocompetence and ability of hatchery and wild fish from both watersheds to resist a secondary stress were evaluated in 1988.

Materials and Methods

From March to June 1987 and 1988, coho were collected about every two weeks in the Humptulips and Chehalis watersheds. Fish were obtained from the Simpson and Humptulips hatcheries and resident and migrant wild coho were collected from a number of locations. The sites and the methods used to obtain fish are the same as those used to collect fish for the smolt quality evaluations described in the previous section. In fact, whenever possible, the same fish were used for physiological and health screenings. Details of the sampling methodology are described in PART II, Hypothesis One. We attempted to collect at least 15 fish on each sampling date from each location; actual sample sizes for fish health evaluations are given in Appendix 1.

Examination of Carcasses

All coho collected for fish health evaluations were killed with a lethal dose of tricaine methane sulfonate (MS-222). Carcasses were transported on ice to the Manchester Field Station for examination of overt disease and incidence of parasites. The

examinations of fish for parasitic infestations focused on *Nanophyetus salmincola* (hereafter referred to as *Nanophyetus*). *Nanophyetus* is a digenetic trematode that utilizes salmonids as a second intermediate host during the completion of a complex life cycle involving the freshwater snail *Juga plicifera* and several mammalian definitive hosts, such as racoons, coyotes, and also fish eating birds (Bennington and Pratt 1960). Attention was focused on this parasite for two reasons. First, it is found in a number of salmon producing streams, including those in the Grays Harbor area (Wilson and Foreyt 1985); in our initial examinations of fish carcasses, we found that many fish were infested with the parasite. Second, heavy loadings of *Nanophyetus* may affect performance of the coho. Baldwin et al. (1967) found that *Nanophyetus* can cause mortality in salmon fry in freshwater while Harrell (NMFS, personal communication) found that heavily infested chinook fry experienced physiological stress and mortality at the time of seawater entry.

To quantify the amount of *Nanophyetus* in coho, the posterior third of the kidney was dissected from the fish and the tissue examined with a dissecting microscope at 10x magnification. The number of metacercarial cysts of *Nanophyetus* were enumerated.

Blood Smears and Histology

Hematological examinations were conducted on fish collected in mid-March by staining blood smears with Diff Quick. Detailed histological examinations of selected organs were conducted on fish collected in late-May. Fish used in histological examinations were fixed in 10% neutral buffered formalin and sections of liver, gill,

heart, and kidney were stained with hematoxylin and eosin, and examined microscopically for histological lesions.

Immunocompetence and Secondary Stress Tests

Background and Rationale for use of Assays. In addition to direct examinations of fish for pathogens and parasites, two additional methods were used to assess the health of collected fish. The first of these evaluated how groups of coho smolts responded to a stress. One of the primary responses of fish to stress (see Figure 2.1) is the activation of the hypothalamic-pituitary-interrenal axis which causes a variety of immediate neuroendocrine responses, including elevated plasma cortisol concentrations (Donaldson 1981). Cortisol is a key component in the response of an animal to stress, as it helps mobilize energy reserves and thus facilitates resistance to the stressor and aids in recovery.

Cortisol levels *per se* may not be a good indicator of general health in cases where fish have been subjected to prior stresses, because some stressors do not evoke heightened cortisol titers. Also, elevated titers can fall back to lower levels after a period of time (Schreck and Lorz 1978: Barton et al. 1986). It has been demonstrated, however, that when a fish experiences multiple acute disturbances, its capacity to secrete cortisol can continue to increase. Consequently, when fish are exposed to a stressor known to induce cortisol secretion, those that have been stressed in the past should have a greater percent change between their resting and post-stress cortisol levels than less perturbed cohorts. Secondary

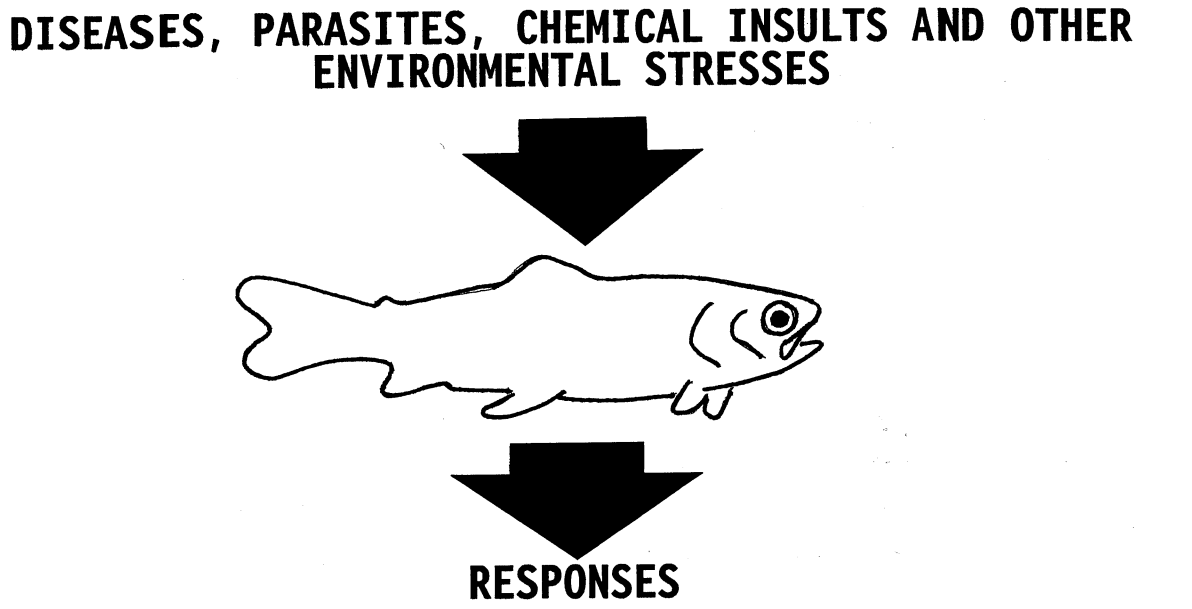

Fig. 2.1. Physiological responses typically exhibited by fish that have encountered stressful events or conditions.

stress tests can thus be used to discriminate between fish from different populations on the basis of their prior history with stress. That is, fish with a recent history of a stressful encounter would be expected to have a larger response in their cortisol secretion than unstressed individuals (Schreck 1990).

The second assay that we conducted was to assess the relative status of the immune system or immunocompetence. This particular assay was chosen because stress is known to adversely influence the immune systems of fish (Wedemeyer et al. 1990). For instance, it has recently been shown that acute stress in chinook salmon reduced the ability of pronephric lymphocytes to generate specific antibodies and hence interfered with the capacity of the fish to resist the pathogen *Vibrio anguillarum* (Maule et al. 1989). Thus, an increase in cortisol titers coincided with a decline in the ability of the fish to resist a disease.

Evaluating immunocompetence is particularly relevant to an anadromous species where the animals immediate environment changes dramatically over a short timespan and holds the potential for challenging the immune system either directly due to the presence of pathogens or indirectly because of poor environmental conditions. During smoltification, salmonids are less able to resist disease than at any other point in their sub-adult life cycle; consequently, a further depression in the immune system at this time can have a profound affect on survival.

Evaluating Immunocompetence and Assessing Stress. The secondary stress test used to evaluate stress levels simply

consisted of measuring resting cortisol levels in a group of fish and then measuring cortisol again after the fish had been exposed to a standardized stress. The standardized stress test involved placing 10 fish into a perforated bucket secured in calm water. The volume of water in the bucket was just sufficient to cover the fish to their dorsal fin. The volume of water was adjusted depending on fish size to normalize for differences and thereby standardize the test. Fish were confined for one hour in the bucket at which time they were killed and bled for plasma samples. Cortisol in 10 μl of plasma was measured in each fish following a radioimmunoassay procedure (Foster and Dunn 1974) modified for coho salmon plasma by Redding et al. (1984).

The immunocompetence of coho was assessed by quantifying the ability of pronephric lymphocytes to produce antibodies in vitro. The method used to accomplish this was the hemolytic plaque assay, described in detail by Tripp et al. (1987). Briefly, this technique consists of preparing lymphocyte suspensions from individual fish and exposing these to an antigen. After a period of incubation, cells secreting antibodies to the antigen are counted, and data are expressed as Plaque Forming Cells/culture (PFCs/culture).

Results

General Fish Health

A total of 922 hatchery and wild coho smolts were examined in 1987 and 1,087 in 1988 (Appendix 1). Only two fish, one from each hatchery, showed overt signs of bacterial kidney disease (BKD).

Necropsies revealed few remarkable lesions other than the two fish with BKD and in fish that had experienced *Nanophyetus* cercarial penetration of the integument and fins (melanomacrophage accumulation at the site of penetration). Microscopic examination of stained sections of kidney, liver, and gills revealed encysted metacercaria but no other evidence of histological change. Microscopic inspection of stained blood smears indicated no abnormalities in erythrocytes or other blood cells. Also, most hematocrit values during these two years were within the normal range for pre-smolting and smolted salmon (see PART II, Hypothesis One).

Nanophyetus Infestation

Metacercarial cysts of *Nanophyetus* were found in coho salmon from all locations sampled in both watersheds. With one exception (the 5/4/87 sample), the mean number of cysts from coho obtained from the two hatcheries in 1987 was low (Fig. 2.2); fish from Simpson Hatchery tended to have slightly more parasites than Humptulips Hatchery fish. Coho were more heavily infested in 1988 than they were in 1987 and the mean number of cysts/fish were comparable in both hatcheries in 1988 (Fig. 2.3). Infestation rates did not appear to change throughout the sampling period in either of the two years.

Cyst levels in resident wild fish varied considerably according to sampling date, year, and location. In 1987 (with the exception of 5/13), resident fish from Stevens Creek had cyst levels greater than or equal to those of resident fish from the

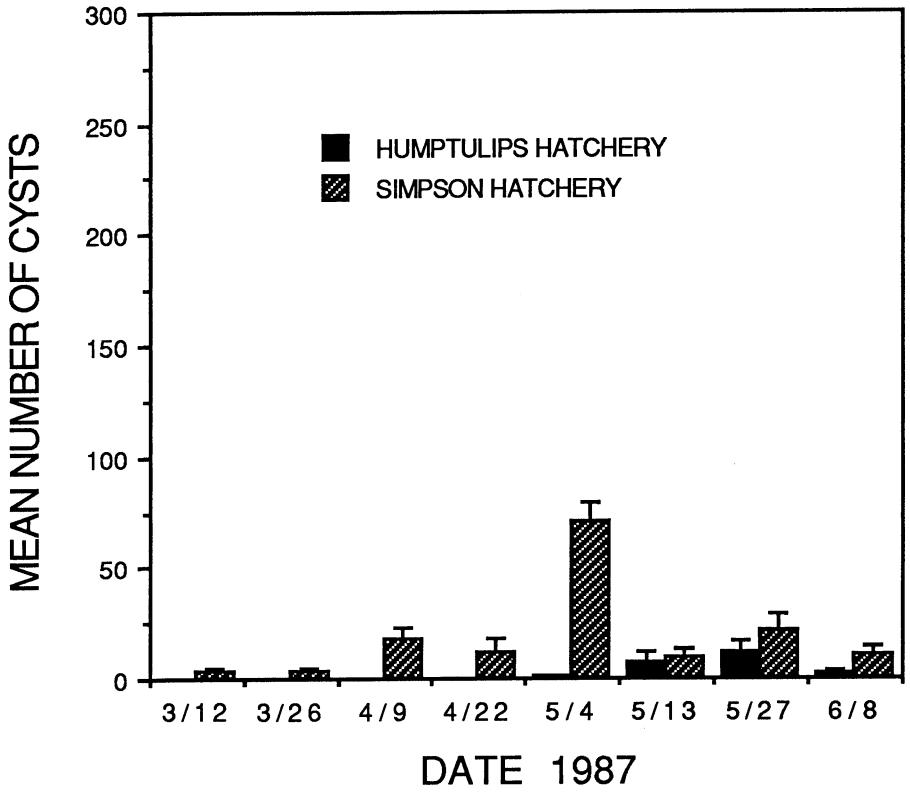

Figure 2.2. Number of cysts of *Nanophyetus* in coho salmon from Humptulips and Simpson hatcheries during 1987. Bars indicate means; brackets indicate ± one standard error.

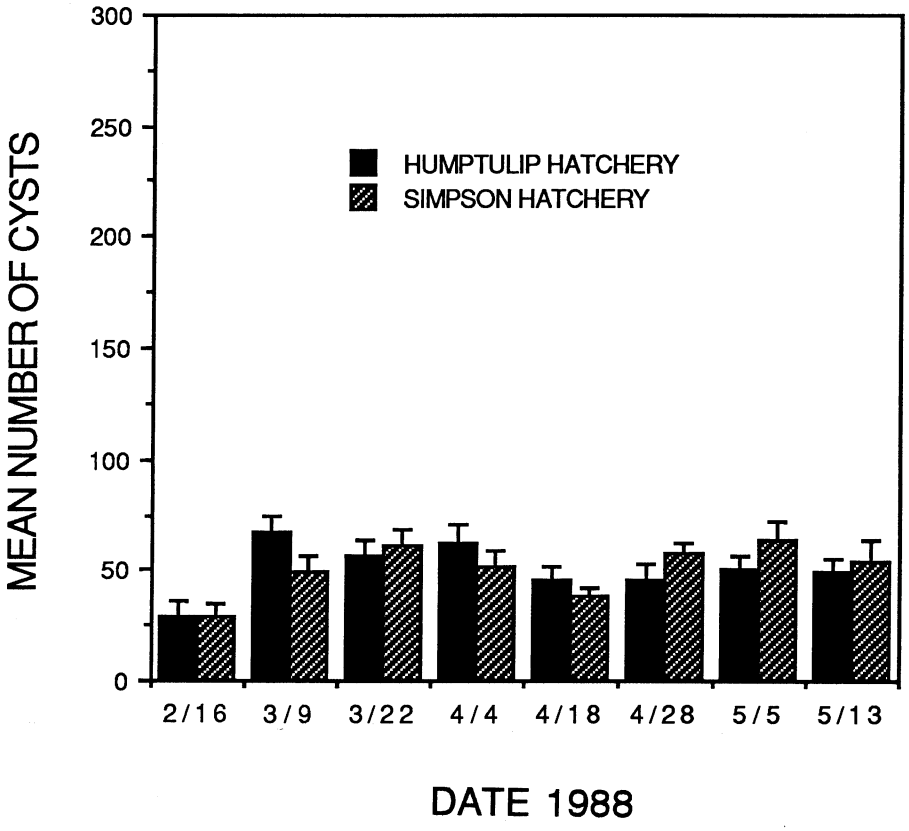

Figure. 2.3. Number of cysts of *Nanophyetus* in coho salmon from Humptulips and Simpson hatcheries during 1988. Bars indicate means; brackets indicate ± one standard error.

Chehalis River (Fig. 2.4). On the other hand, in 1988, resident coho collected from Stevens Creek consistently had lower cyst counts than resident coho from Stillman Creek (Fig. 2.5). However, in the same year, cyst counts made on resident fish from Bingham Creek were generally similar to those observed on wild Stevens Creek fish. Consistent yearly differences in *Nanophyetus* infestation rates did not occur in either watershed. For example, resident fish in Stevens Creek had higher cyst counts in 1987 than 1988, fish from Stillman Creek had higher counts in 1988 than 1987, and there was little difference in infestation rate at Bingham Creek over the two years.

Unlike resident coho, cyst counts in migrant coho from the Humptulips River were generally lower than cyst levels in migrants from the Chehalis River (Fig. 2.6). This was particularly true in 1987 when the cyst levels of migrants from the Humptulips were typically lower than any of the migrant populations sampled in the Chehalis. The relationship was less clear in 1988, as Stevens Creek migrants had comparable levels to migrant populations in the Chehalis River basin on several of the sampling dates (Fig. 2.7) but were substantially lower on other dates.

Data from residents and migrants were compared to ascertain if migrants became more heavily infested with *Nanophyetus* as they migrated downstream. A consistent trend was not apparent. There were instances where it appeared that infestations increased as the fish migrated downstream. For example, at Stevens Creek in 1988, Stillman Creek in 1987, and Bingham Creek in 1987, resident fish

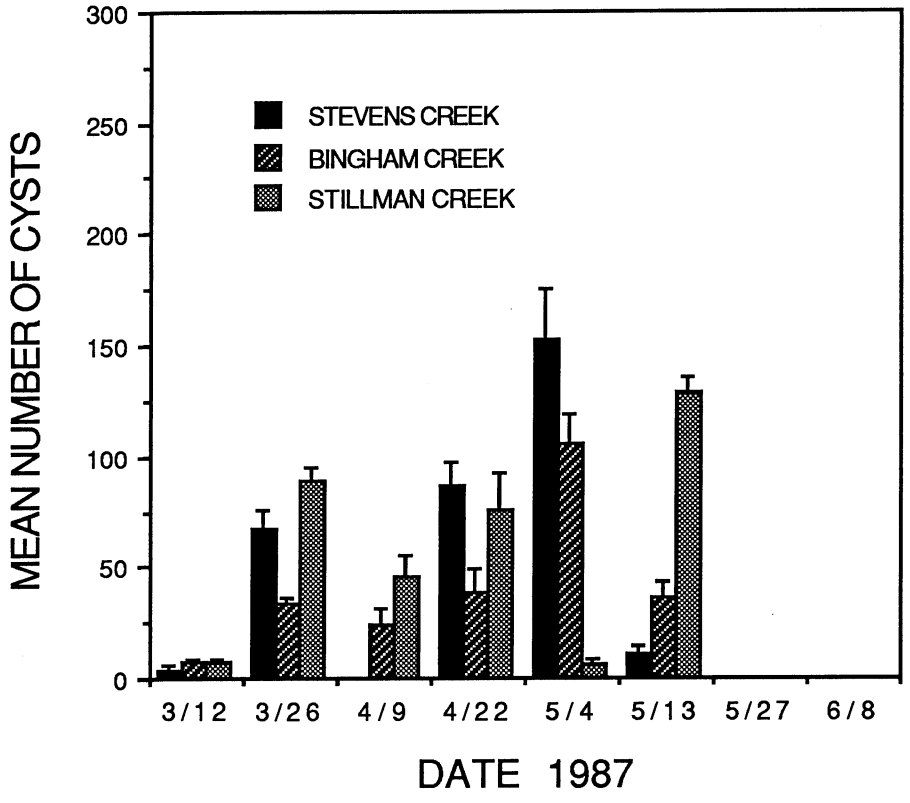

Figure 2.4. Number of cysts of *Nanophyetus* in resident coho salmon from the Chehalis and Humptulips river basins during 1987. Bars indicate means; brackets indicate $\pm$ one standard error.

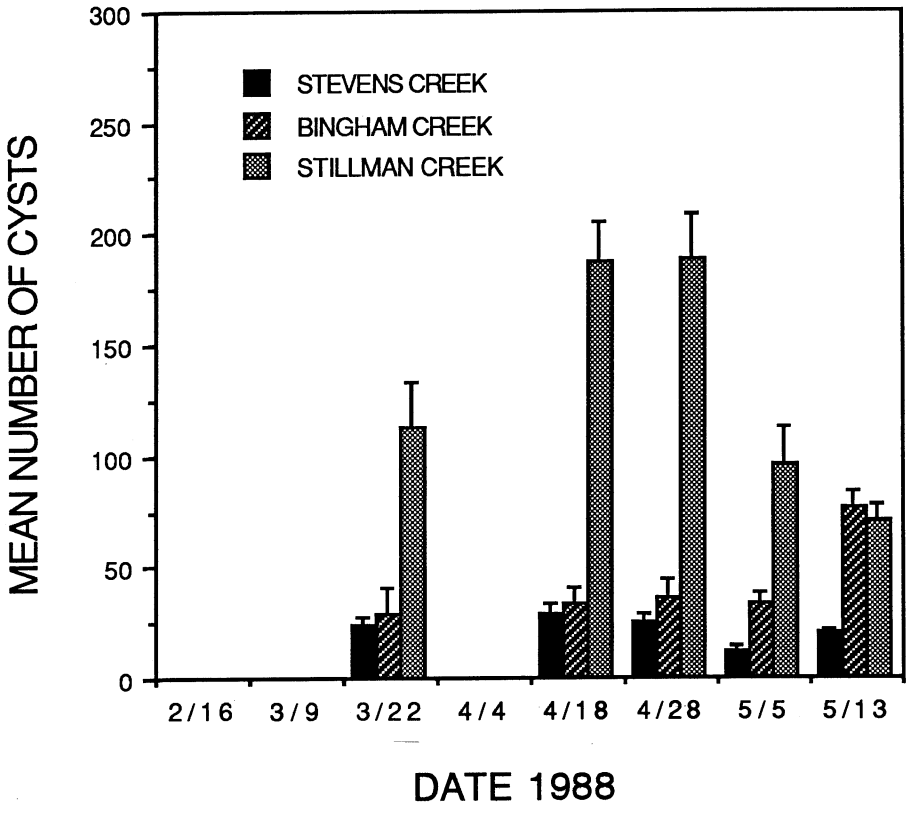

Figure 2.5. Number of cysts of *Nanophyetus* in resident coho salmon from the Chehalis and Humptulips river basins during 1988. Bars indicate means; brackets indicate ± one standard error.

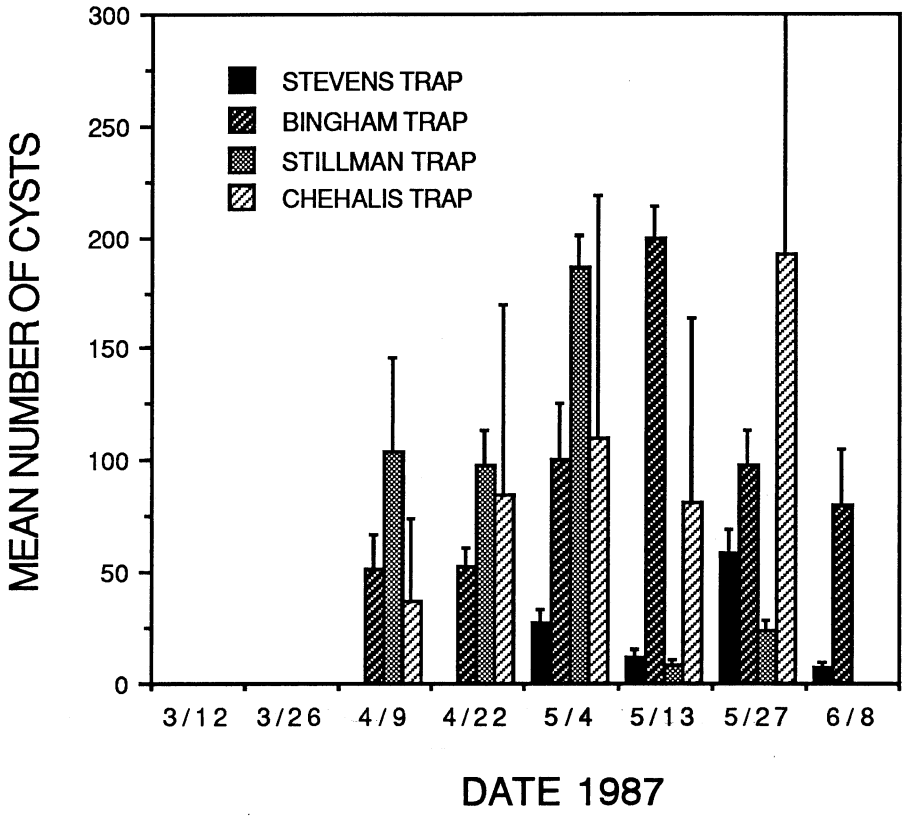

Figure 2.6. Number of cysts of *Nanophyetus* in migrant coho salmon from the Chehalis and Humptulips river basins during 1987. Bars indicate means; brackets indicate ± one standard error.

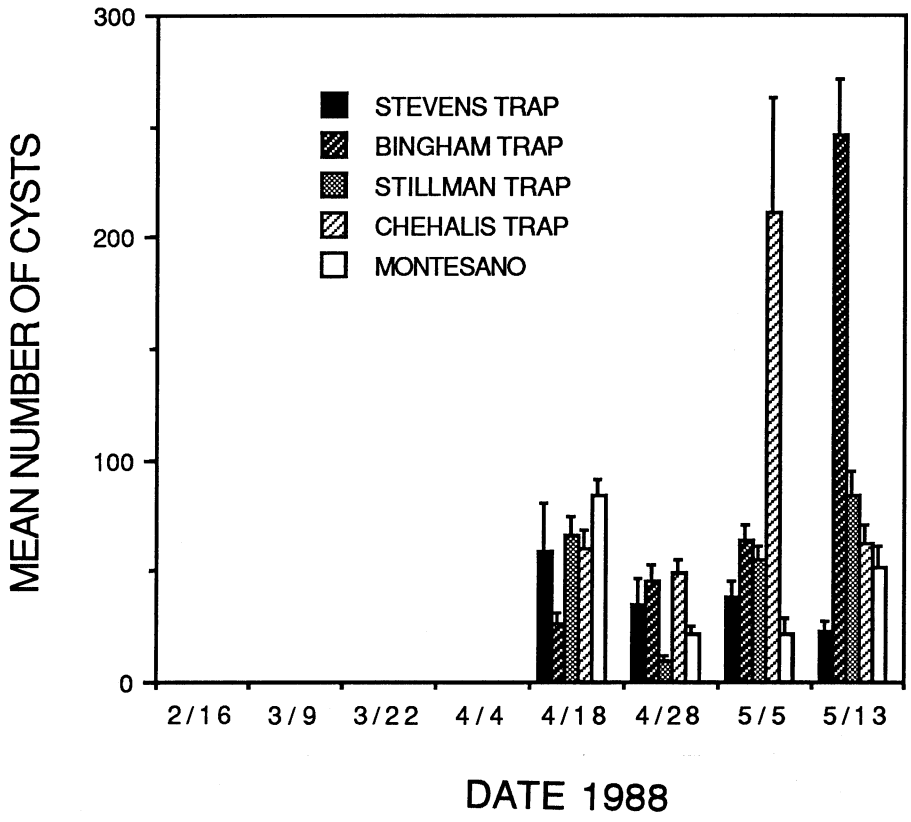

Figure 2.7. Number of cysts of *Nanophyetus* in migrant coho salmon from the Chehalis and Humptulips river basins during 1988. Bars indicate means; brackets indicate ± one standard error.

tended to have lower mean numbers of cysts than migrants (Figs. 2.4-2.7). Also, on some sampling dates (e.g., 5/13/87 and 5/5/88) the fish sampled at the Chehalis trap were more heavily infested than coho at the Stillman Creek trap, which is located about 69 km upstream, suggesting the fish were becoming more parasitized as they migrated further downstream. There were other cases, however, where *Nanophyetus* counts actually decreased or exhibited little change as the fish moved downstream. For example, at Stillman Creek in 1988, resident fish had higher counts than migrants on all but one sampling date (Fig. 2.5 and 2.7).

Secondary Stress Challenge

The results of secondary stress tests conducted at both hatcheries on 4/28/88 indicated that the fish had equivalent stress responses (Fig. 2.8). Control fish at Humptulips Hatchery (from the production pond) had resting levels of cortisol of 25.9 ng/mL while fish subjected to a secondary stress exhibited a mean level of 161.1 ng/mL (a 522% increase over resting levels). At Simpson Hatchery, resting levels of cortisol in control fish were 15.9 ng/mL; when subjected to the secondary stress, mean levels increased to 126.5 ng/mL (695% increase). Responses observed in replicate test groups at both hatcheries were essentially identical.

The results of the stress challenges conducted on wild fish collected at three trap locations (Bingham Creek, mid-Chehalis scoop trap, and Stevens Creek) showed that these fish also had similar stress responses, although they were considerably less than

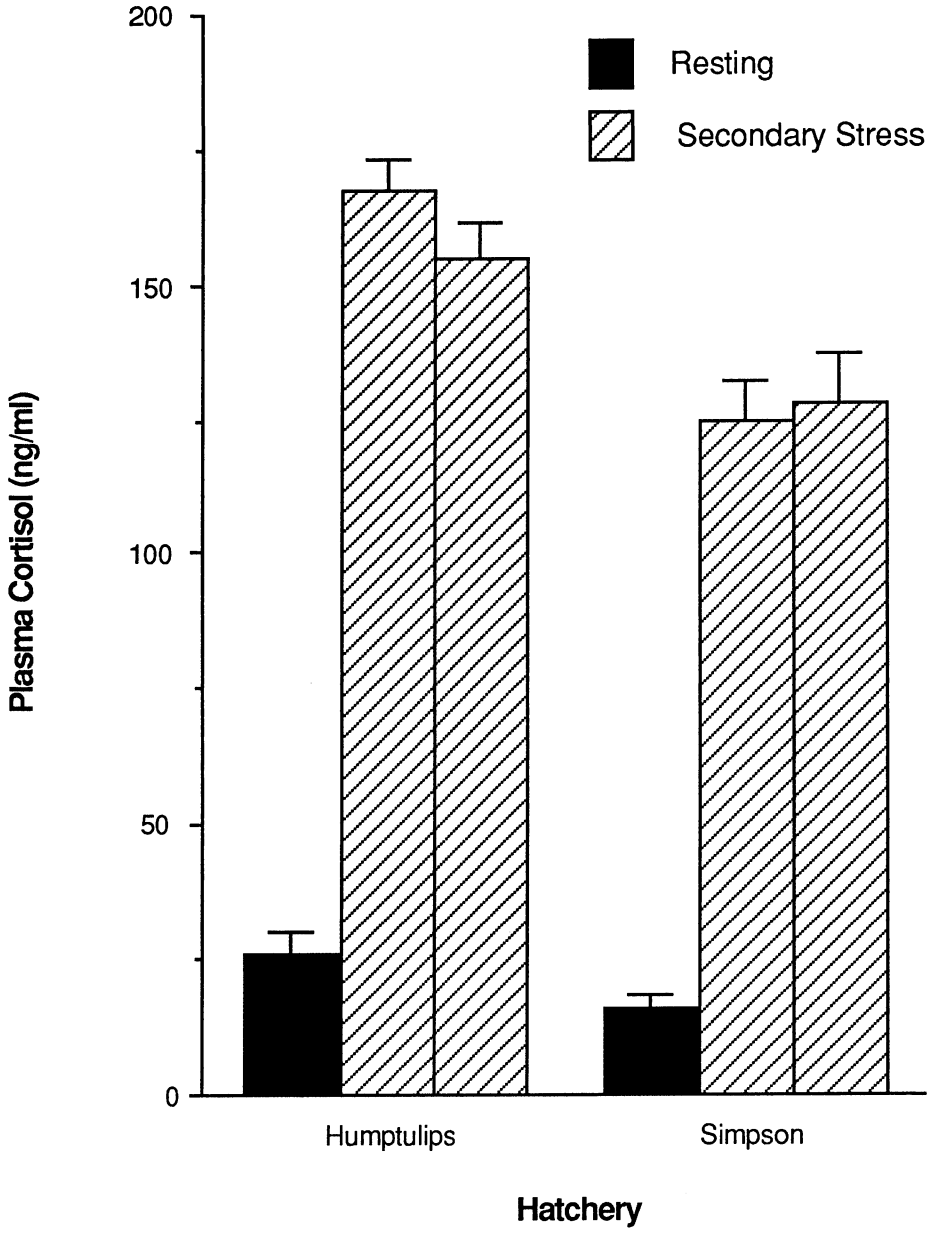

Fig. 2.8. Plasma cortisol concentrations (mean + S.E.) in hatchery coho salmon (production groups) before and after a secondary stress challenge on April 28, 1988. For resting levels n = 15; for secondary stress n = 10 in each of two replicates.

the hatchery fish (Fig. 2.9). Fish collected at the Stevens Creek trap exhibited the greatest magnitude of response, increasing from a resting level of 101.4 ng/mL to 204.8 ng/mL (102% increase) after the secondary stress was applied. Fish obtained at the scoop trap had resting levels of cortisol that were 152.4 ng/mL; after experiencing the secondary stress, their plasma cortisol levels increased to 228.2 ng/mL (50% increase). Finally, plasma cortisol levels in Bingham Creek fish increased from a resting level of 106.4 ng/mL to 155.3 ng/mL after the stress test (46% increase). Responses observed in replicate test groups at all three trap locations were essentially identical.

Immunocompetence

The PFC responses of coho from both watersheds are presented in Figure 2.10. Considerable variability was observed in the PFC response at most locations that were sampled more than once. Wild fish obtained from the Chehalis River watershed appeared to have a better immune system response than wild fish from the Humptulips River basin. At the Bingham Creek trap and the scoop trap, mean PFC responses were 4,088 PFC's/culture and 3,092 PFC's/culture, respectively, while coho from Stevens Creek and the lower Humptulips River had mean PFC's that were ~2,400 PFC's/culture. On the other hand, Humptulips Hatchery fish had a better immune response than fish from Simpson Hatchery. Mean PFCs/culture were nearly 3,000 at Humptulips Hatchery and 808 PFC's/culture at Simpson Hatchery. In 1988, the fish at Simpson were diseased and treated with antibiotics; this may, at least in part, explain the

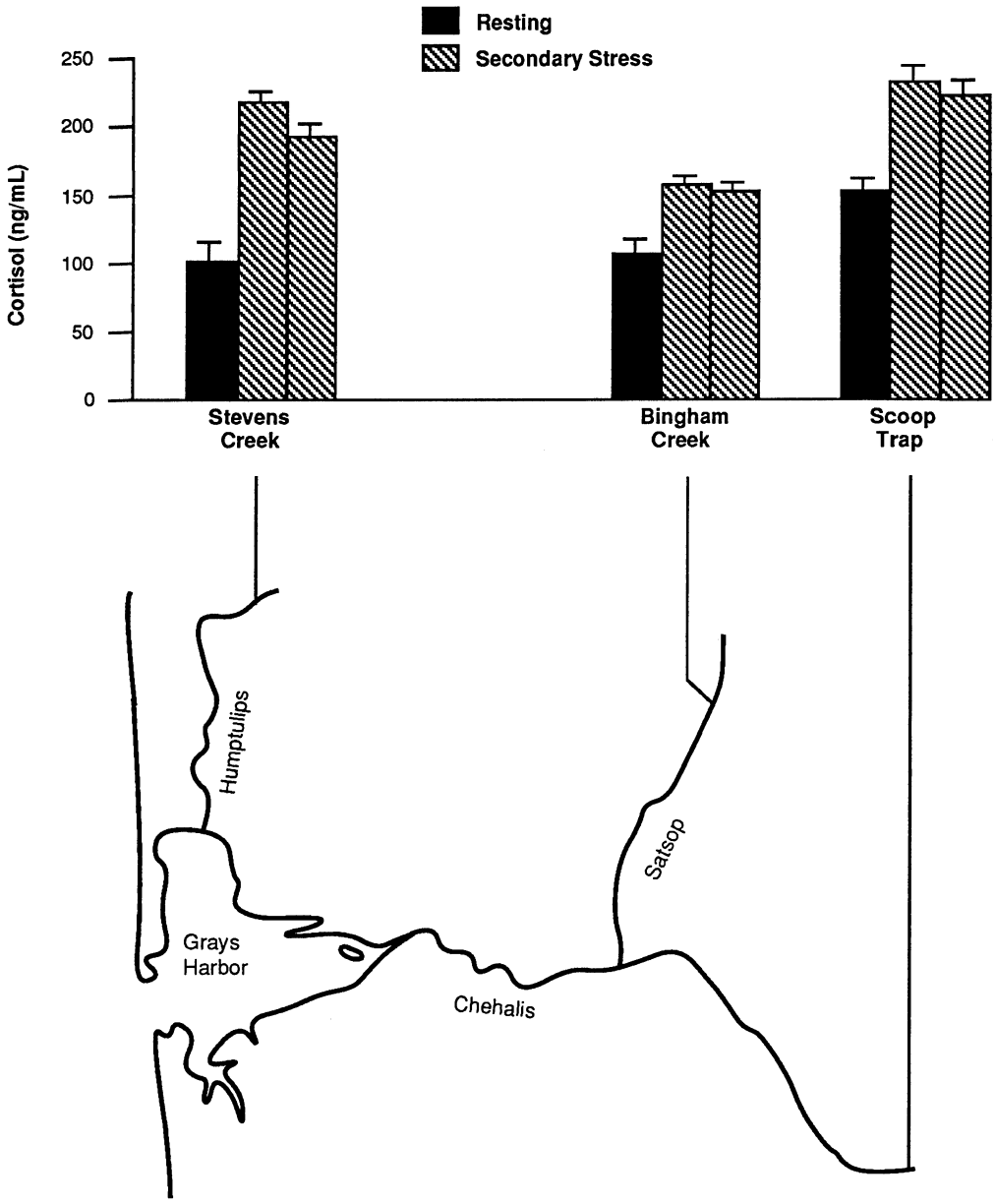

Fig. 2.9. Plasma cortisol concentrations (mean + S.E.) in wild coho salmon before and after a secondary stress challenge. For resting levels n = 10-15; for secondary stress n = 10 in each of two replicates. Stevens Creek was sampled April 30, Bingham Creek on May 1, and fish from the Scoop Trap were tested April 28, 1988.

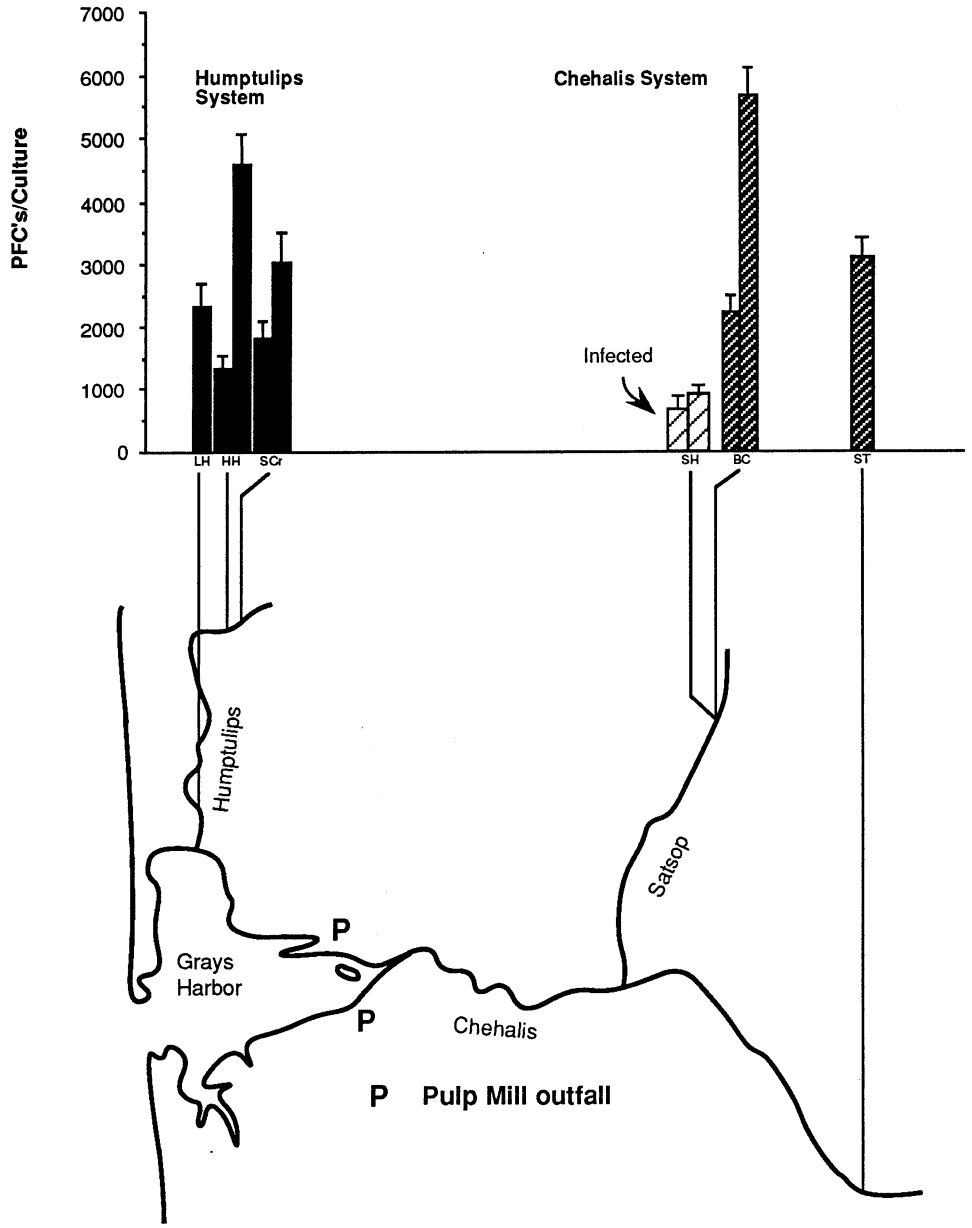

Fig. 2.10. Plaque-forming cell (PFC) response (mean + S.E.) in juvenile coho salmon collected at various freshwater locations and times in 1988. LH = lower Humptulips, May 17 (n = 19); HH = Humptulips Hatchery, April 18 and 29 (n = 20); SCr = Stevens Creek Trap, April 18 (n =14), May 5 (n = 14); SH = Simpson Hatchery, April 18 (n = 19) and April 29 (n = 20); BC = Bingham Creek Trap, April 19 (n = 17) and May 6 (n = 20); ST = Scoop Trap, May 5 (n = 20).

relatively lower PFC response of these fish.

Discussion

General Health

The results of our health screenings indicated that pathogen levels in both watersheds were low. Other available information, however, suggests that pathogen levels in fish from both hatcheries may have been greater than our examinations suggested. The Fish and Wildlife Service (USFWS) found that many of the fish autopsied during the continuous flow bioassay in 1988 and 1989, which were from the Humptulips Hatchery, were infected with BKD (see PART II, Hypothesis Three). They concluded that many of the mortalities in the bioassay were due to BKD. In addition, coho at Simpson Hatchery were infected with pathogens in 1988 to such a degree that WDF pathologists treated the fish with antibiotics. We did not detect the presence of gross BKD lesions, although hematocrit counts were depressed, indicating the fish were diseased.

Our observations and those of the USFWS for Humptulips Hatchery fish are not necessarily inconsistent. BKD could have been present in the fish from Humptulips Hatchery but not at a level that resulted in symptoms of the disease that could be detected by gross examinations of fish carcasses and of hematocrit counts. Fish infected with terminal infections of BKD show gross lesions on the kidney and a depressed hematocrit count. Neither of these conditions were observed. To detect low background levels of BKD requires the use of more sophisticated techniques such as

the fluorescent antibody Technique (FAT) as described by Bullock and Stuckey (1975) and the enzyme-linked immunoadsorbent assay (ELISA) (Pascho and Mulcahy 1987). We did not use these methods for detection of overt BKD in the Grays Harbor studies. During routine sampling at both hatcheries in 1987 and 1988, there were obviously diseased fish, separated from the main population, which were examined and diagnosed as gross BKD. This situation is not uncommon in large salmonid hatcheries in the Pacific Northwest. Gross BKD infections observed in the bioassay fish were probably triggered by the protocols used to conduct the bioassays and the holding period at Marrowstone.

Stress Assays

We were not able to detect differences in the immunocompetence or secondary stress responses of fish originating from the two watersheds. In fact, it appeared that wild fish in the Chehalis produced more PFC's than did wild fish in the Humptulips. Hatchery fish were difficult to compare because those fish at Simpson were diseased when the assay was run in 1988 and this episode undoubtedly depressed their immune systems. Stress responses were also similar as there were no differences between the hatchery fish or among wild fish populations. There were consistent differences between hatchery and wild fish originating from the same watershed, however; hatchery fish had lower resting levels of cortisol than wild fish and a much greater response to the stress. Why this occurred cannot be determined from existing data.

Nanophyetus

The most notable feature of our fish health evaluations was the occurrence of the parasite *Nanophyetus* in hatchery and wild coho from both watersheds (no other parasite was detected in significant numbers). In order for this digenetic fluke to be responsible for the poor survival of Chehalis River coho, two conditions must be met. First, there should be a difference in parasite loadings of fish from the two watersheds; smolts from the Chehalis should have consistently higher infestation rates than coho from the Humptulips River. And second, observed levels of infestation in Chehalis fish should affect their ability to adapt to seawater to a greater extent than the cyst levels present in Humptulips fish.

As can be seen in Figures 2.2 through 2.7, considerable variation exists in *Nanophyetus* infestation rates in wild and hatchery fish from both watersheds. For example, in 1987, resident coho in Stevens Creek (Humptulips drainage) generally had higher infestation rates than resident coho in Bingham Creek (Chehalis watershed). Yet migrants captured from these streams exhibited the opposite trend; fish emigrating from Bingham Creek generally had higher counts of *Nanophyetus* cysts than Stevens Creek coho. In 1988, coho residing in Stevens and Bingham Creeks had fairly comparable parasite loadings but not enough data were collected on the migrants to see if this trend continued. The hatchery fish indirectly corroborate the idea that fish residing in the upper watersheds of both drainages probably have equal opportunities to

become infested. In both 1987 and 1988, Simpson and Humptulips hatchery fish, with one exception, had similar cyst counts suggesting that densities of parasites in the riverine water sources used in each hatchery were comparable.

Plainly, our assessments of parasite loading rates focused on the portions of both watersheds where most coho rear. Once the fish emigrate from their rearing areas we have a much poorer understanding of how their parasite loading rates may change. It is likely that fish traversing through the lower Chehalis River acquire more parasites than those descending the Humptulips. The lower Chehalis is longer and wider than the Humptulips with more backwater (i.e., sloughs) areas, and appears to have a greater amount of habitat preferred by the snail *Juga plicifera*, the first intermediate host for the parasite. In 1988, we examined the incidence of this parasite in coho collected in the inner harbor and North Bay. The results of this survey are presented in PART II, Hypothesis Three and show that smolts entering the inner harbor usually have higher cyst counts than coho entering North Bay.

Whether the apparent differences in *Nanophyetus* infestation rates in coho originating from the Humptulips and Chehalis watersheds are biologically meaningful is another matter. Three pieces of information make it doubtful that *Nanophyetus* by itself is causing Chehalis coho to suffer disproportionately poor survival rates. First, parasite infestation rates vary considerably; fish from both watersheds can have relatively high or low levels of the parasite and these loadings appear to vary unpredictably in space

and time. Next, if the parasite was affecting survival, one might expect to see a linkage between degree of infestation and smoltification. However, smolting indices measured on coho produced from the Chehalis showed that these fish had smolt characteristics that were comparable to fish obtained from the Humptulips. Lastly, the infestation rates observed in our study are not the highest that have been observed in robust coho populations. For example, coho smolts exiting the Deschutes River, Washington, have on average over 340 *Nanophyetus* cysts in the anterior third of their kidneys (NMFS unpubl. data); yet marine survival rates of these fish ranged from 18 to 22% for the past ten years--a rate typical for noninfected populations of Puget Sound coho.

Coho infested with *Nanophyetus* cysts probably experience some degree of stress. From what we currently know, it appears that the amount of stress experienced by infected Chehalis coho is not great enough to destablize smoltification. However, if these fish were to be exposed to additional stressors the accumulated effect could reduce survival (Wedemeyer et al. 1990). It is probable that *Nanophyetus* has reduced the capacity of Chehalis coho to withstand additional stressors. In the next section, the investigations used to determine if such stressors exist in the inner harbor are described.

Summary

In 1987 and 1988, the health of wild and hatchery-produced juvenile coho salmon yearlings in the Chehalis and Humptulips watersheds was evaluated by examining pathogen levels, parasite loadings, histological parameters, hematology, and stress levels of the coho. The results of health screenings indicated that pathogen levels in both watersheds were relatively low. Necropsies performed on 2,009 coho revealed few remarkable lesions on fish other than two hatchery fish that were found to have BKD. The results of secondary stress tests performed at both hatcheries and on wild fish collected at trap sites indicated that hatchery fish had equivalent stress responses as did the wild fish. Considerable variablity was observed in the immune system responses of fish. Wild fish from the Chehalis basin appeared to have a better immune system response than wild fish from the Humptulips basin. Conversely, Humptulips Hatchery fish had a better response than Simpson fish, perhaps because the fish from Simpson were diseased and undergoing antibiotic therapy.

The most notable feature of our fish health evaluations was the occurrence of the parasite *Nanophyetus salmincola* in hatchery and wild fish from both basins. Infestation rates of individual fish ranged from 0 to 500 parasites in the posterior third of the kidney. We could discern no consistent differences in parasite loadings between the two watersheds. A major reason for this was that there was considerable spatial and temporal variability in the infestation rates of *Nanophyetus*. Our assessments of parasite

loadings focused on the rearing areas (i.e., upper portions of the watersheds). We have a much poorer understanding of infestation rates once the fish leave these areas and migrate downstream. Data obtained in the estuary shows that smolts entering the inner harbor have higher cyst counts that coho entering North Bay. Whether this difference translates into a difference in survival is another matter, however. We do not believe that *Nanophyetus* by itself can account for the survival difference between the two watersheds because: 1) infestation rates are highly variable both within and between watersheds, 2) there appears to be no linkage between infestation and survival in the absence of additional stressors, and 3) other coho populations that have high survival rates have higher levels of the parasite.

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HYPOTHESIS THREE: EXAMINING THE EFFECTS OF INNER HARBOR WATER QUALITY ON EMIGRATING COHO SMOLTS

Introduction and Background

The trucking experiment performed in 1985 (see Part I) showed that Humptulips Hatchery coho transported and released into the lower Chehalis River contributed to ocean fisheries at approximately $1/20^{th}$ the rate of cohorts that had been similarly hauled but liberated into the Humptulips River. The only difference between these groups was that one had migrated through the lower Chehalis and its estuary while the other exited through North Bay. One explanation for why fish liberated into the Chehalis survived so poorly is that conditions in the lower river and estuary interfered with smoltification or reduced the immunocompetence of these fish to such an extent that they succumbed before they could contribute to ocean fisheries. This notion that degraded environmental conditions in the lower Chehalis River and inner harbor reduce survival of emigrating coho juveniles became the foundation for hypothesis three.

Eight studies were performed to examine whether poor water quality and pathogens in the lower Chehalis River and inner harbor caused deleterious physiological effects on smolting coho salmon. First, coho migrants were serially collected in the inner harbor and North Bay during 1987 and 1988. The migration rate, smoltification, and health status of these fish was examined and compared. Second, wild coho were equipped with acoustic tags and tracked in 1988 and 1989 to determine their migration speed and

residency in the inner harbor.

Results of the tracking investigations were used to determine the duration of four bioassays. Two of these tests were performed *in situ*. First, in 1988, smolting coho from the Humptulips Hatchery were barged through the inner harbor in a manner that generally mimicked their usual migration route. A control population was simultaneously barged through North Bay. The second *in situ* assay took place in 1989 and involved placing fish in one of seven live-boxes anchored in various lower river, inner harbor and North Bay locations.

Two continuous-flow bioassays were also performed. In 1988, effluents from the two pulp mills and two sewage treatments plants, Chehalis River water, and positive and negative control waters were continuously introduced into tanks containing smolting coho salmon. The fish were exposed to effluents for five days and then transported to a laboratory where seawater growth and survival were evaluated for an additional seven months. A similar assay was conducted in 1989. In this instance, coho were exposed to pulp mill effluents and negative control waters for either five or fourteen days. During this continuous-flow assay some fish were also exposed to mixtures of pulp mill effluents. After the exposure periods had been completed, the treated animals were again transported to saltwater rearing locations to assess growth, survival and resistance to a disease challenge.

In the seventh study, pulp mill effluent was introduced into swimming chambers and its effect on swimming speed, endurance and

body weight was evaluated. The final study used two-choice mazes to determine if coho could detect pulp mill effluents and whether exposure to these waste waters would interfere with their olfactory acuity. In the sections that follow descriptions of how each of these studies were performed and summaries of the results obtained are presented.

Beach Seine Studies

Study objectives 1987

Coho migrants were collected in Grays Harbor during 1987 and 1988. The objectives for each sampling year were slightly different. In 1987, a major objective was to determine how and where to capture fish. Once this was established, six sites in the inner harbor were regularly sampled to assess how rapidly coho emigrated through this portion of Grays Harbor. Some captured fish had CWTs; they were used to ascertain if Humptulips fish resided or emigrated into the inner harbor.

Sporadic sampling also took place in North Bay, primarily to determine appropriate sampling locations. However, coho possessing CWTs were also captured and consequently it was possible to discern if any tagged fish from the Chehalis basin had been captured in this portion of Grays Harbor. Finally, on two occasions, ATPase samples were obtained on coho collected from North Bay and the inner harbor. These samples were compared to see if there were any differences in how well the fish were adjusting to seawater.

Study objectives 1988

In 1988, coho emigrants were routinely sampled in the inner

harbor and North Bay to meet two objectives. Most importantly, fish were collected so that it would be possible to monitor and compare their gill ATPase activities and capacity to resist disease, i.e. their immunocompetence. Secondly, an effort was made to collect fish with CWTs to once again determine whether fish originating from the Humptulips or Chehalis drainages utilized the same estuarine areas.

Fish Collection and Sampling Locations

A 33 m floating beach seine (Miller et al. 1977; Fresh et al. 1981; Simenstad and Eggers 1981) and a 66 m by 6 m purse seine were tested in the inner harbor to determine which of these gears would most effectively capture coho emigrants. Purse seine catches were consistently lower than those obtained with the beach seine. Moreover, the purse seine was difficult to use because of sunken debris and the high current and windy conditions that often prevailed in the harbor. Consequently, beach seining was the method chosen for fish collection in 1987 and 1988.

Six inner harbor locations were sampled in 1987; five were located in the North Channel (Cow Point, East Rennie Island, West Rennie Island, Moon Island, and Buoy 30) and one was situated at the western end of the South Channel (Fig. 3.1). In 1988, the two sites on Rennie Island were not sampled. Instead, a site located just at the eastern entrance of the South Channel and another situated approximately two km upstream of Cosmopolis on Sand Island were used (Fig.3.2).

In 1987, nine sites in North Bay were sampled: four were in

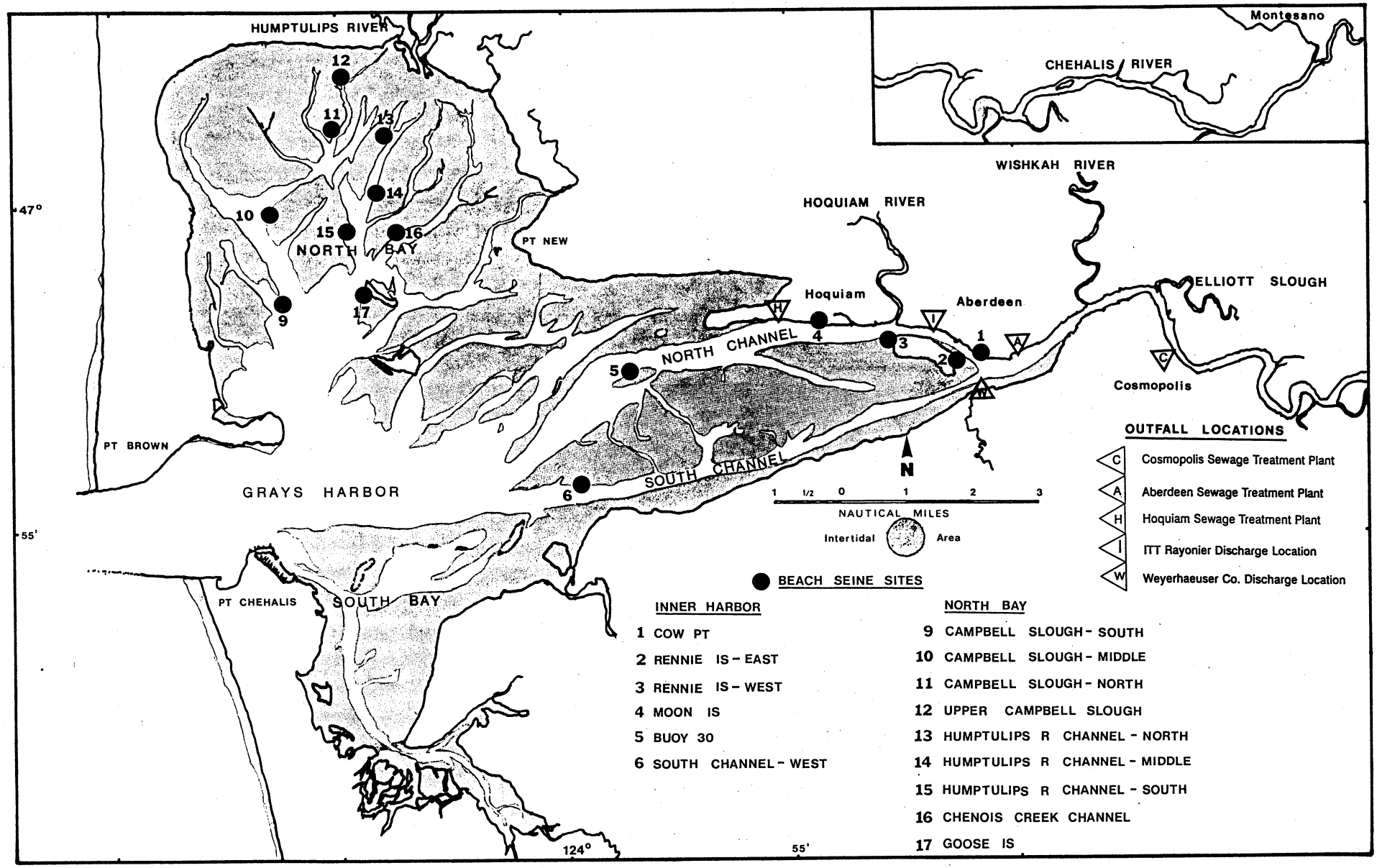


Fig. 3.1. Grays Harbor beach seining locations used in 1987. The inner harbor sites are numbered 1-6, while the North Bay locations have numbers 9-17.

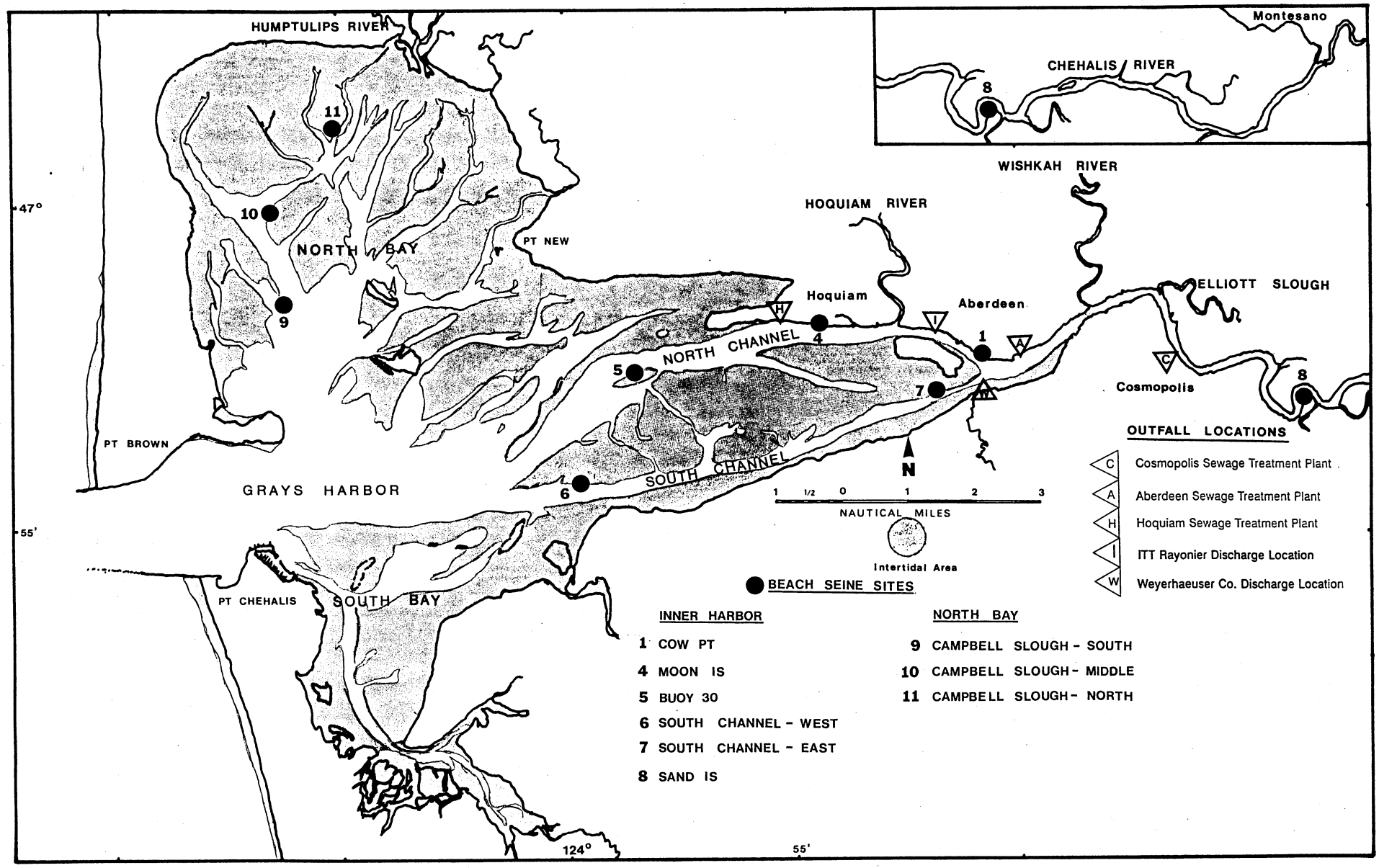


Fig. 3.2. Grays Harbor beach seining locations used in 1988. The inner harbor sites have numbers 1, 4, 5, 6, 7, and 8 while the North Bay locations are numbered 9-11.

the Campbell-Oyhut channels, three in the Humptulips River channel, one in Chenois Creek channel and another on Goose Island (Fig. 3.1). Because the highest catches of coho occurred in the Campbell Slough area, three of those sites (Fig. 3.2) were regularly sampled in 1988.

Results of the Beach Seining Surveys

Beach seining occurred from late April through May in both years. Altogether 228 hauls were made and 1,354 coho were captured. Approximately 4% of these fish (58/1,354) possessed CWTs.

Migration Patterns. The principal objective of the beach seining work conducted in 1987 was to determine the migration speed of coho as they moved through the inner harbor. It was hoped that temporal and spatial changes in Catch-Per-Unit-of-Effort (CPUE) might reflect how rapidly the fish moved through portions of the inner harbor. The CPUE values obtained are shown in Fig. 3.3. In this figure catches made at Cow Point and eastern Rennie Island have been combined and represent the Cow Point area.

CPUE of coho was lowest at all sites on the first sampling date and then increased substantially seven days later. Catches peaked in mid May and remained high over the next several weeks; after which CPUE values declined until they reached a value of less than five coho/haul by the last sampling date. Considerable variation occurred, although the highest catches were generally obtained in the Cow Point area and lowest at the western end of the South Channel.

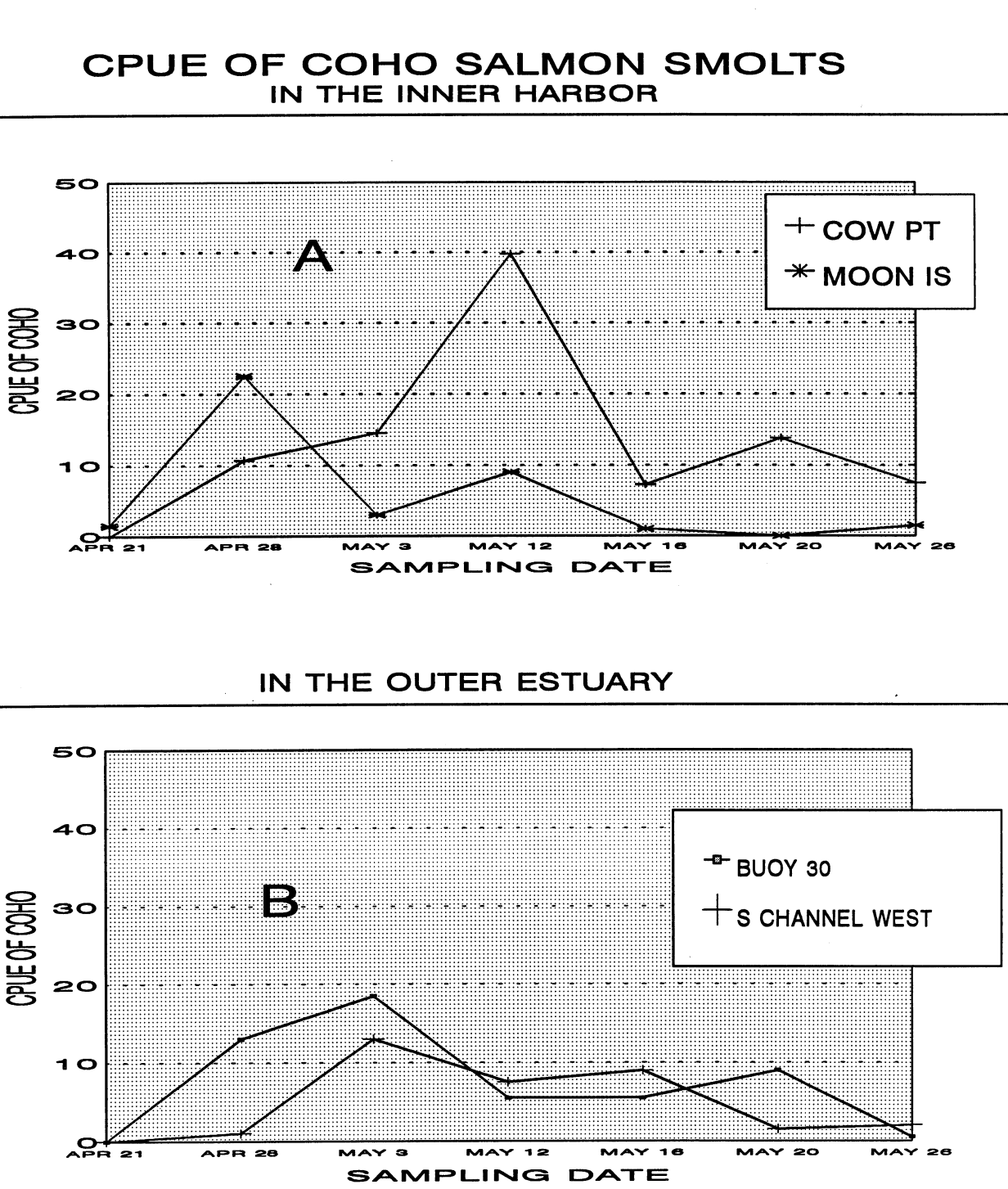


Fig.3.3. Temporal changes in the CPUE of coho salmon smolts in the Chehalis River estuary, 1987. The upper graph (A) shows catches at Cow Point and Moon Island while the lower graph (B) compares the outermost sites.

No consistent pattern of change occurred in CPUE values and thus it was not possible to estimate how rapidly fish were moving through the inner harbor. We feel that migration speed varies among coho because of body size, smolt development and environmental conditions; such variation would clearly make it difficult to detect pulses of fish moving through the estuary. These results made it plain that an understanding of migration speed or residency in the inner harbor could only be ascertained by using uniquely tagged or marked fish.

Some information, however, was obtained on how long it took coho to migrate through North Bay. On 5/9/87, ≈220,000 coho smolts were released from the Humptulips Hatchery and about seven percent of these fish possessed CWTs. Despite sampling in the estuary ten days later, just five coho were captured in ten seine hauls; only one was a tagged fish from the hatchery. Nineteen days after the release, eleven seine hauls were made and ten additional coho were captured. None of these fish were tagged. Since CPUEs were small, and only one tagged fish from the Humptulips Hatchery was caught, its likely that the fish cleared North Bay within several days after being released.

Data collected on coho possessing CWTs were also used to see if these fish moved extensively throughout Grays Harbor before commencing on their ocean migrations. In 1987, 35 coho or approximately 5% of all fish captured had CWTs. An additional 23 coho with tags were recovered in 1988. Of the tagged fish recovered in 1987, only two were captured outside of the estuary

they would have initially encountered while migrating seaward. One was a Humptulips fish captured in the Cow Point area while the other was an upper Chehalis River fish recovered in outer North Bay. Similar results were obtained in 1988, although in this year no Humptulips fish were caught in the inner harbor but three were obtained at the western mouth of South Channel. These data indicate that once an individual enters an estuarine area it tends to remain there until it migrates into the open sea.

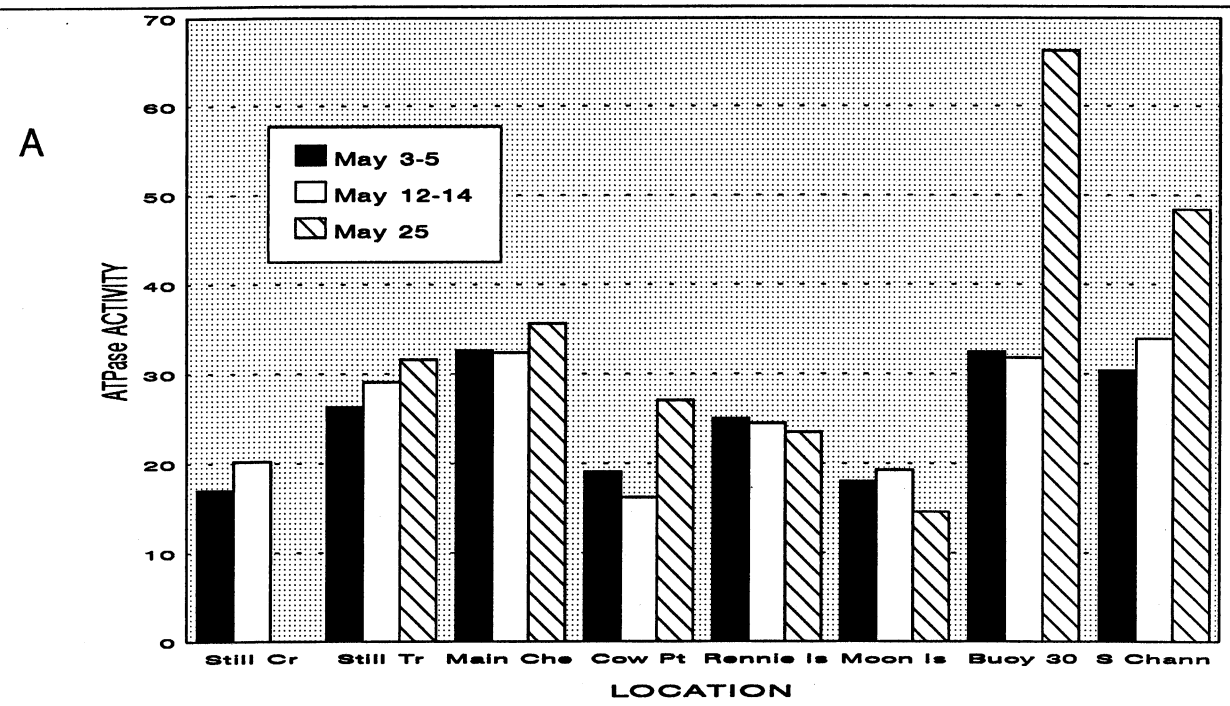
This important finding is probably directly linked with how coho complete the final steps of seawater adaptation. The estuarine areas they first encounter obviously contain waters with varying salinities. While in this environment, fish are free to move within the salinity gradient until they become fully adjusted to seawater. Once this has occurred, forays into neighboring estuaries are probably avoided because the fish would have to readjust themselves to brackish waters. Regardless of what induces Grays Harbor coho to predominately utilize estuarine areas adjacent to their natal streams, it causes fish from the Chehalis and Humptulips drainages to experience unique estuarine conditions.

ATPase Values of Beach Seined Fish. Two physiological evaluations, gill ATPase activity and immune response, were used to compare the smolt status and stress levels of fish beach seined from the inner harbor and North Bay. As mentioned elsewhere (Part II, Hypothesis 1) the level of gill ATPase activity gives a general indication of a fish's ability to adapt to seawater. ATPase levels in coho collected in Grays Harbor were expected to be similar, or

higher than those of migrant smolts since exposure to seawater normally elevates levels of this enzyme.

In 1987, ATPase values were procured from fish captured from five of the six sites sampled (too few fish were caught at the sixth site to be included). Values were consistent from one date to another, but there were clear differences among sites. For instance, fish at Buoy 30 and the western end of South Channel had ATPase values > 30 on all dates. Yet, fish sampled at Cow Point had values < 20 on two out of three dates while those sampled at Moon Island had values < 20 on all three dates. Fish sampled at Rennie Island had values between 20 and 25. Since coho sampled from the scoop trap (main Chehalis River site on Fig. 3.4) had values > 30 on these dates, the low ATPase values collected on fish obtained at the three innermost harbor sites were unexpected. In comparison, ATPase values of fish obtained in late May at three sites in North Bay approached 50, a level more characteristic of fish adapting to seawater (Fig. 3.4).

These results prompted a more intensive sampling effort in 1988. Like 1987, fish in the inner harbor had generally lower ATPase values than those secured at the western edge of the inner harbor (Fig. 3.5). For example, coho at Cow Point and Moon Island had values that ranged from 15 to 20, however, on 5/15/88 fish captured at Cow Point had values of around 10. Unlike 1987, the ATPase values of fish captured in North Bay were quite variable. They were commonly <15 to 30 at the North- and Mid-Campbell sites and 20 to 25 at Pile 7. Values significantly lower than these were



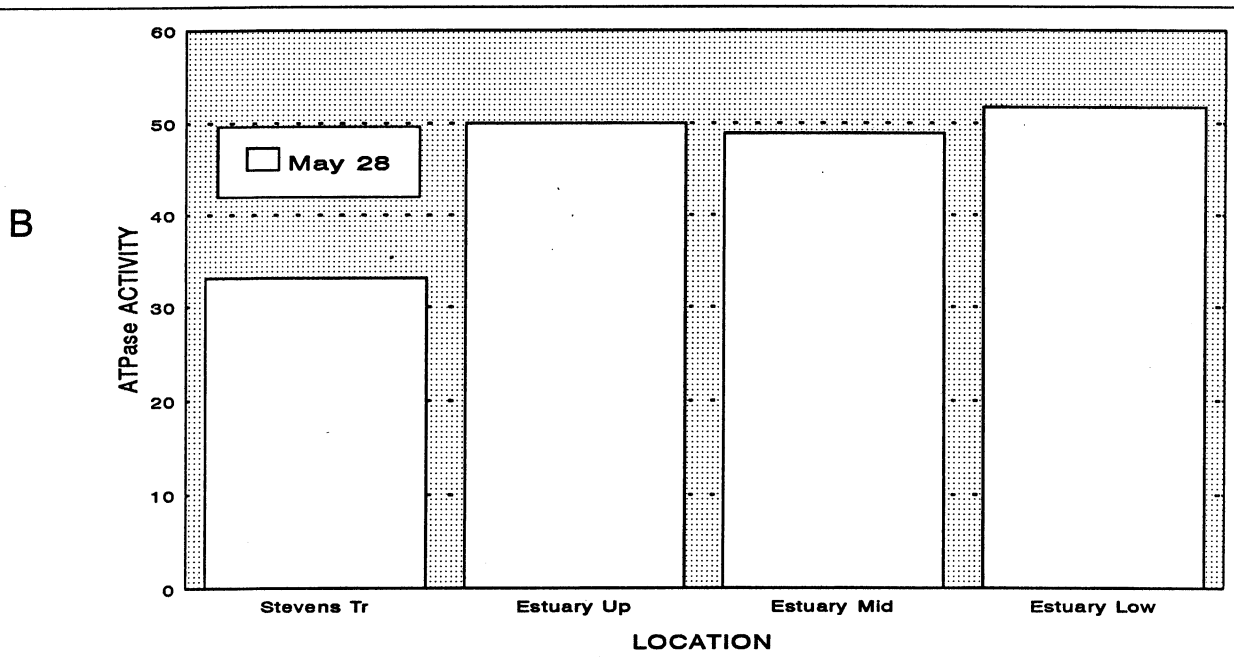


Fig. 3.4 A comparison of ATPase activities from coho salmon captured in 1987 at freshwater locations in the Chehalis River watershed and in the inner harbor (A). The bottom figure (B) shows ATPase activities from coho captured in the Humptulips drainage and North Bay.

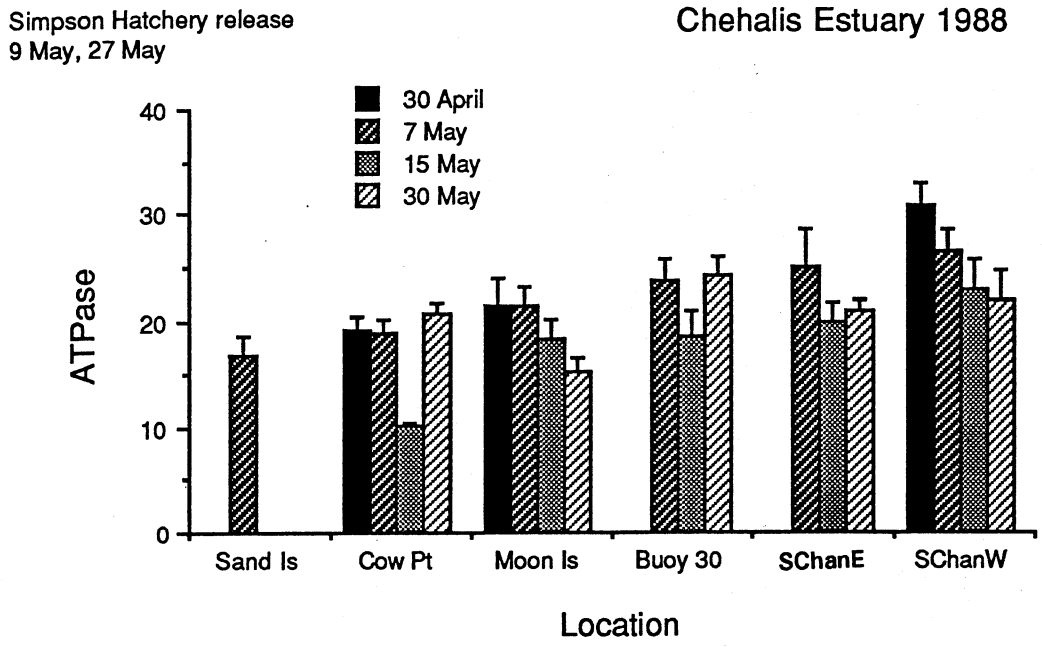


Fig. 3.5. ATPase values obtained from beach seined coho salmon captured in the Chehalis estuary in 1988. Coho were liberated from the Simpson Hatchery on 5/9-16/88 and 5/17-27/88.

found on 4/30/88 at Upper-Campbell and on 5/30/88 at Upper- and Mid-Campbell (Fig. 3.6). These low values were apparently caused by a large influx of fish from Humptulips Hatchery.

Other than the above two dates, the gill ATPase values obtained on coho sampled in North Bay and the western edge of the inner harbor were similar or higher than those found in wild migrant fish captured in the Humptulips and Chehalis Basins. Thus, their ATPase levels were elevated enough to allow them to easily adjust to seawater. The ATPase values obtained from fish collected in the inner harbor were more complex. For example, Fig. 3.5 shows a progressive east-to-west increase in ATPase from coho collected on 4/30/88 and 5/7/88. This pattern appears to reflect a natural response to more saline waters. This trend, however, was not repeated on the last two sampling dates in 1988. For a variety of reasons, it is difficult to establish a causal link between the ATPase data collected in the inner harbor and conditions the fish encountered there.

First, the origin and residency of beach seined individuals can be quite variable. In particular, both watersheds possess hatchery and wild populations of coho, and as the freshwater ATPase evaluations showed, hatchery fish consistently had lower levels of this enzyme than wild fish (see Part II, Hypothesis One). Hatchery fish, along with any wild fish with low ATPase values, may preferentially reside in areas with low salinities until they become more fully adapted to seawater. This behavior pattern could cause a disproportionate number of fish with low ATPase values to

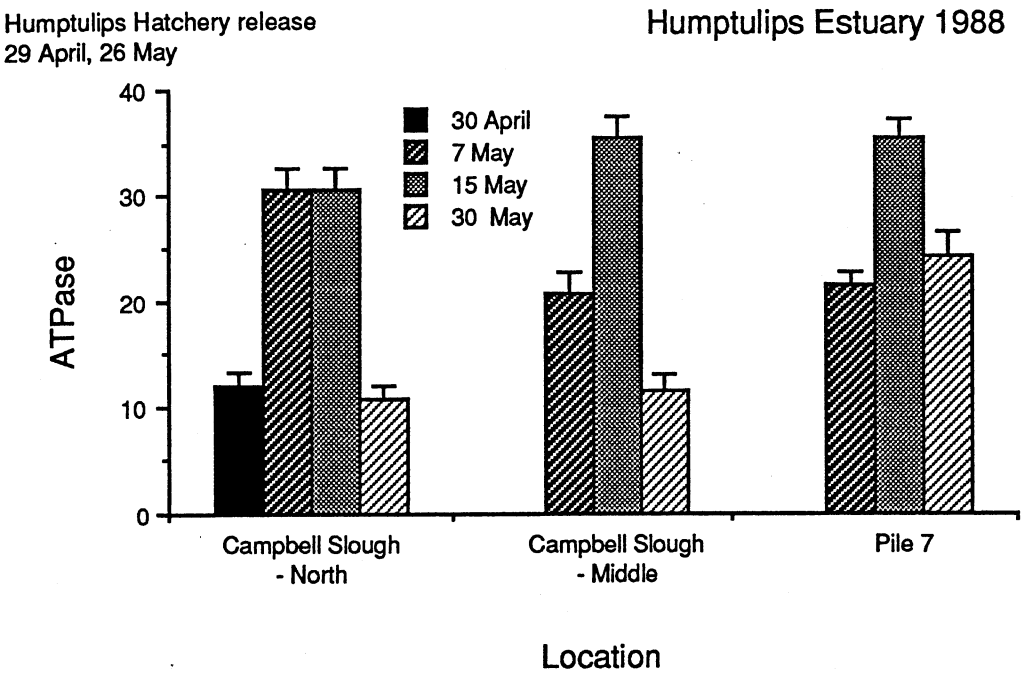


Fig. 3.6. ATPase values obtained from beach seined coho salmon captured in North Bay in 1988. Coho were released from the Humptulips Hatchery on 4/30/88 and 5/26/88.

accumulate in the inner harbor. Conversely, it is possible that environmental conditions within the harbor are deleterious enough to destablize smoltification and thereby reduce ATPase levels. For instance, it has been shown that certain heavy metals and pesticides can inhibit ATPase activity fairly quickly (Davis and Wedemeyer 1971; Leadem et al. 1974; Lorz and McPherson 1976; Beckman and Zaugg 1988). If contaminants in the inner harbor impair ATPase production then a preponderance of fish with low values of this enzyme would be expected.

It is not possible to simply use data gathered on beach seined fish to evaluate these or other likely hypotheses. This was recognized in 1987, and thus part of the 1988 field work was directed toward discovering why fish with low ATPase values occurred in the inner harbor. Three further evaluations were performed. Two of these were part of the *in situ* bioassays that were conducted in 1988 and 1989. The results of these tests will be discussed in subsequent subsections. The third element consisted of evaluating the immunocompetence of beach seined fish.

Immune Response Values of Beach Seined Coho. As indicated earlier, the immunocompetence of smolting salmonids is lower than at any other time during their juvenile life. Maule (pers. comm.) recently speculated that the hormonal/physiological events that accompany smoltification change the animal so dramatically that the immune system may fail to recognize host tissues. Consequently, to avoid attacking itself, the immunocompetence of a smolt is reduced, until the physiological changes the fish is experiencing have

stabilized. Plainly, further reductions in immunocompetence will increase the risk of death via disease or parasites. Such reductions are possible if the fish encounter stressful environmental conditions.

Briefly, salmonids respond to stress by activating the hypothalamic-pituitary-interrenal axis which drives a number of immediate neuroendocrine events (see Part II, Hypothesis 2; Donaldson 1981). Among these, is the release of cortisol which is used by the fish to metabolize energy reserves and thus overcome the short-term consequences of a stressful event. The physiological cost of using cortisol in this manner is that it reduces the immunocompetence of a fish.

The linkage between stress, cortisol secretion, and reduced immunocompetence is one that can be used to determine whether fish have experienced poor environmental conditions. Recall that cortisol titers, secondary stress responses, and immunocompetence were all evaluated on fish collected in hatcheries and various freshwater habitats. Similarly, in 1988, coho emigrating through the inner harbor and North Bay were collected by beach seine on four separate occasions. These fish were designated as belonging to either the Humptulips or Chehalis watersheds and were compared to fish obtained from the Simpson Hatchery which were known to be infected with a pathogen.

As before, the relative health, or competence of the immune system of each fish was assessed by using the passive hemolytic plaque assay (Tripp et al. 1987). The results of such tests are

measured by the number of plaque-forming cells (PFC) produced; healthy immune systems yield higher PFC responses than depressed ones.

The PFC responses observed in juvenile coho collected by beach seine from Grays Harbor in 1988 are presented in Fig. 3.7. Considerable variability in the PFC response was observed at most locations sampled more than once. In general, fish collected throughout the Humptulips system exhibited a relatively high PFC response (mean PFC's/culture >2000) regardless of location. In contrast, fish sampled in the Chehalis system exhibited a marked decrease in the PFC response as they moved from the upper reaches of freshwater into the estuary. For example, fish sampled at the Bingham Creek trap and the scoop trap exhibited mean PFC responses of 4088 PFC's/culture and 3092 PFC's/culture, respectively. This response decreased to 1138 PFC's/culture near the eastern mouth of South Channel and 1233 PFC's/culture at Cow Point; and was approximately one-third of their up-river counterparts (Bingham and scoop traps). Fish sampled in the North Channel exhibited intermediate mean responses; 1718 PFC's/culture at Moon Island and 2421 PFC's/culture at Buoy 30. Pathogen-infected fish at Simpson Hatchery exhibited a mean response of 808 PFC's/culture.

These results suggest juvenile coho salmon moving through the inner harbor are less healthy when compared with either their own up-river counterparts or fish sampled from any of the sites in the Humptulips system. It would appear then, that coho emigrating from the Chehalis basin experience an immunosuppression upon entering

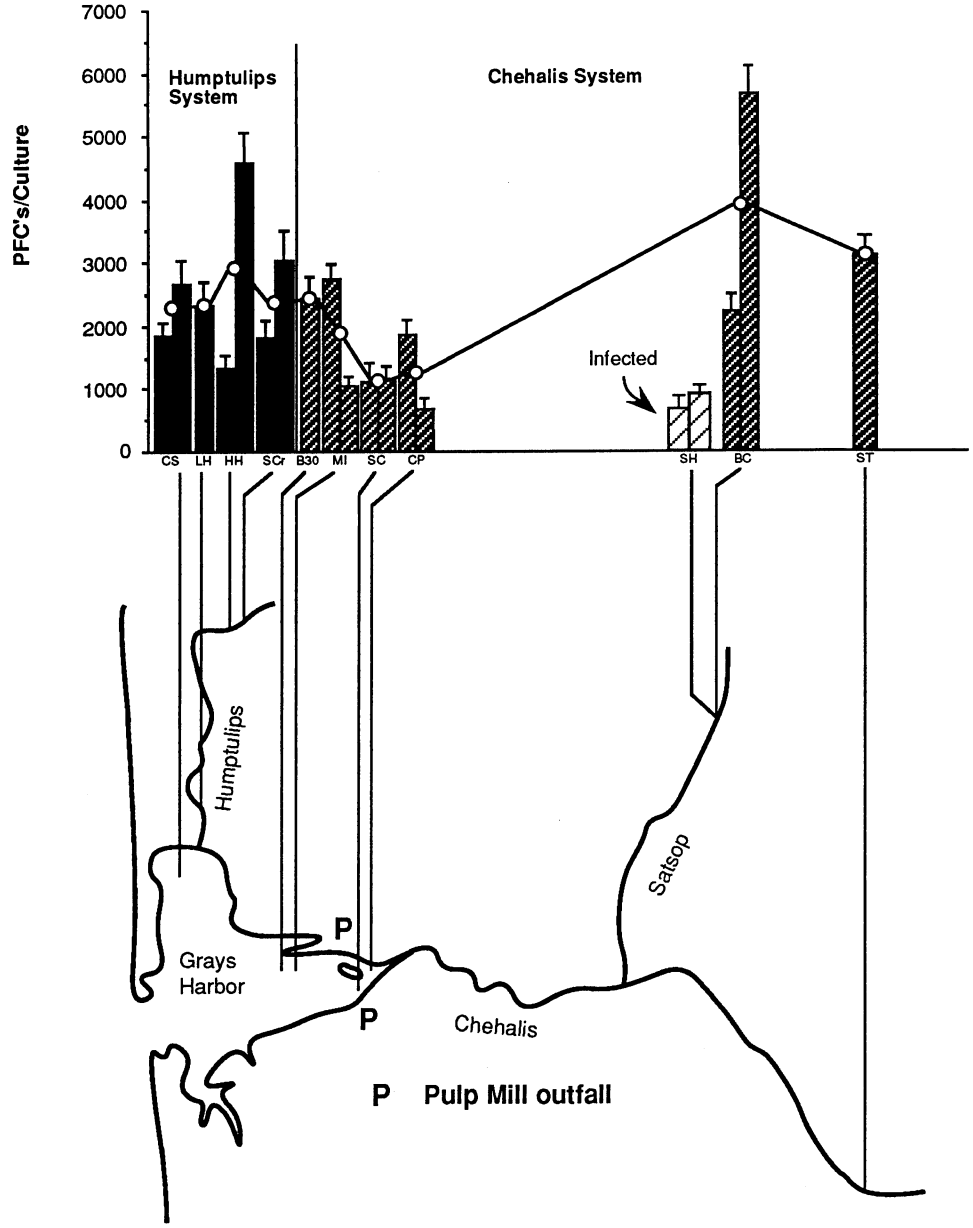


Fig. 3.7 Plaque-forming cell (PFC) response (mean + S.E.) in juvenile coho salmon collected at various locations and times, 1988. CS = Mid-Campbell slough, May 8 (n = 16) and May 16 (n = 19); LH = Lower Humptulips, May 17 (n = 19); HH = Humptulips Hatchery, April 18 and 29 (n = 20); SCr = Stevens Creek Trap, April 18 and May 5 (n = 14); B30 = Buoy 30, May 7 (n = 19); MI = Moon Island, May 7 (n = 13) and May 15 (n = 20); SC = South Channel/Weyerhaeuser Outfall, May 7 (n = 3) and May 15 (n = 19); CP = Cow Point, May 7 (n = 18) and May 15 (n = 20); SH = Simpson Hatchery, April 18 (n = 19) and April 29 (n = 20); BC = Bingham Creek Trap, April 19 (n = 17) and May 6 (n = 20); ST = Scoop Trap, May 5 (n = 20). The line with open circles connects mean values for samples collected on different dates at the same site.

the inner harbor.

It is tempting to treat these data like the ATPase values obtained on fish collected in the inner harbor, but two factors make this approach invalid. First, coho collected in the inner harbor before fish were liberated from the Simpson Hatchery (5/9-16/88) clearly showed a depression in their immune competence. Second, the 332,000 fish liberated from the hatchery in mid May were not production fish but were late-run coho. Anecdotal evidence from the hatchery staff and WDF pathologist reports indicate that these fish were not diseased or stressed when released (Tami Black, WDF, pers. comm.). Additionally, none of the production fish, which had impaired immune systems, were released until the immunocompetence evaluations in the inner harbor had been completed. Thus, even though it is possible that late-run coho from the Simpson Hatchery were beach seined in the inner harbor, it is unlikely that they affected the results of the immune response assays.

In 1988 and 1989 two *in situ* bioassays were performed in the inner harbor to avoid the confounding effects of hatchery releases and the unknown history of beach seined fish. One objective of these assays was to determine how residence in various portions of the inner harbor affected smolting coho. Before these tests could be performed, it was necessary to ascertain how long the bioassays should run. Ideally, the duration of such tests should approximate the time fish typically spend in the inner harbor. In 1988 and 1989 smolt tracking studies were performed to gather the

information used to set the exposure times for all bioassays conducted during this study.

Fish Tracking Studies

The estuarine migration behavior of Pacific salmon has been mainly determined by tag recapture data and hence is poorly understood. However, with the development of miniaturized radio and ultrasonic transmitters, it is now possible to track movements of individual fish in both freshwater and estuarine environments. To estimate how long smolting coho reside in the inner harbor, fish were tagged with ultrasonic transmitters and tracked while they emigrated from Cosmopolis to Moon Island or down a comparable length of the South Channel. Migration parameters collected on each fish were used to create residency estimates.

Tag Assessments

Before performing field tagging and tracking work, miniature transmitters were evaluated in the lower Chehalis River and inner harbor. Radio tags could not be used in this area because their signals were attenuated when salinities exceeded 1 ‰ (WDF/NMFS 1988). Consequently, all fish tracked in the inner harbor were tagged with ultrasonic transmitters. Two such tags were tested. The Vemco V2B-1L was found to have a range of approximately 500 m in Grays Harbor. The other tag, a prototype developed by the Ministry of Agriculture, Fisheries, and Food, Lowestoft, England, had a range < 50 m.

Laboratory tests were conducted to assess the effects of these tags on fish buoyancy, behavior, and swimming performance (Moser et

al. 1990) Yearling coho salmon from 150-221 mm were tagged with dummy transmitters which corresponded to the dimensions of the Vemco (33 x 9 mm) and English prototype (17 x 7 mm) transmitters. Observations on time to recover buoyancy, feeding and aggressive behavior were made on tagged and untagged fish. Neither tag significantly altered fish behavior, although coho with mock Vemco tags took longer to recover their buoyancy (Moser et al. 1990). Swimming performance, tested by using modified Blazka respirometer-stamina chambers (Smith and Newcomb 1970), was also unaffected by dummy tags of either size (Moser et al. 1990).

The results of these assessments indicated that: 1) Vemco tags were the best transmitters to use in the inner harbor, 2) smolts $\geq$ 160 mm should be used to minimize impacts of the tags on behavior, and 3) tagged fish should be allowed to recover for at least 24 hrs before being released. These protocols were followed throughout the duration of the estuarine tracking studies.

In both 1988 and 1989 coho smolts were tracked in the lower Chehalis River and inner harbor from April to June, a time that coincides with the normal out migration period of these fish in Grays Harbor. As previously indicated, the principal objective of the coho tracking studies was to determine the residency of migrating smolts in the inner harbor. This information was gathered to establish how long fish should be exposed to inner harbor waters or effluents in our bioassays. Tracking data collected in 1989 were not used to determine exposure periods because these observations were performed after, or simultaneously

with the bioassays. Instead, the 1989 study was conducted to see if yearly variation occurred and to ascertain how various environmental factors might influence migration patterns. A comprehensive account of the estuarine tracking investigations can be found in Moser et al. (1991). What follows here is a brief description of the methods, results, conclusions, and caveats associated with the tracking work.

Study Location and Fish Handling

All tagged fish were wild coho migrants obtained from a weir located on Scatter Creek, a tributary of the Chehalis River. The fish were held in live boxes located in the inner harbor for no more than six days (Fig. 3.8). The 1.5 m^3, 4 mm mesh pens were supplied with wooden baffles to protect the smolts from strong currents.

Prior to tagging, smolts were lightly anaesthetized with MS-222 (50 mg/*L*, 2-4 min). Transmitters were then gently pushed down the esophagus until they were no longer visible. The fish were allowed to recover for at least 24 hrs before being released.

Types of Tracking Data Obtained

In 1988, two mid-channel release sites were used, one was located just downstream of the Chehalis River bridge (lower) while the other was situated about three km upstream (upper)(Fig. 3.8). The first five fish were released at the upper site and the remaining ten at the lower site. In 1989, all eight fish were released at the lower site. Transmitter signals were detected with a directional hydrophone and tunable receiver (Vemco Ltd) from a

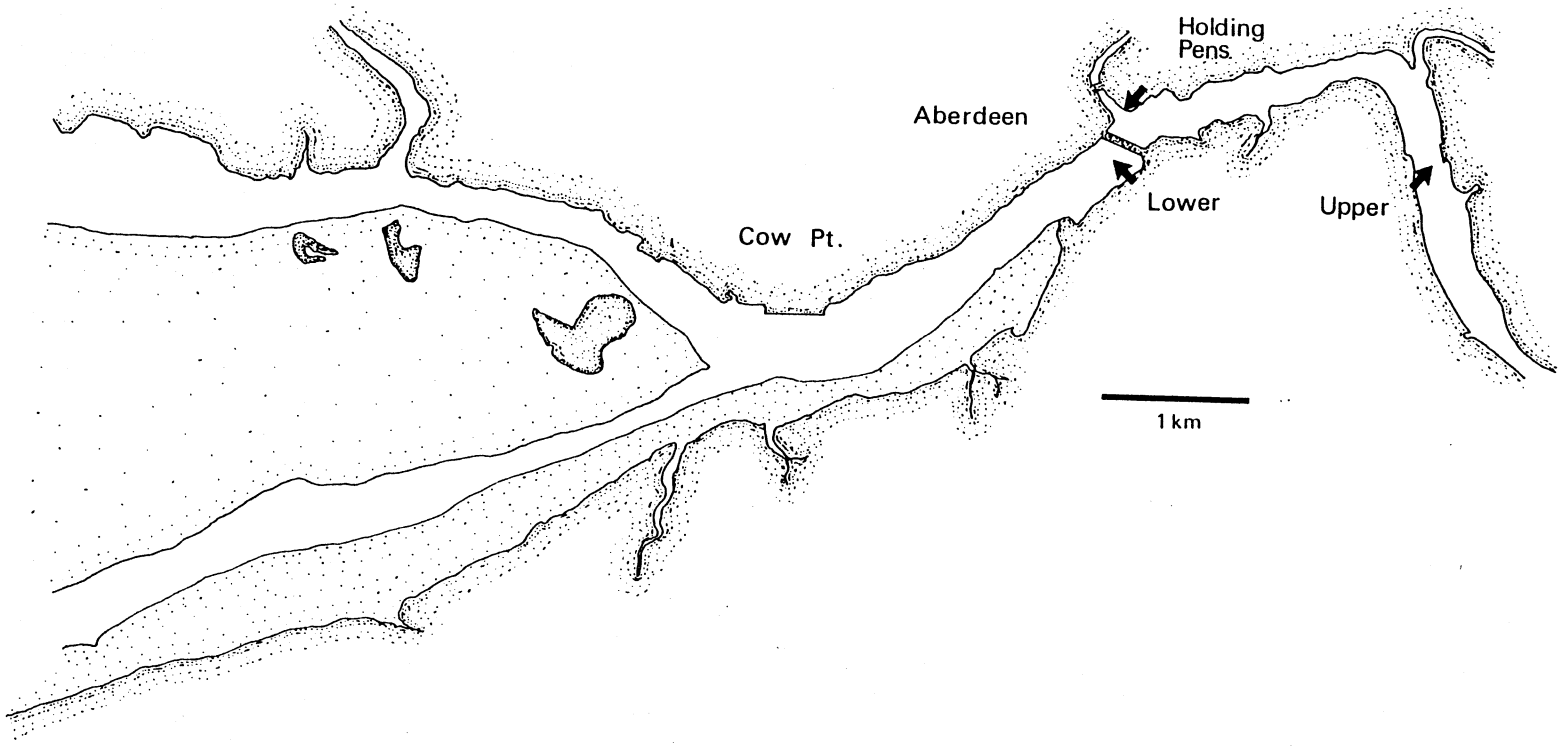


Fig. 3.8. Locations of holding pens, upper and lower release sites and pulp mill discharge points in the inner harbor, Chehalis River estuary.

small boat, and smolt locations were determined (± 50 m) using a combination of triangulation and signal strength.

Once a fish was released it was continuously tracked for up to 16 hrs. During continuous tracking the position of each fish was plotted on a map at ten minute intervals. These data were used to calculate the average ground speed (distance traveled/by time observed) of each tagged fish. Current velocity, direction (AquaMeter No. 660), salinity and temperature measurements (Hydrolab Scout) were made every 30 minutes to ascertain how the fish responded to varying environmental conditions in the inner harbor. For instance, the percentage of time the fish moved in the same direction as surface currents was routinely noted. This parameter did not measure how long a fish was observed swimming, but indicated whether it traveled in the direction of prevailing currents when it did move. Gross distance and net distance traveled were also calculated. Gross distance equaled the total distance a fish traveled, both up and downstream, while net distance equaled the distance a fish traveled downstream from its release point.

After a continuous tracking episode was completed, additional fish locations were determined by routinely sampling the inner harbor with the hydrophone. Because tag signals were transmitted on four different frequencies it was possible to identify each relocated fish. In many instances, continuously tracked and relocated fish remained relatively stationary for periods of time. The percentage of time this occurred was calculated by using the

following formula: %H =(100)(t_h)/t_t, where %H = percent holding time, t_h = the amount of time a smolt spent in one location for at least 1 h and t_t = the total time the smolt was tracked (Moser et al. 1991). One other migration parameter, total time observed was also calculated. This value equaled continuous tracking time plus the hrs accrued up to the last time a fish was observed. For instance, suppose a fish was continuously tracked for 10 hrs and then observed one last time 24 hrs after its continuous tracking episode had been completed, its total observation time would then equal 10 + 24 or 34 hrs. All of the migration parameters were only calculated on fish that had been tracked for at least one hr.

Between year differences in these parameters were assessed by using the Mann-Whitney U test (Zar 1974). Additionally, the effects of fish size and release date on gross and net distance traveled were examined by using Kendall correlation tests (Zar 1974). Mann Whitney U tests were also used to compare yearly differences in current velocity and direction, salinity, and water temperature. Additionally, Kendall tests were used to check for correlations between ground speed and current velocities.

Results of Tracking Studies

Fifteen smolts were tracked in 1988 and eight smolts in 1989; however, two fish in 1989 were lost after less than one hr (Table 3.1). Ten fish in 1988 and three fish in 1989 were tracked beyond the divergence of North and South channels in Grays Harbor. Six fish in 1988 migrated seaward via the North Channel and four used South Channel. In 1989, two fish moved between both channels and

Table 3.1 Information obtained in 1988 and 1989 from acoustically tagged coho salmon juveniles which were emigrating through the Chehalis River estuary.

Year	Fish #	Fish Size (mm FL)	Time (hr) Tracked	% Time Moving w/Current	Gross: Net Distance (km)	% Time Holding
1988	2	193	15.3	100	10.6	20.3
	3	173	127.0	100	3.1	62.5
	4	169	32.5	100	9.8	71.7
	5	179	11.0	100	1.8	72.7
	6	163	31.5	45	1.7	75.2
	7	198	183.3	78	1.3	96.6
	8	192	134.0	100	3.4	94.2
	9	170	57.8	100	3.5	90.8
	10	173	10.0	100	1.4	15.0
	11	171	23.8	100	2.1	58.0
	12	183	285.3	100	1.2	99.5
	13	215	17.3	100	2.3	85.6
	14	210	31.5	100	1.3	89.8
	15	209	68.3	86	2.2	95.9
	16	212	3.3	100	1.0	90.9
	$\bar{x}$	187	68.7	94	3.3	74.6
1989	1	160	1.0	100	1.0	-
	2	183	12.2	62	3.2	27.0
	3	182	4.0	100	1.0	62.5
	4	160	7.0	100	4.9	31.4
	5	172	8.5	100	1.2	52.9
	6	159	1.0	100	1.0	-
	7	160	8.5	100	3.3	78.8
	8	166	9.7	92	3.5	27.8
	$\bar{x}$	168	6.5	94	3.2	46.7

one fish exited through South Channel. In both years several smolts passed through pulp mill effluent fronts.

In both 1988 and 1989 water temperature did not vary more than 1° C during periods of continuous tracking; however, the tidal cycle caused both longitudinal and vertical changes in salinities. For example, at high slack tide, surface salinities at Cow Point ranged from 10 to 22 ‰ yet at a sampling site only five km to the east, they ranged from 5 to 8 ‰. Similar changes in vertical salinities also occurred, for instance, at high tide there was a 21 ‰ difference from top to bottom at Cow Point (Fig. 3.9). Thus, coho emigrating through the inner harbor have a wide range of salinity options. Fish could experience highly variable salinities by remaining at one depth and position throughout a tidal cycle. Or, they could remain in a fairly uniform salinity by either moving back and forth with tidal currents or by moving up and down while generally remaining in the same location. How coho may have responded to shifts in salinity was not discerned during this study because the transmitters used to track the fish did not provide vertical distribution data.

The tracking data indicated that tagged coho were positively rheotactic (i.e. they oriented themselves into currents) but that their movements generally followed the direction of tidal currents (Figs. 3.10 and 3.11). Because of this propensity to move back and forth with tidal currents, the gross distance traveled by continuously tracked fish over a tidal cycle was greater than net downstream distance. Ratios of gross to net downstream distances

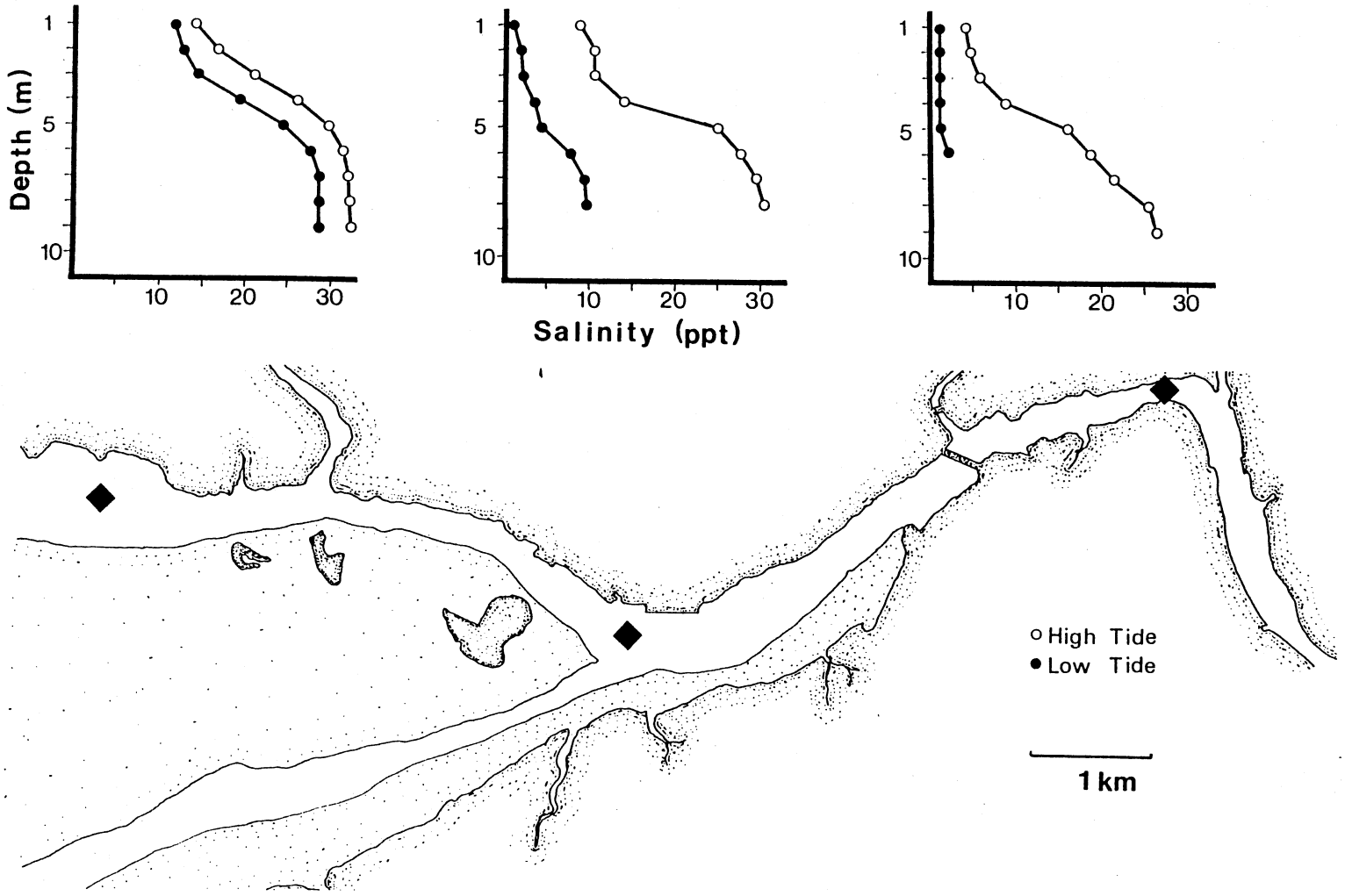


Fig. 3.9. Salinity readings at different tidal stages and depths in three regions of the Chehalis River estuary. White circles represent salinities at high slack water and black circles represent salinities at low slack water.

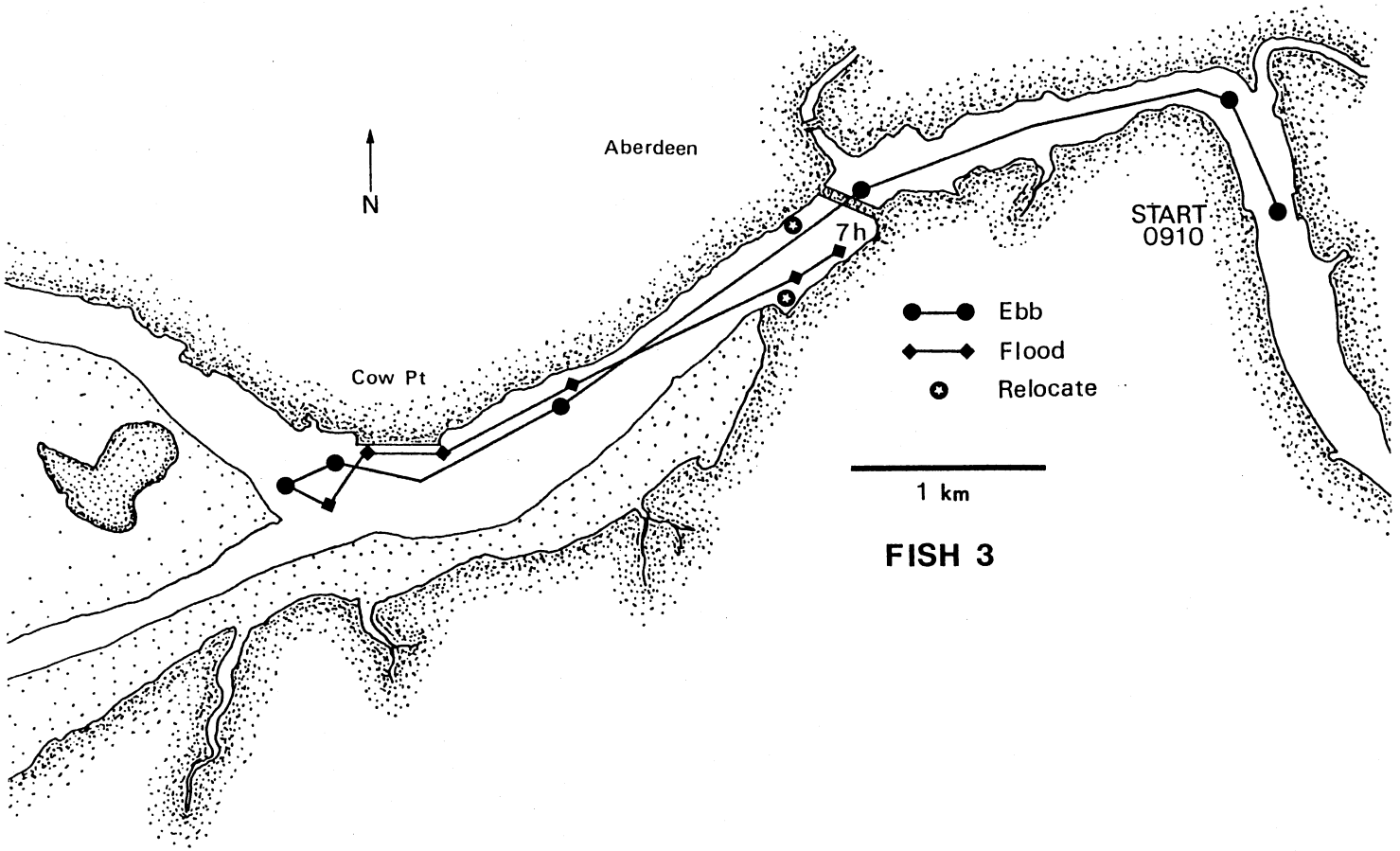


Fig. 3.10. Location of fish # 3 (1988) at 30 min intervals during ebbing (circles) and flooding tides (squares). The length of time spent (h) at a site is also shown along with where the fish was relocated (starred circle).

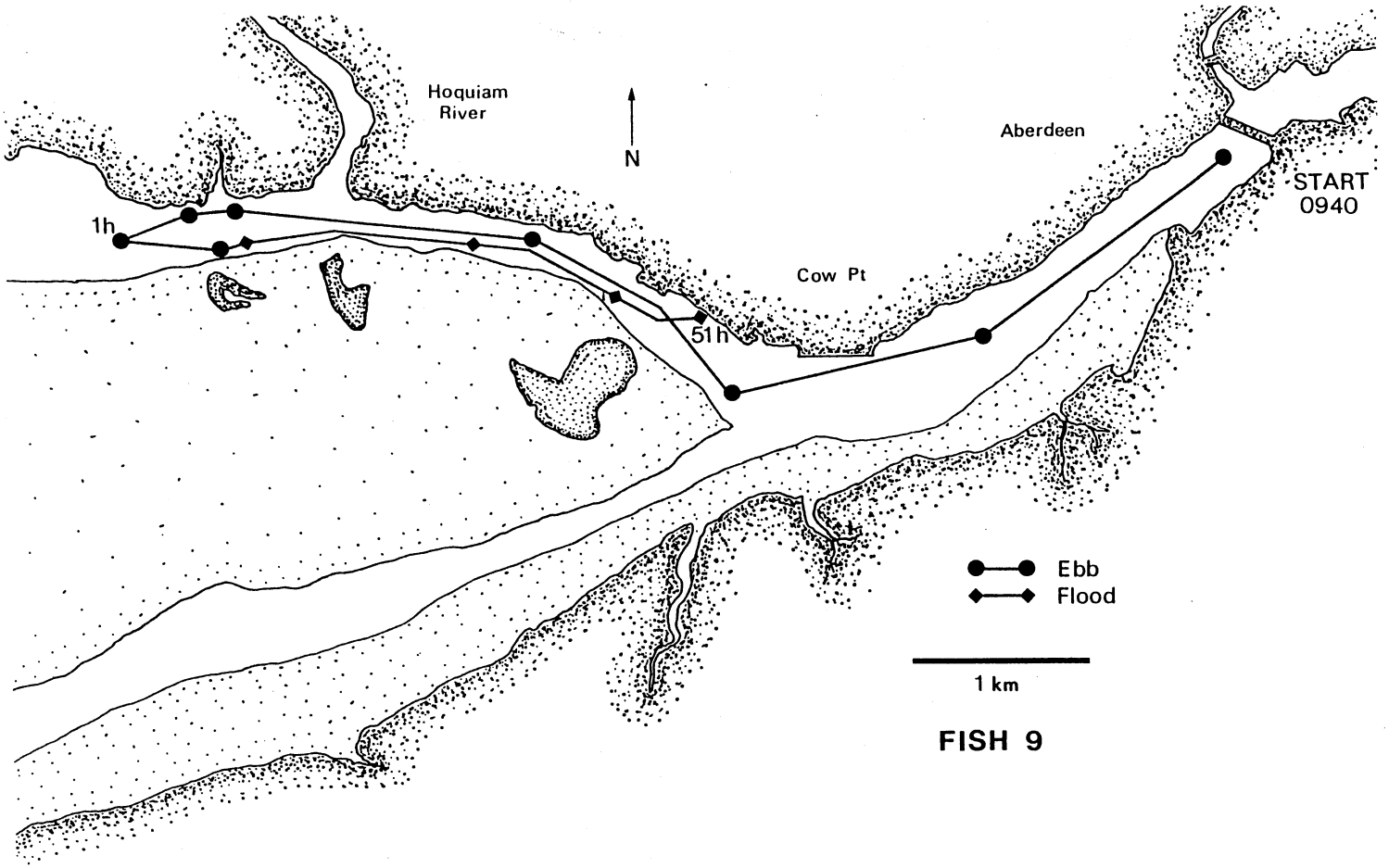


Fig. 3.11. Location of fish # 9 (1988) at 30 min intervals during ebbing (circles) and flooding tides (squares). Time (h) spent holding at a site is shown the map.

averaged 3.3 and 3.2 in 1988 and 1989. These ratio values, which were not statistically different from one another, reflected that fish often oscillated over the same areas of the inner harbor. Moreover, many fish commonly remained in the same location for prolonged periods of time (Table 3.1).

Mean ground speed was positively correlated with current speed in both years and mean current speed during the smolt migration in 1988 (45.7 cm/s) was greater than mean current speed in 1989 (24.1 cm/s). Hence, mean ground speed was higher in 1988 (42.9 cm/s) than in 1989 (29.9 cm/s). In both years fish spent a great amount of time holding in areas with low current velocity, particularly in the area around Cow Point. In fact, of the fish tracked to the confluence of North and South Channels, 70% in 1988 and 100% in 1989 held for extended periods at Cow Point.

Mean ground speed, % holding, and the gross/net distance ratio were used to estimate an average residency time in the inner harbor for each year. In 1988, average ground speed equaled 1.5 km/hr {[(.4 m/s)(60 s)](60 minutes) $\simeq$ 1.5 km/hr} and each fish swam for about 6 h/day [(1.0 - % holding time or .75)(24 h) = 6} therefore total gross distance traveled equaled 9 km [(1.5)(6) = 9]. The gross/net distance ratio for this year was 3.3 so total net downstream distance traveled averaged 2.7 km. The distance from Cosmopolis to Moon Island is approximately 16 km, hence, on average it took approximately 6 days for a smolt to travel through the inner harbor (16 km/2.7 = 5.9). In 1989, the mean ground speed was lower (1 km/hr), % holding time was less (47%) and the gross/net

distance ratio equaled (3.2) so in this year estimated residency averaged about 4 days. Another possible way to assess residency in the inner harbor is to examine the total hours fish were observed in the inner harbor (see Table 3.1). In 1988 the average fish was observed for about 69 hours while in 1989 this value equaled 6.5 hours. However, these values did not provide reliable residency esitmates because battery life on the tags was rated for 6 days, signals became attenuated when the fish located themselves behind obstructions or in shallow mud flat areas, and in many instances weather or other factors prevented a complete search of the inner harbor (Moser pers. comm.) Consequently, we assumed that smolting coho generally resided in the inner harbor for about 5 days.

Clearly some individuals may exit more rapidly than this but others probably remain for several weeks. For example, one fish remained for at least twelve days and another resided near Cow Point for over a week in 1988 (Table 3.1). Because of the proclivity of some fish to hold for prolonged periods we extended the length of several of our bioassay treatments to 2 wks to account for this behavior.

Conclusions from the Coho Tracking Studies

In general, it took about 5 days for smolting coho to emigrate through the inner harbor. Their migration routes and rates of travel did not differ significantly during the two study years. Fish entered both North and South channels as they moved seaward. Migration behavior was characterized by fish moving back and forth with tidal currents even though they exhibited positive rheotaxis

and, in 1988, by extensive periods of holding in areas of low current velocity. Ultrasonic tracking of Atlantic salmon smolts (*Salmo salar*) also revealed that smolts moved in the direction of tidal currents (Fried et al. 1978; Tytler et al. 1978) and in some cases exhibited extended periods of holding (Tytler et al. 1978).

In both years, fish holding was most common around pilings and docks, particularly near Cow Point. This area is located at the confluence of the two major inner harbor channels. Because of the configuration of Cow Point and the numerous structures present at this location, current flow is complicated, with numerous back eddies and regions of slack water. Since coho smolts orient to current flow they may have accumulated at this site because of its hydrography. Similarly, prey organisms may concentrate in this location, which could result in fish feeding aggregations. Finally, water quality at Cow Point may be affected by nearby pulp mill and sewage discharge points, resulting in altered fish behavior.

These migration data were used to establish the duration of four bioassays, two of which were performed *in situ*. In the following three sections, background information about these assays and their results are presented.

In Situ Bioassay 1: Barging Coho Through the Inner Harbor and North Bay

Introduction and Background

Previous investigations that examined the effects of inner harbor water quality on salmonids used fixed live boxes and

compared mortality rates from one location to another (Deschamps and Phinney 1971; Jeane 1973; DOE 1975). These studies, however, lacked suitable controls (all the live boxes were situated in the inner harbor) and no attempts were made to evaluate the consequences of sublethal stressors. The barging study was designed to circumvent these problems. First, each barge was equipped with a specially designed box that protected the fish from excessive currents but still exposed them to surrounding waters. Second, to mimic the environmental conditions an emigrating fish would typically experience, the barges were moved back and forth with tidal currents and then moored for periods of time. Third, to serve as a source for comparisons, one barge was berthed and moved through the inner harbor while another was treated the same way in North Bay. To determine if fish barged in North Bay and the inner harbor were differentially affected, ATPase, cortisol, immunocompetence, and secondary stress response data were collected and compared. Additionally, some barged fish were reared in seawater net pens and exposed to a natural outbreak of *Vibrio* to appraise the long-term effects of being barged in different areas.

Descriptions of the Barges

A modified, standard-sized, floating incline trap (scoop trap) was used to move fish through the inner harbor (Seiler et al. 1981). This gear has two 10.9 m by 0.9 m by 0.9 m steel pontoons spaced 2.1 m apart with 1.8 m by 3.9 m steel decks fore and aft. The aft end of the scoop trap was enlarged to accommodate a pilot house; a motor mount and anchor boom were also installed. To test

the maneuverability of the barge, field trials in the inner harbor were conducted in the late summer of 1987. These trials showed that the barge could be successfully piloted and that water turbulence in the holding box was minimal when the barge was under way.

A smaller barge was used in North Bay. It was 2.7 m wide by 6 m long and also had two pontoons, which were approximately 0.5 m in diameter. Unlike the inner harbor barge, this vessel was not powered by its own outboard motor and therefore had to be towed throughout North Bay.

Each barge had a steel framed, plywood box that was suspended between the two pontoons. These boxes protected barged fish from excessive currents but still allowed a free exchange of water. The box placed in the inner harbor barge measured 3.6 m long by 1.8 meters wide by 2.4 m deep. Ten, 0.6 m by 1.2 m high openings were cut in the box, four on each side and one on each end. Stainless steel panels perforated by 3 mm holes (5.3 holes/cm^2--40% open area) were placed over each opening. Additionally, the outside edge of each opening was fitted with guides that allowed a sheet of plexiglas to be inserted over each hole if further baffling was required. The box was sealed with two coats of Fiberglas resin to prevent release of potentially toxic glue compounds. Two net pens (1.8 m X 1.8 X 1.8 m) constructed of 3 mm soft nylon seamless bobinet were hung inside the box to hold hatchery coho. A smaller, (0.6 m X 1.2 m X 1.2 m deep) wooden framed box surrounded by 6 mm vexar was inserted between the two net pens to hold wild coho.

A smaller plywood box was built for the barge used in North Bay. It was 3 m long by 1.2 m wide and 1.8 m deep and had four 0.6 m by 1.2 m holes cut into it, one on each side and one at each end. Like the box in the inner harbor barge, these openings were baffled with perforated stainless steel screening and plexiglas doors. Two 1.2 m by 1.2 m by 1.8 m deep net pens constructed of 3 mm mesh were hung in the box and a wooden framed, vexar covered box identical to the one used in the inner harbor barge was inserted between the two net pens to hold wild coho.

Methods Used to Conduct the Barging Bioassay

Coho from the Humptulips Hatchery were used to stock the net pens in both barges. Approximately 380 fish were placed in each pen located on the inner harbor barge. Fewer fish (170/pen) were used in the smaller North Bay barge to ensure that fish densities in each barge were comparable. However, the same number of wild coho (92) were placed in each barge since the containers used to hold these fish were identical. Wild fish caught at the scoop trap (mid-Chehalis River) were deposited in the inner harbor barge which was moored at the mouth of the Wishkah River. Migrating coho obtained from Stevens Creek (mid-Humptulips River) were utilized in the North Bay Barge which was berthed at the Ocean Shores Harbor Marina. Once placed in the barges, the fish were allowed to acclimate for seven days.

After the acclimation period, the barges were moved in their respective estuaries for an additional five days. Generally, the barges traveled in the same direction as tidal currents for about

eight to eleven hrs/day. In North Bay the barge was towed back and forth between Damon Point and Campbell Slough and then moored at the Ocean Shores Marina. In the inner harbor, barge movements were more complex. On days one and two, the barge was moved back and forth between Cosmopolis and Cow Point and then moored at the mouth of the Wishkah River. During days three and four it was moved back and forth in the North Channel between Cow Point and Moon Island and secured at the Army Corps of Engineers dock (≃3.8 km west of the Wishkah site). On the last day, the barge was piloted east of Cow Point and then moved west down the South channel for 2.6 km before being returned to the Wishkah docking site.

While the barges were being moved, a number of environmental parameters were collected. Ecology personnel, for example, regularly used a Hydrolab 8000 and a Beckman induction salinometer to measure D.O., pH, temperature and salinity. In addition, a continuous-flow centrifuge with XAD resin columns was used to concentrate suspended solids and hydrophilic compounds in the areas the barges passed through. This equipment was either stationed on the barges or in smaller vessels and was operated by EPA staff (see Part III for the results of these water quality assessments).

Physiological Assessments Made on the Barged Fish

ATPase, plasma cortisol, secondary stress and immunocompetence evaluations were made on the barged fish. In addition, hatchery fish from both barges were trucked to the NMFS Manchester Field Station and held in saltwater net pens to monitor long-term growth and survival. Fish were initially sampled approximately twelve hrs

before a barge was first moved; in Table 3.2 this sampling period is referred to as day zero. Additional fish were collected at the end of each day the barges were moved. The types of samples obtained depended on the barge the fish were in, and whether they were hatchery or wild coho (Table 3.2). No attempts, for example, were made to rear wild fish in saltwater net pens because they do not readily feed on commercially available diets.

Results of the Assays

ATPase Evaluations. Gill tissues were collected from barged fish to see if residence in different areas of Grays Harbor affected ATPase activity. No obvious trend in ATPase values occurred in the wild fish in either barge or in the hatchery fish located in the inner harbor barge (Fig.3.12). However, hatchery fish placed in the North Bay barge, increased their ATPase values over time. Furthermore, these values were consistently higher than those obtained from hatchery fish located in the inner harbor barge. These results may have occurred because of the greater salinities found in North Bay. In general, it appears that the areas the barged fish were moved through did not adversely affect their capacity to produce ATPase.

Cortisol, Secondary Stress, and Immunocompetence. Cortisol levels found in coho held in both barges are shown in Fig. 3.13. Plasma cortisol levels in all fish held in both barges increased from day zero to day five. However, both the magnitude of this increase, as well as the absolute levels of circulating cortisol, were approximately twice as great in fish sampled from the inner

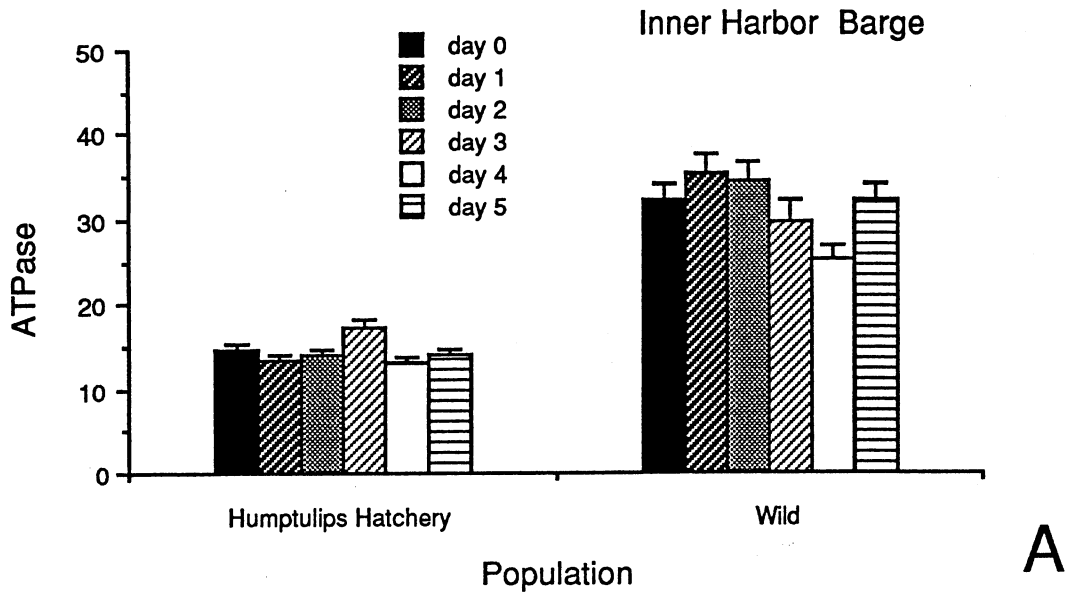
Table 3.2 Schedule followed for the collection of ATPase, plasma cortisol, secondary stress, immunocompetency and marine survival data collected on wild and hatchery coho salmon barged through the inner harbor (5/31-6/4) and North Bay (6/3-7) in 1988.

HATCHERY FISH

	Inner Harbor Day Collected						North Bay Day Collected					
ASSAY	0	1	2	3	4	5	0	1	2	3	4	5
ATPase	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Cortisol	Yes	-	-	Yes	-	Yes	Yes	-	-	-	-	Yes
Secondary Stress	Yes	-	-	-	-	Yes	Yes	-	-	-	-	Yes
Immunocompetence	Yes	-	-	Yes	-	Yes	Yes	-	-	-	-	Yes
Marine Survival (≃100 fish/sample)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	-	-	-	-	Yes

WILD FISH

	Inner Harbor Day Collected						North Bay Day Collected					
ASSAY	0	1	2	3	4	5	0	1	2	3	4	5
ATPase	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Cortisol	Yes	-	-	Yes	-	Yes	Yes	-	-	-	-	Yes
Secondary Stress	-	-	-	-	-	-	-	-	-	-	-	-
Immunocompetence	Yes	-	-	-	-	Yes	Yes	-	-	-	-	Yes
Marine Survival (≃100 fish/sample)	-	-	-	-	-	-	-	-	-	-	-	-



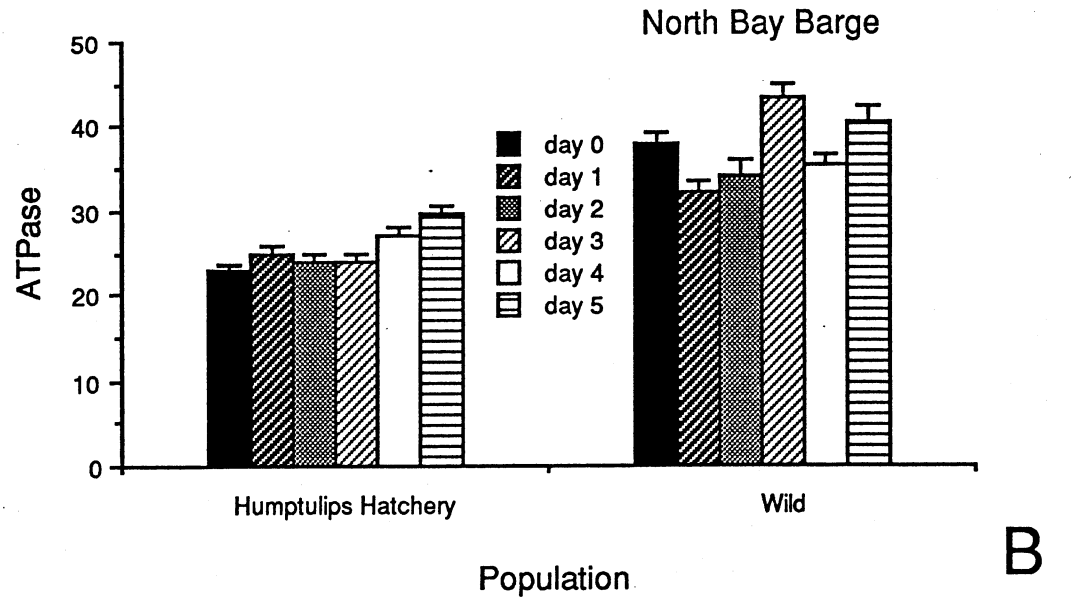


Fig. 3.12. ATPase values for wild and Humptulips Hatchery coho salmon maintained in net pens on barges that were moved either through the inner harbor (A) or North Bay (B).

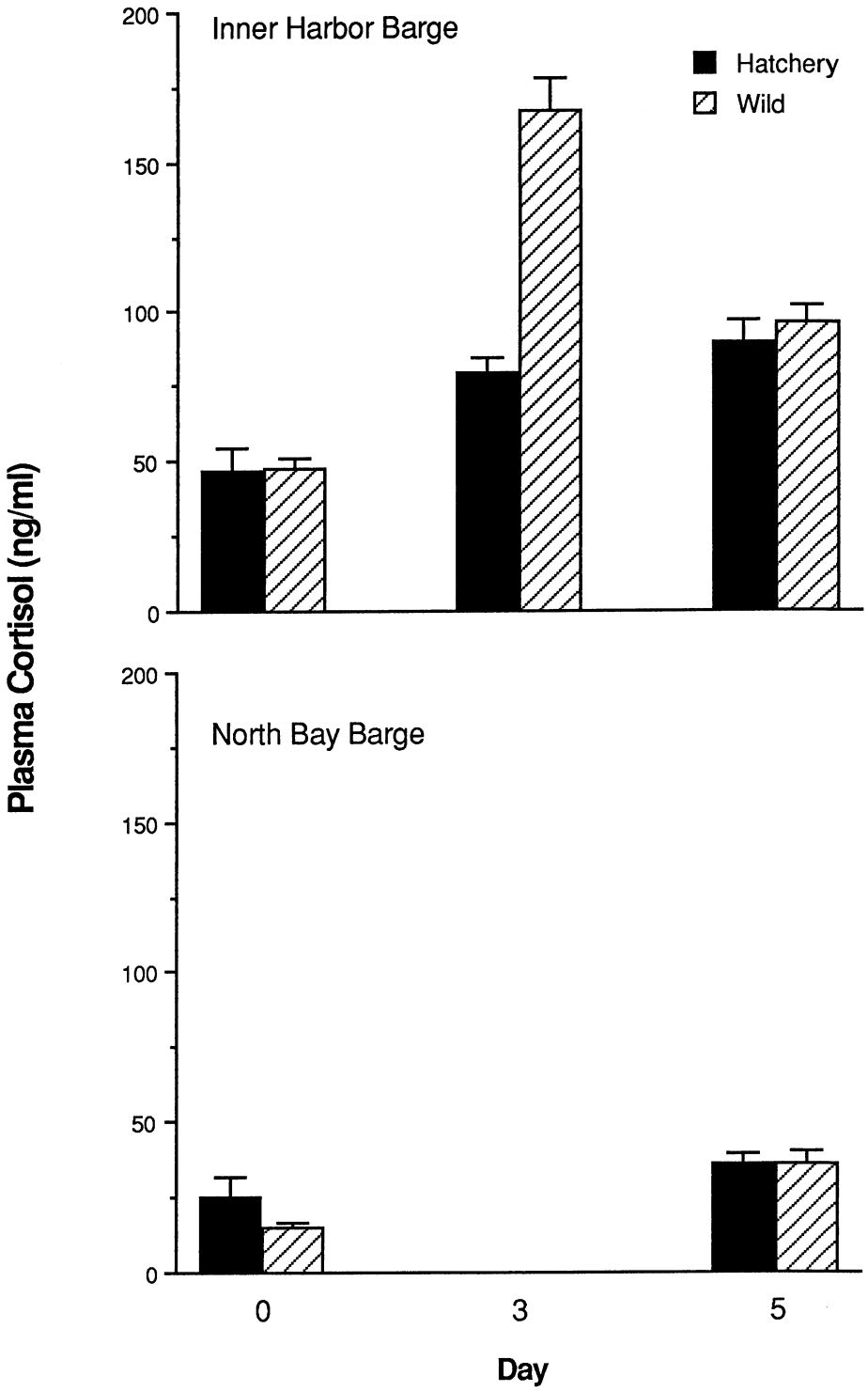


Fig. 3.13 Plasma cortisol concentrations (mean + S.E., n = 14-15) in wild and hatchery juvenile coho salmon held on the inner habor and North Bay barges. The inner harbor barge was sampled May 30-June 4; the North Bay barge was sampled June 1-7, 1988.

harbor barge as in fish collected from the North Bay barge. For example, hatchery fish on the North Bay barge had mean cortisol levels of 24.7 ng/ml on day zero and 36.1 ng/ml on day five, a 46% increase. Hatchery fish from the inner harbor barge had mean cortisol levels of 46.8 ng/ml at day zero; on day five these levels were 89.2 ng/ml, a 90% increase. Plasma cortisol levels in wild fish held on both barges were similar to those observed in the hatchery-reared fish. On day three, however, wild fish held on the inner harbor barge exhibited the highest mean plasma cortisol levels observed during the barging bioassay (167ng/ml).

The cortisol levels of barged fish that experienced secondary stress tests are shown in Fig. 3.14. The magnitude of the stress response appeared to be greater on the North Bay barge; but hatchery fish on both barges showed a decreased magnitude of response on day five compared to day zero. Hatchery-reared fish held on the North Bay barge demonstrated a stress response (plasma cortisol levels in stressed fish compared to controls) of 821% on day zero, this value decreased to 446% by day five. Whereas hatchery fish on the inner harbor barge had a stress response of 439% on day zero which decreased to 145% by day five. At first glance it would appear that the fish on the North Bay barge were undergoing more stress than those held on the inner harbor barge. However, fish collected from the inner harbor barge actually had greater resting levels of cortisol prior to the tests. This higher resting level effectively reduced the stress response the fish exhibited. Indeed, as Fig. 3.14 shows, these fish actually had

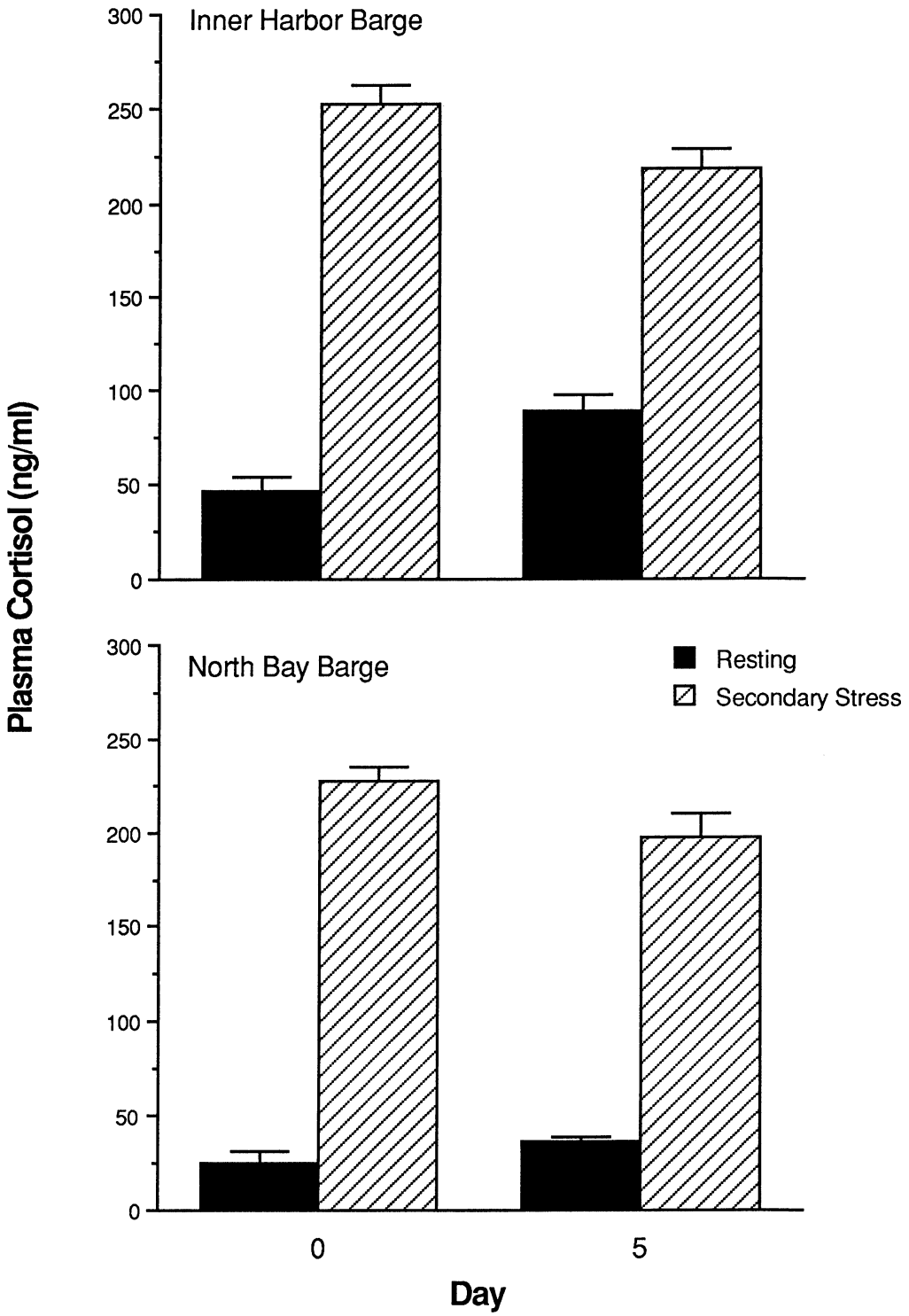


Fig. 3.14 Plasma cortisol concentrations (mean + S.E.) in hatchery coho salmon held in barges in 1988 before and after a secondary stress challenge. N for resting levels equaled 15; for the secondary stress evaluations it equaled 9 to 10.

higher cortisol titers after completing a stress test than their North Bay counterparts.

Immunocompetence was appraised as before, by the passive hemolytic plaque assay (Tripp et al. 1987). The changes observed in PFC response for both wild and hatchery fish held on the barges are shown in Fig. 3.15. Generally, the PFC response of all fish held on the barges decreased over time. The magnitude of this decrease was greater for fish held on the inner harbor barge. For instance, on day five, hatchery and wild fish retained on the North Bay barge exhibited PFC responses that were 51% (339/671) and 39% (608/1577) respectively, of that observed prior to barging. These values were 15% (307/2021) for hatchery fish and 23% (757/3364) for wild coho held on the inner harbor barge. This reduced immunocompetence is not surprising because fish barged through the inner harbor had consistently higher cortisol titers than those moved through North Bay.

Survival and Growth in Seawater. On day zero and at the completion of each barging day thereafter, approximately 100 hatchery fish from the inner harbor barge were transported to NMFS's Manchester Field Station and released into saltwater net pens. Similar collections of hatchery fish were made on day zero and day five from the North Bay barge (Table 3.2). Fish were held in seawater for six months in 1.2 m by 2.1 m by 1.5 m deep pens and their growth and survival were monitored. Little mortality occurred in the net pens for the first 45 days. In mid July however, a natural challenge of *Vibrio* occurred at Manchester and

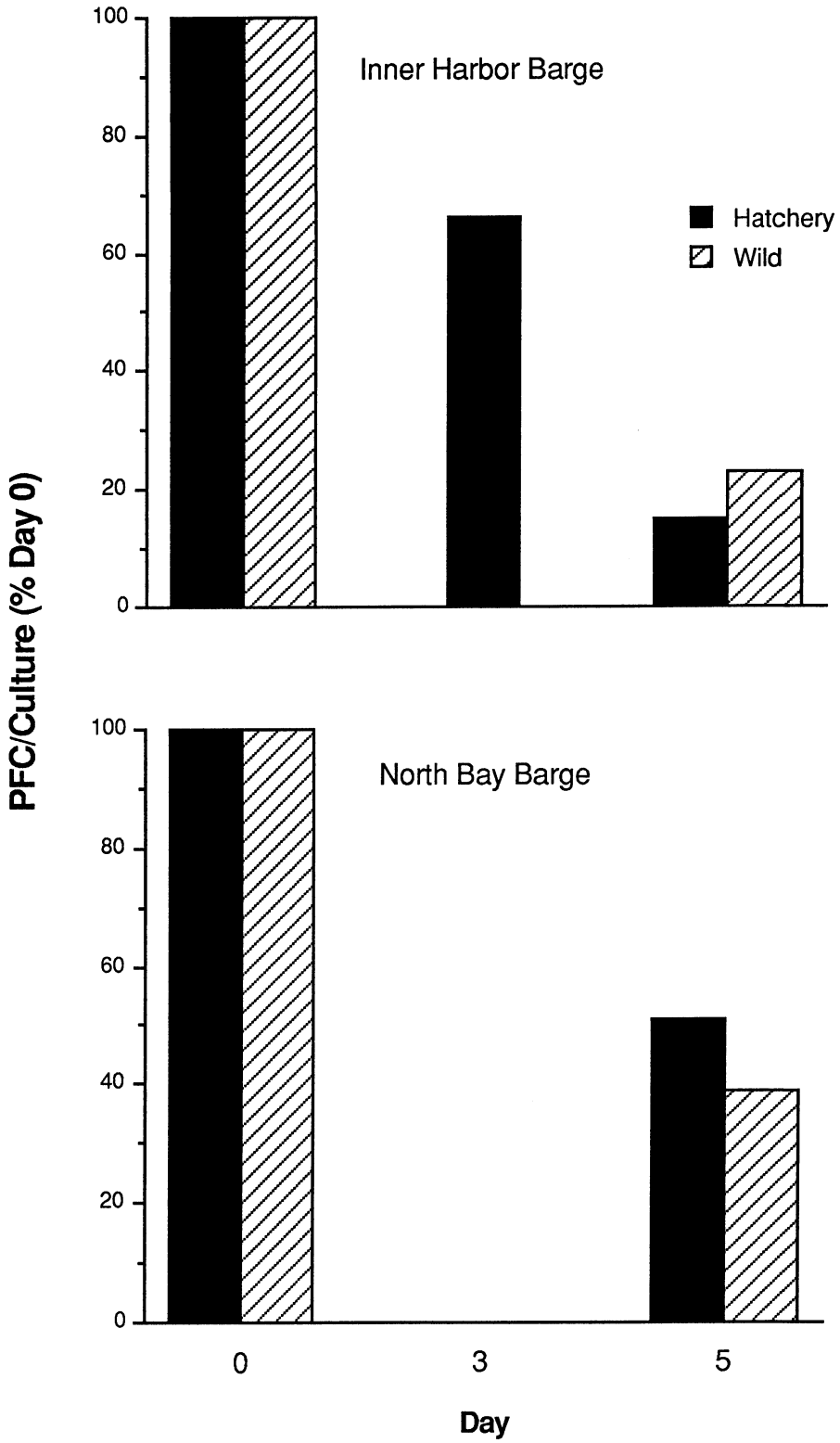


Fig. 3.15 Changes in plaque-forming cell response (PFC) in hatchery and wild juvenile coho salmon held on barges that were moved through the inner harbor or North Bay. Note that data are presented as the percentage of the response observed on Day zero - Inner Harbor Barge: Hatchery fish = 2021, Wild fish = 3364; North Bay Barge: Hatchery fish = 671, Wild fish = 1577).

over 80% of the fish that had been barged through the inner harbor perished while only 23% died that had been exposed to North Bay water.

Mortality rates of fish maneuvered through the inner harbor did not depend on how long they had been barged (Table 3.3). Instead, whatever affected the survival of these fish appears to have impacted them sometime during the acclimation period. The growth rates of the fish barged in both estuaries were very similar. At the end of the rearing period, the average weight of fish held in the inner harbor barge was 57 grams while those from the North Bay barge weighed approximately 55 grams.

Conclusions

The physiological data presented above indicate that fish from the inner harbor barge experienced greater stress than fish barged in North Bay because they had higher levels of circulating cortisol and lower immunocompetencies. Moreover, their ability to survive a secondary stressor like the *Vibrio* outbreak at Manchester was clearly diminished. A number of factors besides simply being exposed to inner harbor waters may have influenced these results. First, fish on the inner harbor barge were inadvertently stressed on barging day five. While attempting to move the barge into the South Channel it became stuck on submerged pilings located at the eastern end of Rennie Island. To free the barge, the holding box had to be lifted up which clearly decreased the volume of water available and probably stressed the fish. Second, because two separate crews removed fish from each barge, it's possible that

Table 3.3 Mortality and growth rates of smolting coho barged through the inner harbor and North Bay in 1988. After being barged, the fish were held in seawater netpens located at NMFS's Manchester Field Station.

INNER HARBOR BARGE

	Collection Date						Totals
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	
No. of Fish Collected	92	109	109	83	97	82	572
No. Died	74	80	91	71	96	68	480
% Dead	80.4	74.3	83.5	85.5	98.9	82.9	83.8
Wt. at End of Rearing period (g)	58.0	57.9	57.7	50.0	75.0	59	57.2

NORTH BAY BARGE

	Collection Date						Totals
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	
No. of Fish Collected	102					100	202
No. Died	27					20	
% Dead	26.5					20.0	23.3
Wt. at End of Rearing period (g)	55.0					54.0	55.7

coho destined to be transported to Manchester were removed prior to those collected for cortisol samples. If this occurred, the activity associated with fish removal certainly could have stressed the remaining hatchery fish and caused them to have artificially high cortisol values.

Before giving these factors an unduly amount of weight however, it is also important to consider data gathered from the barges that were not affected by these activities. Recall that on day zero, cortisol titers of hatchery and wild fish from the inner harbor barge were 89 and 192% higher, respectively, than their counterparts on the North Bay barge. Additionally, wild fish were not subjected to any possible stress caused by fish removal since none of these fish were transported to Manchester. Thus, the cortisol levels of wild fish originating from the two barges can be legitimately compared. On day three, wild fish removed from the inner harbor barge had 351% more cortisol than wild coho taken from the North Bay barge on day five. The fish rearing study at Manchester, also clearly indicated that fish collected on days zero through four from the inner harbor barge were simply not as immunocompetent as North Bay fish. All of this evidence consistently points to the conclusion that fish held in the inner harbor were more stressed and less healthy than those moved through North Bay.

In Situ Bioassay 2: Live Boxes in the Inner Harbor and North Bay

Introduction and Background

Several aspects of the barging bioassay suggested another

approach was needed to evaluate, *in situ*, how coho were affected by inner Grays Harbor waters. Barging the fish through the inner harbor made it impossible to separately evaluate the effects of different areas. Furthermore, temporal variability in water quality could not be assessed. For example, if the assays had been conducted at another time, they may have yielded different results because of dissimilar river flows, tidal cycles and effluent composition. Finally, clear effects were observed on fish that had been acclimated for seven days at the mouth of the Wishkah River. There was need to further refine when the onset of these impacts occurred and if they manifested themselves in other areas of the harbor. To accomplish these objectives, smolting coho were held for varying periods of time in live boxes anchored throughout the inner harbor and in North Bay. Physiological parameters similar to those obtained from the barged fish were measured and long-term seawater survival was also appraised.

Locations and Description of the Live Boxes

Five live boxes were moored in the Chehalis River and its estuary (Fig. 3.16). One was located at river km 24 directly under the Montesano bridge (State Route 107). The water level at this site was influenced by tidal cycles, but saltwater was not present. All the remaining sites were either in salt or brackish water areas. The least saline water site was situated in Elliott Slough, which empties into the eastern end of the inner harbor. A live box was also anchored 0.5 km west of the mouth of the Wishkah River, a location that was relatively close to where the inner harbor barge

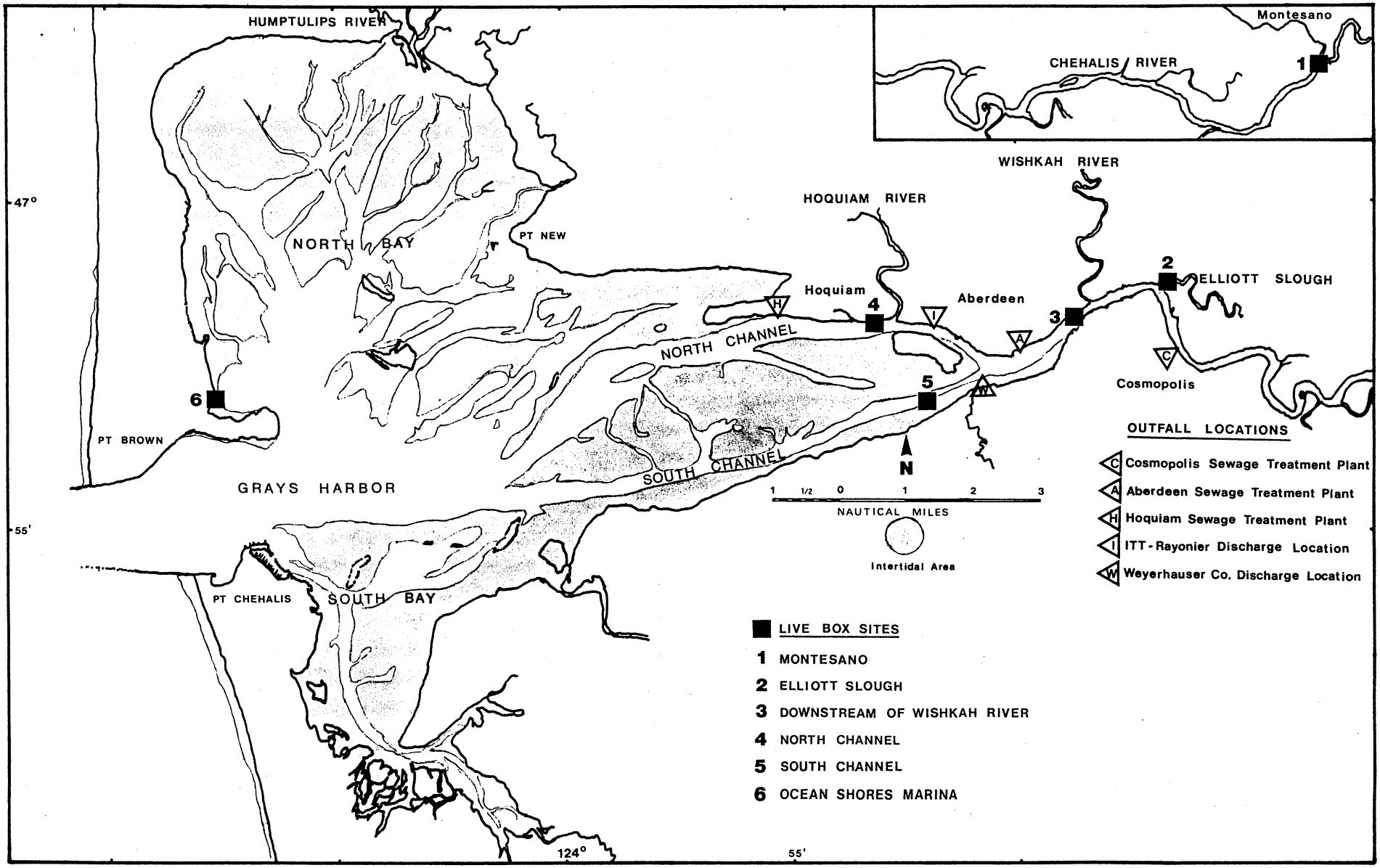


Fig. 3.16. The locations of the live boxes used to hold coho smolts in the inner harbor and North Bay in 1989.

had been moored. The other inner harbor boxes were stationed 2 km west of the ITT Rayonier discharge point in North Channel; and in South Channel, approximately 2 km west of the Weyerhaeuser wastewater diffuser. The remaining two boxes were berthed at the Ocean Shores Harbor Marina in North Bay.

The live boxes were wooden framed 5.5 m by 3.9 m rectangles that were floated by six styrofoam-filled tires attached to the perimeter of the frame. Each box held two, 2.4 x 2.1 by 1.8 m deep nylon-mesh net pens covered with hinged lids. To fully suspend the nets, seven kilogram weights were placed at each corner. In addition, plexiglas baffle plates were attached to the ends of each box to protect the fish from currents (Fig. 3.17). The boxes were either directly moored to existing docks (North Bay sites), or tethered and anchored between one or two adjacent pilings.

Methods Used to Conduct the Bioassay

Smolting coho were introduced into the live boxes on three separate occasions, hereafter referred to as Series 1, 2, and 3. In Series 1 and 2, coho from the Humptulips Hatchery were used. In each live box, one of the net pens received ≃270 fish while the other was loaded with ≃175 coho. Initial pen densities therefore did not exceed 1.75 kg/m^3, which is well below normal aquacultural densities. In Series 3, ≃23 Humptulips Hatchery coho that had been reared in seawater net pens at Westport were placed into every live box to observe how their ATPase levels responded to the waters surrounding each live box.

The duration and sampling schedule of Series 1 and 2 were

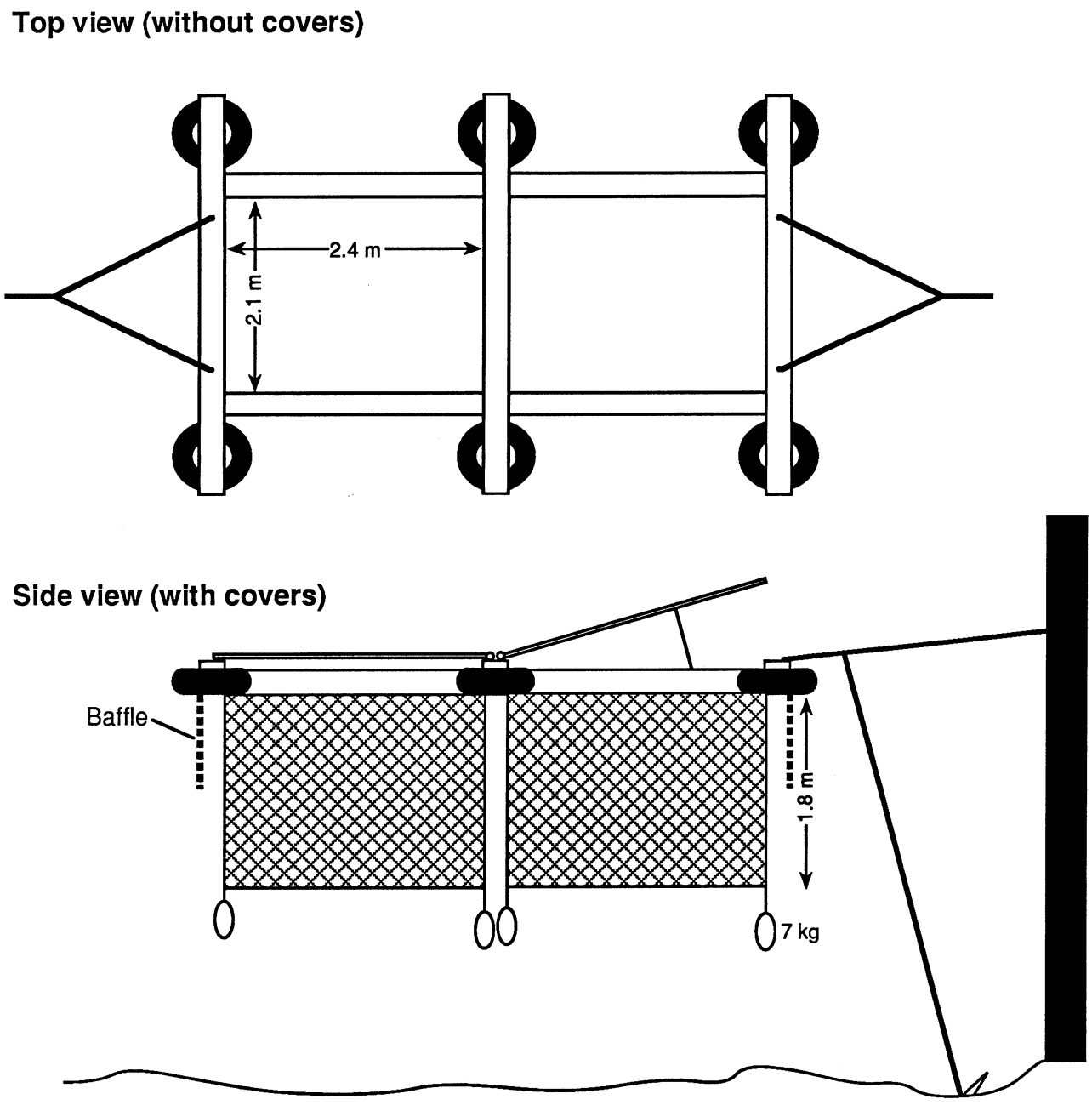


Fig. 3.17 Top and side views of a live box showing the dimensions of the box and the placement of baffle plates and anchor and tether lines.

different than those used in Series 3. During Series 1 and 2, samples of hatchery fish were taken one day prior to moving the fish into their live boxes. Five and nine days after being placed into a live box, samples of fish were obtained from the net pen that originally was stocked with 270 fish. On day 14, fish were sampled from the other pen. Any coho remaining in the net pens after day 14 were removed. In Series 3, fish were held in each live box for eight days.

During sampling, care was taken to ensure animals destined for immediate assays were collected first. Coho scheduled for long-term rearing were then removed and transported to the NMFS's Manchester Field Station and placed into seawater net pens.

Because immunocompetence assays are time consumptive, it was not possible to fill all the live boxes used in Series 1 and 2 simultaneously. Instead, coho were placed into the seven boxes over a three day period. Consequently, Series 1 began on 4/22/89 and finished on 5/8/89 (a 17 day period) and Series 2 ran from 5/12/89 through 5/28/89. In series 3, it was possible to fill the live boxes in one day, so the entire assay was completed over an eight day period that ran between 5/4/89 and 5/11/89.

Water quality evaluations similar to those performed during the barging bioassay were performed. EPA characterized the waters surrounding five of the live box sites (only the Elliott Slough site was not sampled) by collecting water, suspended solids and XAD resin samples for one 24 hr period during Series 1. An additional 24 hour sampling episode at the South Channel live box occurred

during Series 2. The objective of this work was to provide some initial clues as to which pollutants or pollutant classes may be responsible for any observed biological effects. The results of this screening work are presented in the water quality portion of this report (see Part III).

Physiological Assessments Made on Coho Situated in the Live Boxes

The periodicity and types of samples procured during each livebox series are shown in Table 3.4. As this table shows, ATPase, plasma cortisol, immunocompetence, mixed function oxidase, and long-term seawater survival assessments were made on fish collected during Series 1 and 2. Only ATPase evaluations were obtained from coho collected at the completion of Series 3. As alluded to above, "pre-treatment" or baseline levels for each physiological parameter measured were obtained by sampling hatchery fish (Series 1 and 2) or Westport net pen coho (Series 3) one day prior to their placement in the live boxes.

Except for the mixed function oxidase test, the assays listed above are nearly identical to those used to characterize barged fish. The mixed function oxidase test determines the activity of cytochrome P-450 1 A, which is a liver enzyme that catalyzes the biotransformation of a wide variety of xenobiotics in fish. P-450s usually proliferate in the liver once a fish has been exposed to certain chemical contaminants. Consequently, the relative levels and catalytic activities of these enzymes can provide information on exposure of fish to chemical contaminants. Moreover, the biotransformation of certain chemicals by cytochrome P-450s can

Table 3.4 Schedule followed for the collection of ATPase, plasma cortisol, immunocompetency, mixed function oxidase, and marine survival data on fish held in live boxes located in the inner harbor and North Bay during the spring of 1989.

	Series 1 and 2			
	Collection Date			
Assay	Day 0	Day 5	Day 9	Day 14
ATPase	Yes	Yes	Yes	Yes
Plasma Cortisol	Yes	-	Yes	Yes
Immunocompetence	Yes	-	Yes	Yes
Mixed Function Oxidase	Yes	Yes	-	Yes
Marine Survival	Yes	Yes	Yes	Yes

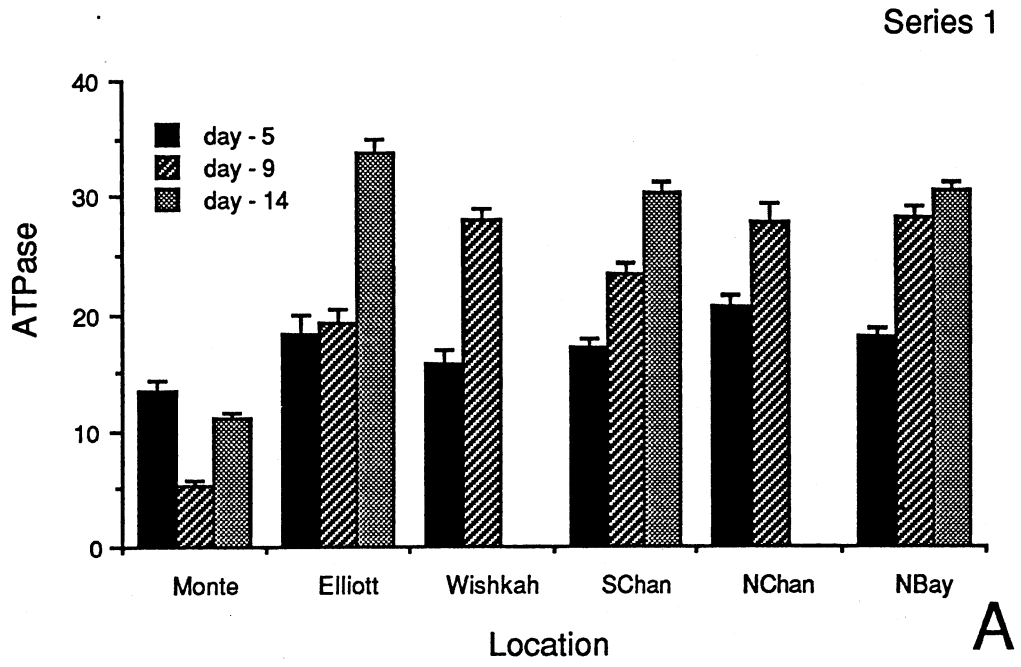
	Series 3	
	Collection Date	
Assay	Day 0	Day 8
ATPase	Yes	Yes

create toxic metabolites which have been associated with impaired physiological functions (Mattison and Nightingale 1980; Landner et al. 1985; Andersson et al. 1988; Johnson et al. 1988). Thus, increased or induced activity of these enzymes may help elucidate the biochemical links between exposure to toxicants and long-term physiological consequences.

Mixed function oxidase samples were obtained from fish in each live box on days five and 14 during Series 1 and 2. During a sampling period, 16 fish were removed from each live box and immediately killed. Their livers were then removed and pooled into four groups (four livers/group) and frozen in liquid nitrogen. Samples were transferred to a -80° C freezer until hepatic microsome preparations were made by following the methods of Collier et al (1986). Protein concentrations in the resulting microsomal suspensions were determined (Lowry et al. 1953) along with aryl hydrocarbon hydroxylase (AHH) (Collier et al. 1986) and 7-ethoxyresorufin-O-deethylase (EROD) activities (Prough et al. 1978), both of which are measures of mixed function oxidase activity. Because the EROD assay proved to be more sensitive to pulp mill effluents (Andersson et al. 1988; Lindstrom-Seppa and Oikari 1990), only results of this assay are reported here.

Results of the Assays

ATPase evaluations. The effect on ATPase activity of holding Humptulips Hatchery fish in different areas of Grays Harbor is shown in Fig. 3.18. Fish in all locations, except the Montesano site, steadily increased their ATPase values from day five through



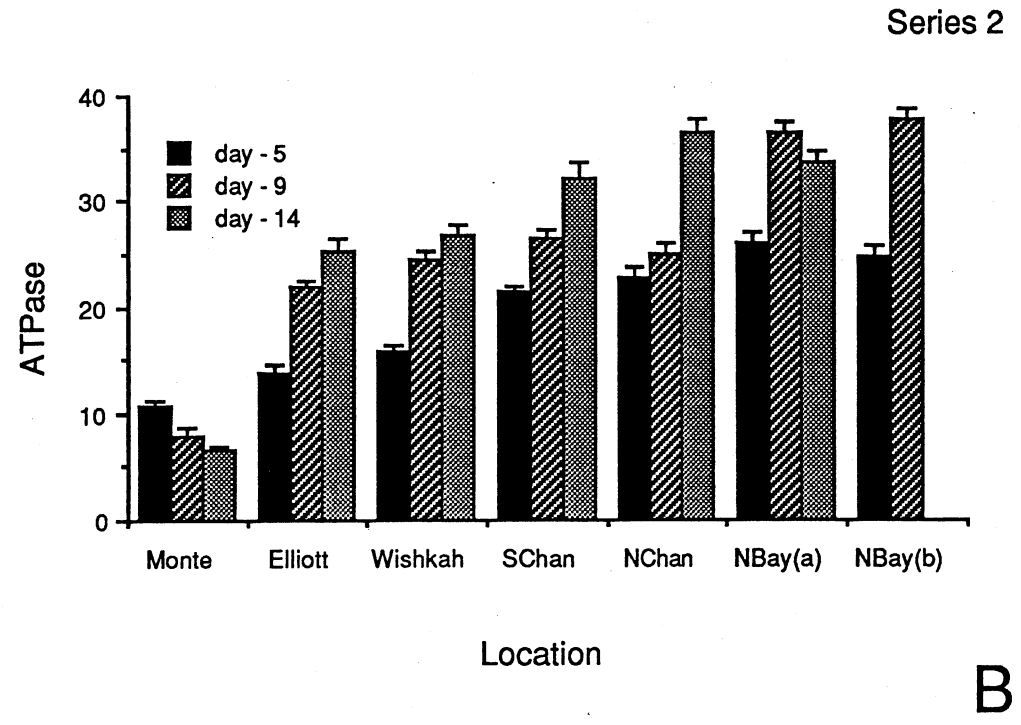


Fig. 3.18. ATPase values obtained from Humptulips Hatchery coho held in live boxes ancohored in the Chehalis River, inner harbor, and North Bay. The fish were sampled after five, nine, and 14 days of residence in the boxes. Day zero ATPase values equaled 9.9 and 9.0 for Series 1 and 2. Series 1 (A) started on 4/22/89 and was completed 5/8/89 while Series 2 (B) took place from 5/12-28/89.

day 14. Like the barging evaluations, the greatest increases in this enzyme occurred in fish held in the most saline areas (e.g. North and South channels and North Bay). The decreasing values observed at the Montesano location probably reflect that fish were reverting back to parr since they were unable to enter seawater.

In some of our experiments, the effects of environmental conditions on the ability of fish to secrete ATPase were difficult to determine because pre-treatment levels of this enzyme were low. Consequently, in Series 3, sea water adapted coho were used, since these fish were expected to have ATPase values in excess of 30. The results of this assay demonstrated that high ATPase values ($\geq$ 30) occurred in all sampled fish except those held at the Montesano site (Fig. 3.19), where ATPase levels decreased from 36.6 to 15 during the eight day holding period. Again, this decrease was probably caused by the reversion of the fish to parr because they were forced to reside in freshwater. In general, these assessments showed that waters surrounding the inner harbor and North Bay live boxes were not interfering with the production of ATPase.

Cortisol and Immunocompetence. Cortisol samples were taken from fish placed in each live box on days nine and 14 during both Series 1 and 2. The natural log transformation was used on the subsequent cortisol values to improve their homoscedasticity and thus make them amenable to parametric statistical tests. If the values obtained on the two sampling dates were not statistically different from each other they were pooled and used in a number of ANOVAs (Analysis of Variance). These statistical procedures were

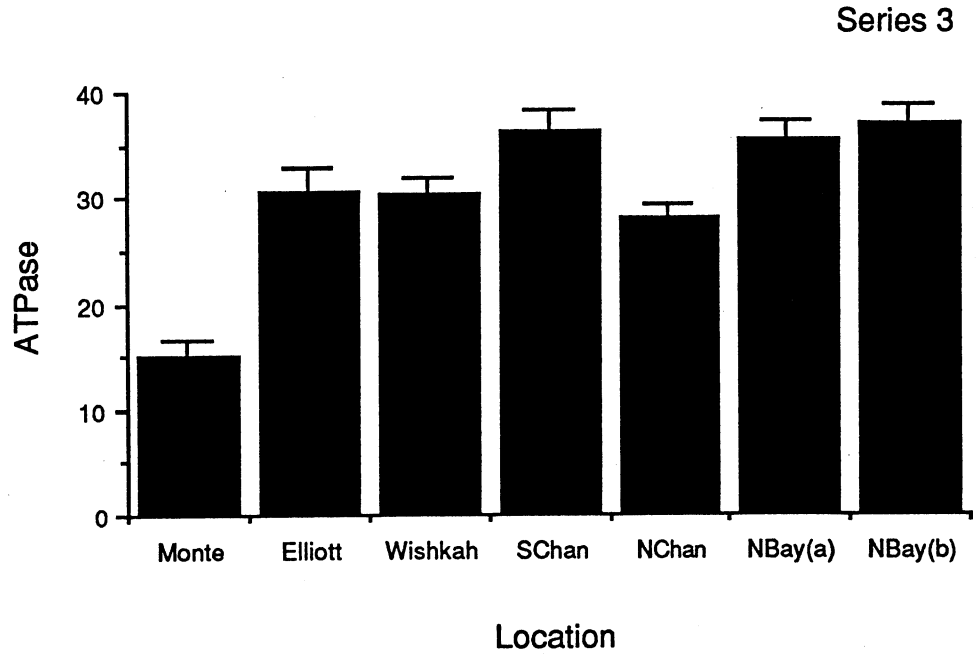


Fig. 3.19. ATPase values obtained from coho salmon reared at Westport that had been placed in live boxes anchored in the inner harbor, North Bay, and Chehalis River. The fish were sampled after eight days of residency in the boxes. The day zero ATPase value equaled 36.3 and the test (Series 3) took place on 5/3-11/89.

used to ascertain: 1) if fish from any of the live box locations had different cortisol titers than pre-treatment fish, and 2) whether differences existed among fish held in North Bay, the inner harbor, and the Chehalis River (i.e. the Montesano site).

The plasma cortisol levels of fish sampled in Series 1 are shown in Fig. 3.20 while those obtained during Series 2 are depicted in Fig. 3.21. Unfortunately, during Series 1, fish held for 14 days at the North Channel and Wishkah sites were lost from the net pens.

In Series 1, nine-day fish from Wishkah and 14-day fish from the Montesano site had cortisol titers lower than pre-treatment fish collected at the Humptulips Hatchery. Additionally, none of the inner harbor sites had statistically higher cortisol titers than the North Bay fish. The cortisol data collected from fish during Series 2 were slightly different. First, no samples were lost and all the nine and 14 day values from each site could be pooled. The ANOVAs performed on these data showed that fish held in North Channel had higher cortisol titers (59.8 ng/ml) than those from the control or North Bay boxes (28.4 ng/ml).

Immunocompetence information was simultaneously collected on the fish used in the cortisol determination study by once again using the passive hemolytic plaque assay (Tripp et al. 1987). As in the cortisol analyses, raw data were transformed to natural logarithms to improve their homoscedasticity before ANOVAs were performed. Moreover, nine and 14 day samples were pooled if they were not statistically different from one another. These analyses

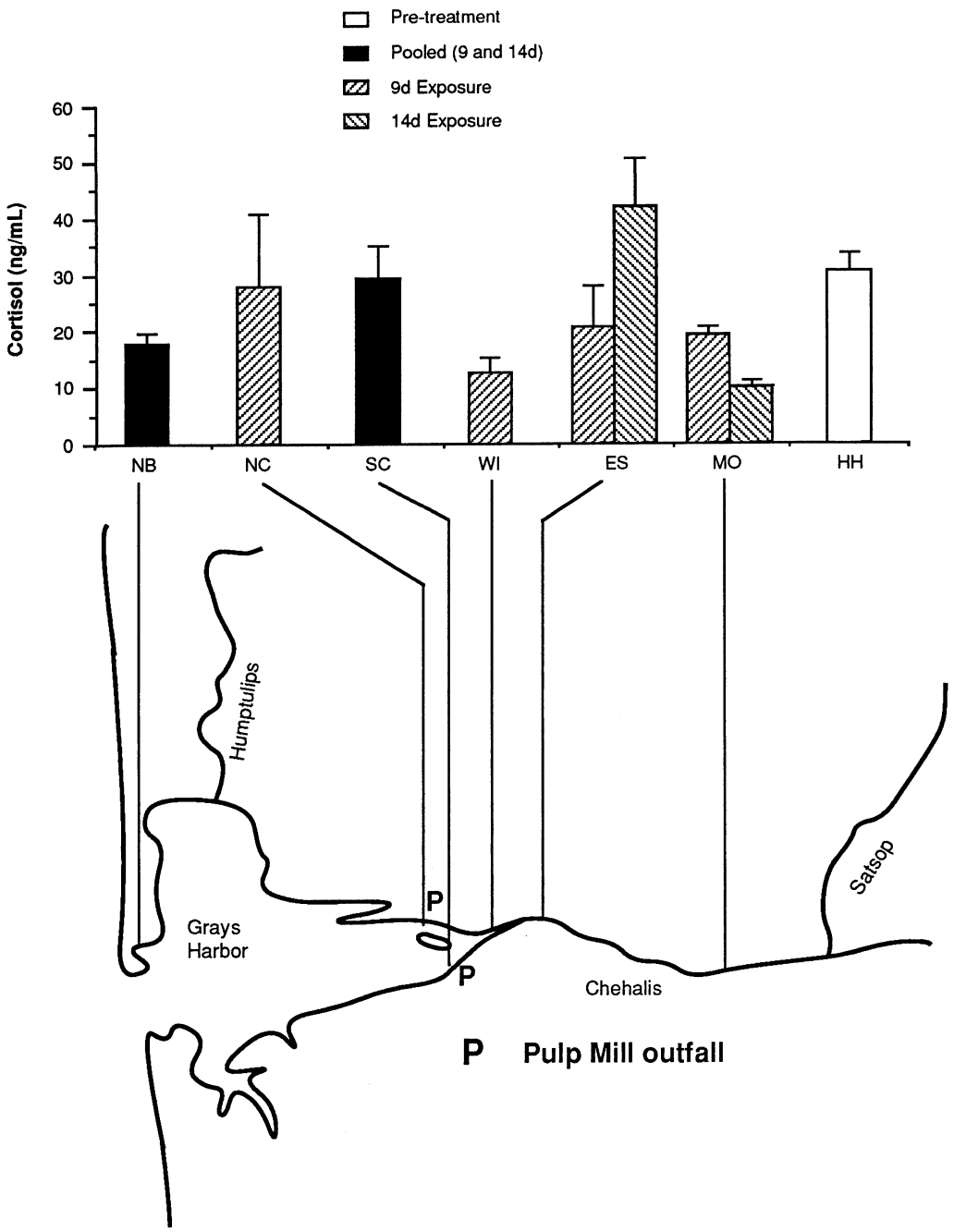


Fig. 3.20 Plasma cortisol concentrations (mean + S.E., n = 15) in hatchery juvenile coho salmon sampled after nine and 14 days from live boxes at various locations and from the general hatchery population during Series 1, 1989. NB = North Bay, May 1 and 6; NC = North Channel, May 3; SC = South Channel, May 3 and 8; WI = Wishkah River mouth, May 1; ES = Elliott Slough, May 2 and 7; MO = Montesano, May 2 and 7; and HH = Humptulips Hatchery, April 21.

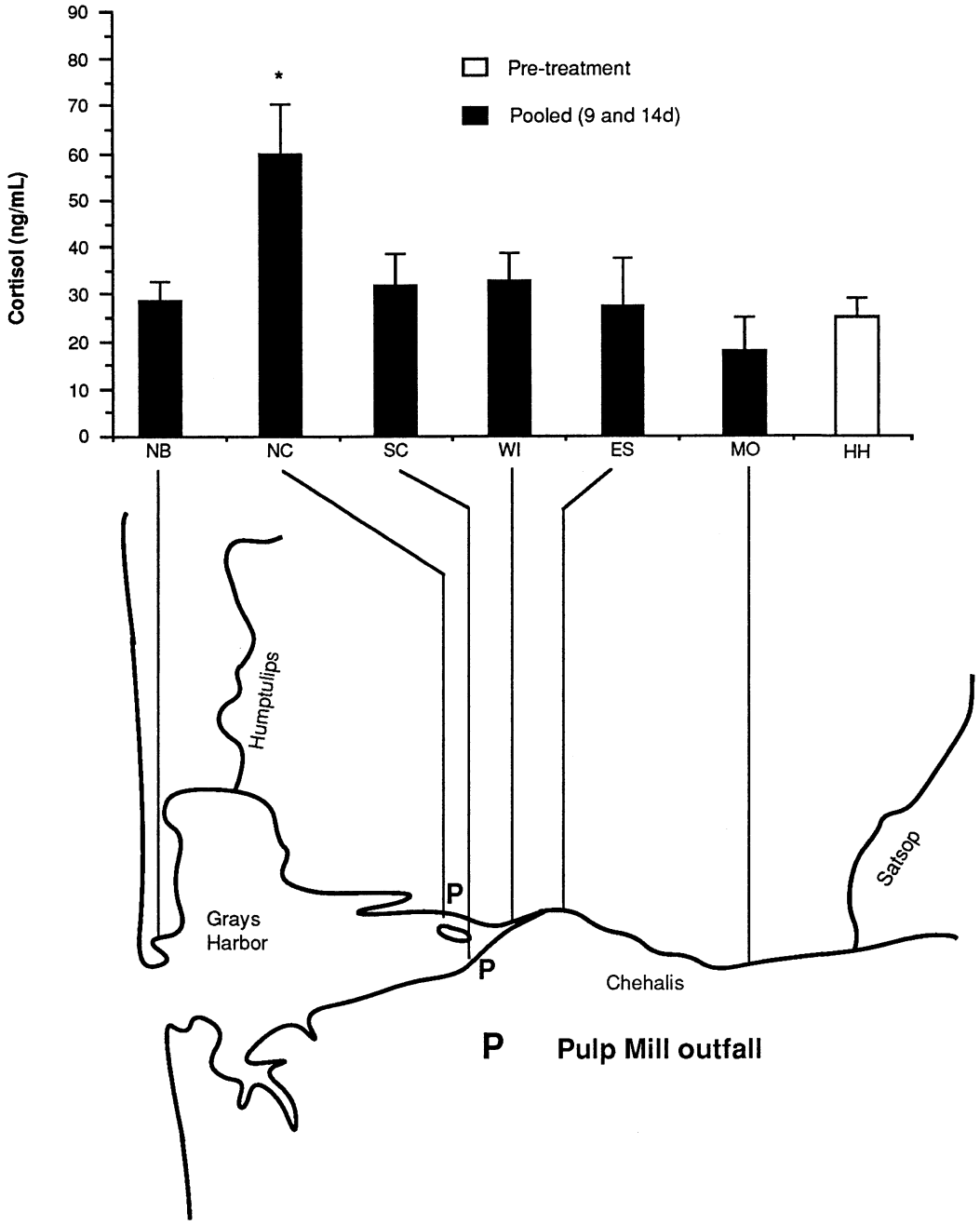


Fig. 3.21 Plasma cortisol concentrations (mean + S.E.) in hatchery coho salmon sampled after nine and 14 days from live boxes at various locations and from the general hatchery population during Series 2, 1989. NB = North Bay, May 21 (n = 30) and 26 (n = 15); NC = North Channel, May 23 (n = 15) and 28 (n = 15); SC = South Channel, May 23 (n = 15) and 28 (n = 15); WI = Wishkah River mouth, May 21 (n = 14) and 26 (n = 15); ES = Elliott Slough, May 22 (n = 15) and 27 (n = 15); MO = Montesano, May 23 (n = 15) and 27 (n = 15); and HH = Humptulips Hatchery, May 12 (n = 15).

evaluated: 1) whether fish from the six live box locations had different immune responses than those obtained from Humptulips Hatchery coho collected just prior to the commencement of the live box tests, and 2) if fish sampled from inner harbor live boxes had different immunocompetencies than those from the North Bay site.

The immune responses of fish collected during Series 1 and 2 are shown in Figs. 3.22 and 3.23. In Series 1, all livebox sites contained fish with immune responses (range = 1374-2026 PFC's/culture) that were greater than the pre-treatment sample taken at the Humptulips Hatchery (516 PFC's/culture). Moreover, the immune responses of fish from the inner harbor sites were not significantly different from those at the North Bay site.

In Series 2, however, four of the six live box sites (Montesano, Wishkah, North Channel, and South Channel) possessed fish with significantly lower immune responses (range = 998-1778) than the pre-treatment fish collected at the Humptulips Hatchery (3378 PFC's/culture); the immune response of fish from the North Bay site was similar to the pre-treatment sample. Fish from the North and South Channel locations had the lowest PFC responses, but only the immune response of the South Channel (998 PFC's/culture) fish was significantly lower than fish held at North Bay (2419 PFC's/culture).

Interpretation of the Cortisol and Immune Response Data. Clearly, the plasma cortisol levels and immune responses differed between the two series. During Series 1, the loss of two of the 14-day populations and the inability to pool some cortisol samples

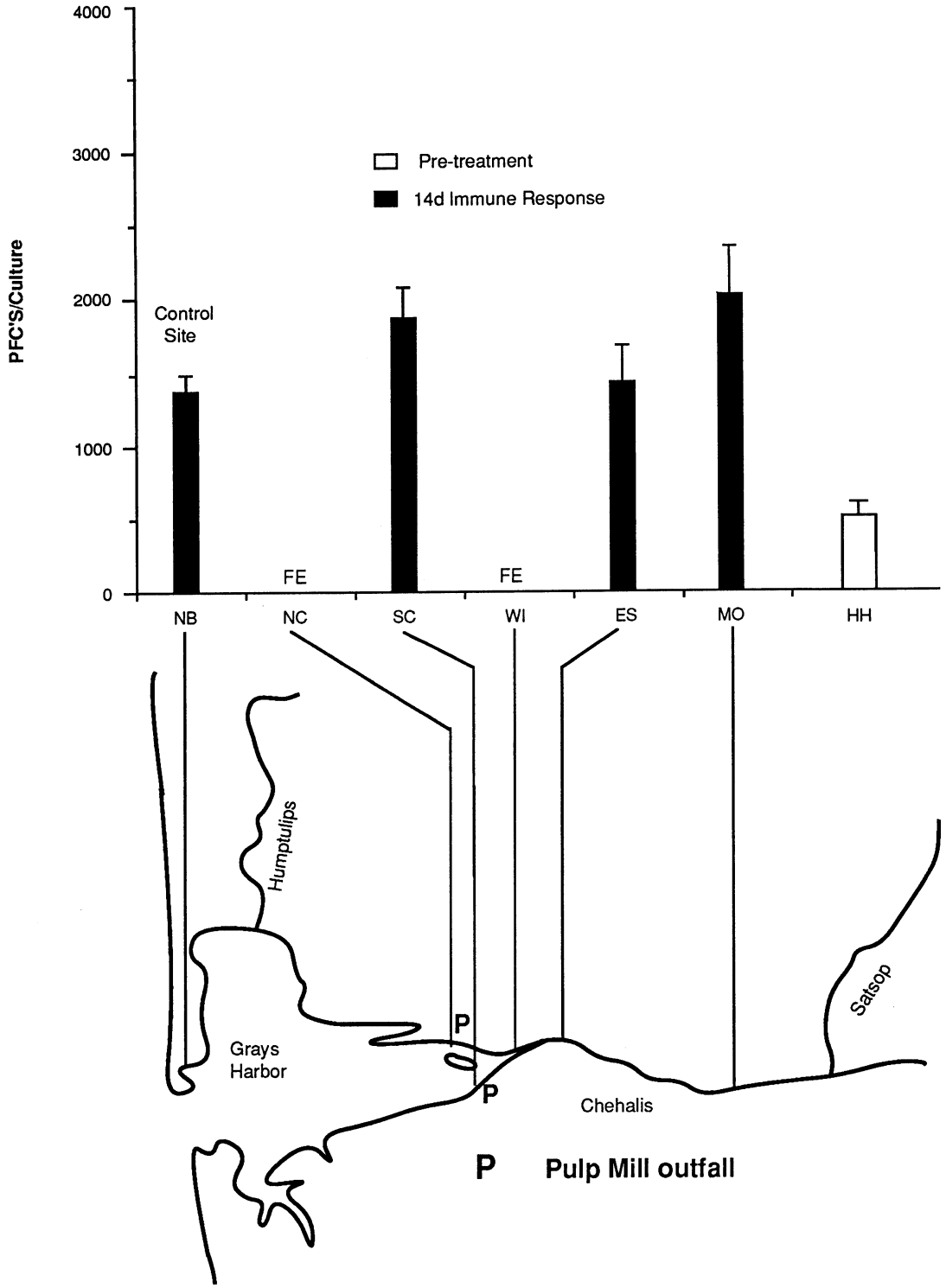


Fig. 3.22 Plaque-forming cell response (PFC) (mean + S.E.) in hatchery juvenile coho salmon sampled after 14 days from live boxes at various locations and from the general hatchery population during Series 1, 1989. FE = Fish escaped from the live box. NB = North Bay, May 6 (n = 18); NC = North Channel, FE; SC = South Channel, May 8 (n = 17); WI = Wishkah River mouth, FE; ES = Elliott Slough, May 7 (n = 19); MO = Montesano, May 7 (n = 19); and HH = Humptulips Hatchery, April 21 (n = 20).

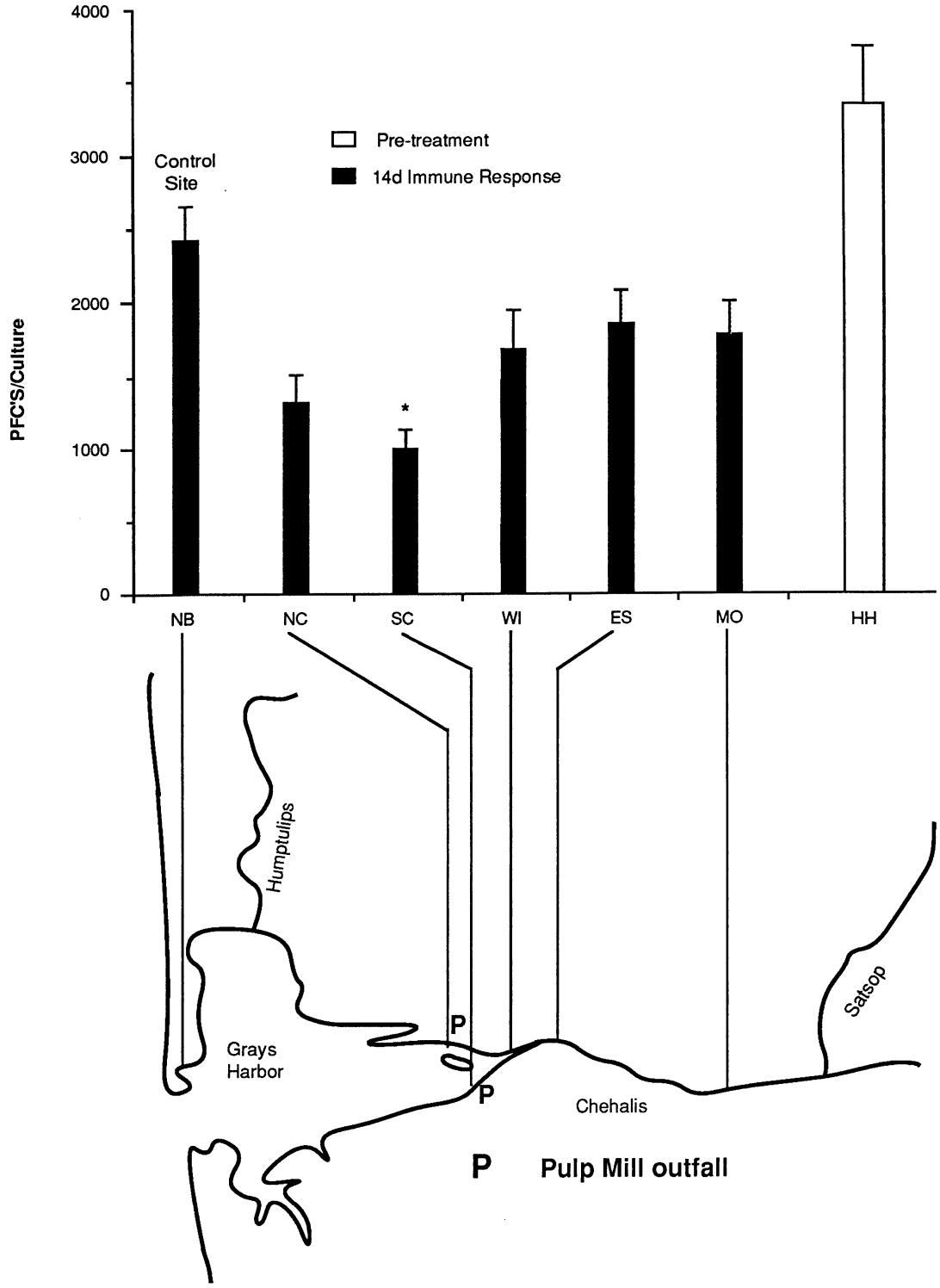


Fig. 3.23 Plaque-forming cell response (PFC) (mean + S.E.) in hatchery juvenile coho salmon sampled after 14 days from live boxes at various locations and from the general hatchery population during Series 2, 1989. NB = North Bay, May 26 (n = 20); NC = North Channel, May 28 (n = 19); SC = South Channel, May 28 (n = 20); WI = Wishkah River mouth, May 26 (n = 20); ES = Elliott Slough, May 27 (n = 18); MO = Montesano, May 27 (n = 19); and HH = Humptulips Hatchery, May 12 (n = 19).

hampered interpretation of the data. Basically, there did not appear to be any noticeable trends or site-specific differences in either immune competence or plasma cortisol levels.

On the other hand, data collected during the Series 2 showed that plasma cortisol levels acquired from fish held in the North Channel were more than twice as high as those obtained from coho held in the control boxes moored in North Bay (Fig. 3.21). Also the immune responses of fish held in the North and South channel boxes during this second series were substantially less than those of fish placed in the North Bay boxes. Thus, the Series 2 results indicated that an immunosuppression was experienced by fish if they were confined at inner harbor locations. This result is consistent with those obtained from the immunocompetence assays performed during the barging bioassay.

Moreover, a significant, inverse relationship between plasma cortisol levels and numbers of plaque-forming cells was revealed (Fig. 3.24) once data from both series were pooled by live box location (i.e. all nine and 14 day cortisol titers and 14 day immune responses/location were combined). This correlation analysis indicated that on average, fish from the South and North channel sites had lower immune responses and higher cortisol titers than fish from other live box locations. This inverse relationship is not without precedence. Maule et al. (1988) demonstrated that acute stress in chinook salmon elevated plasma cortisol titers, diminished the immune response, and reduced the ability of the fish to resist the pathogen *Vibrio anguillarum*. This relationship

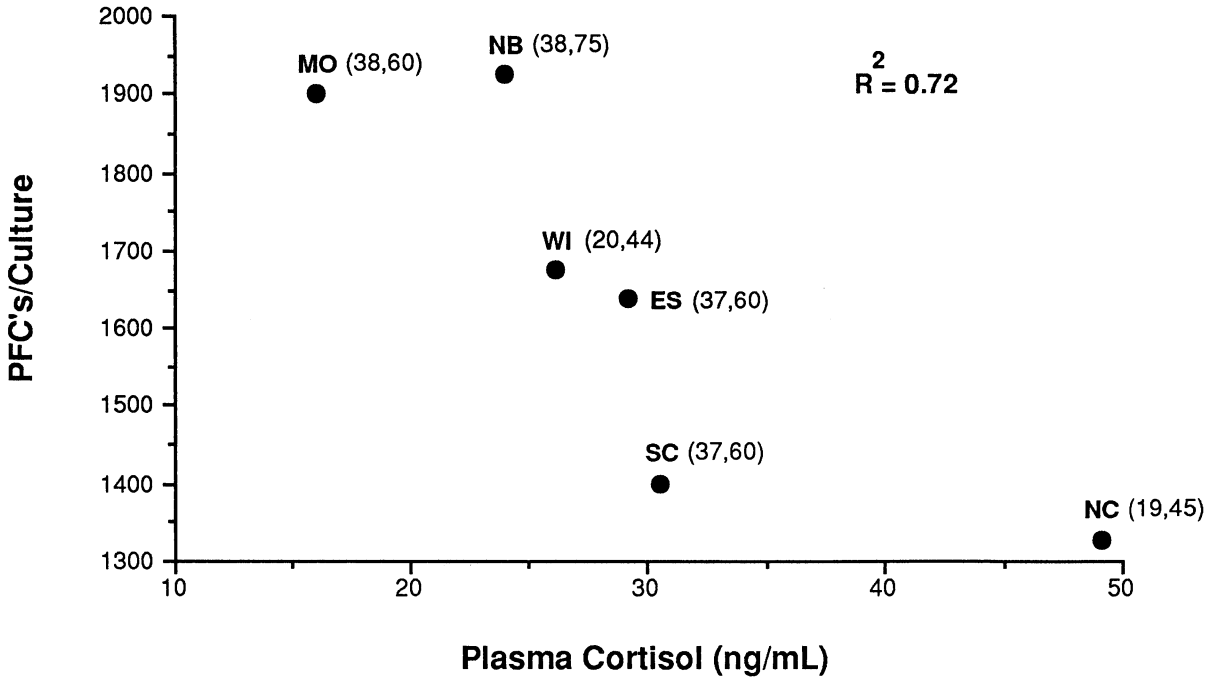


Fig. 3.24 The inverse relationship between the numbers of plaque-forming cells and plasma cortisol concentrations found in juvenile coho salmon held in live boxes located in Grays Harbor during Series 1 and 2. NB = North Bay; NC = North Channel; SC = South Channel; WI = Wishkah River mouth; ES = Elliott Slough; MO = Montesano. Numbers in parentheses are sample sizes, the PFC sample size is placed ahead of the plasma cortisol sample size.

suggests that juvenile coho from the two inner harbor sites are more stressed and less capable of resisting pathogens than counterparts held in other locations in the harbor.

Mixed Function Oxidase Evaluations. Hepatic EROD activities were measured on all samples collected during Series 1 and 2. The two-way ANOVA conducted on data collected during Series 1 revealed that fish held in North Channel, South Channel and Wishkah live boxes had significantly higher EROD values than those situated in North Bay, Montesano, and Elliott Slough on both sampling days. Besides this site effect, a significant day effect was also observed. Generally EROD values decreased from day five to day 14. For instance on day five, coho sampled at South Channel had a mean EROD value of 5.63 pmol/mg protein/minute but by day 14 this value had significantly decreased to 3.18 pmol/mg protein/minute.

During Series 2 this temporal trend of decreasing EROD values continued as values collected on fish sampled on day five were consistently higher than those obtained on fish held for 14 days. The ANOVA performed on these data also showed that on day five, fish from the North Channel site had higher EROD values than North Bay fish and that, in general, fish collected from inner harbor live boxes had greater EROD values (but not always significantly so) than those located elsewhere. On day 14, fish from the Wishkah site had the highest EROD values and these were significantly different than those obtained from coho held at the Montesano location. All other populations had relatively similar values.

Why decreasing levels of EROD were detected in fish held at

North Channel, South Channel, and Wishkah from the beginning of Series 1 through the end of Series 2 is unknown. A number of factors, such as salinity, tidal mixing effects, multiple sources of contaminants and changes in contaminant concentration, may individually or in combination have influenced the level of EROD activity in coho salmon during the live box study.

In summary, the EROD assay results showed that xenobiotics capable of inducing cytochrome P-450 monooxygenease activities in the liver of coho salmon were present in inner Grays Harbor. However, it was not possible to determine sources of the xenobiotics from these data alone. Interestingly, the five-day values obtained on fish held in the three inner harbor sites during Series 1 (North Channel 6.78; South Channel 5.63; and Wishkah 3.25 pmol/mg protein/minute) were comparable to the activity levels found in fish exposed to 5% Weyerhaeuser effluent for five days (4.9 ± 1.1 pmol/mg protein/minute).

Survival in Seawater

During Series 1 and 2, 100 fish were removed from the live boxes on days five, nine, and 14 (Table 3.5). These fish were transported to the NMFS's Manchester Field Station and released into 1.2 m by 2.1 m by 1.5 m deep nylon mesh net pens and reared for 19 (Series 1) or 16 (Series 2) weeks in seawater. Dead fish were removed daily and examined for bacterial pathogens and counts were made of the number of metacercarial cysts of the fluke *Nanophyetus salmincola* in the posterior third of the kidney.

As previously mentioned, coho held for 14 days at the Wishkah

Table 3.5 The dates Humptulips Hatchery coho were placed into their live boxes and subsequently transferred to the Manchester Field Station in 1989 for seawater rearing. Each group consisted of 100 fish.

Livebox Location	Day Livebox Filled	When Transfers To Manchester Occurred Day 5	Day 9	Day 14
		Series 1		
North Bay (a)	4/22	4/27	5/1	5/6
North Bay (b)	4/22			5/6
Wishkah	4/22	4/27	5/1	
Montesano	4/23	4/28	5/2	5/7
Elliott Slough	4/23	4/28	5/2	5/7
North Channel	4/24	4/29	5/3	
South Channel	4/24	4/29	5/3	5/8
		Series 2		
North Bay (a)	5/12	5/17	5/21	5/26
North Bay (b)	5/12	5/17		
Wishkah	5/12	5/17	5/21	5/26
Montesano	5/13	5/18	5/22	5/28
Elliott Slough	5/13	5/18	5/22	5/27
North Channel	5/14	5/19	5/23	5/28
South Channel	5/14	5/19	5/23	5/28

and North Channel sites could not be collected during Series 1. Moreover, at North Bay, predation by great blue herons was great enough to prevent complete replication of some treatments (Table 3.5). However, by pooling fish from both North Bay pens it was possible to collect single lots of 100 fish held for five, nine, and 14 days during each series.

The cumulative mortality of each group of fish collected during Series 1 and 2 are shown on Fig.s 3.25 and 3.26. In both series, fish held at the Montesano site died more rapidly and had lower survival values than any other group. Besides this clear trend, no association between seawater survival and live box location could be discerned. Furthermore, the length of time a fish was held, was not always correlated with how well it survived. For instance, in Series 2, fish held for five, nine, and 14 days at North Bay had 66%, 93% and 32% mortality rates, respectively. Plainly, whatever caused the fish to die at such disparate rates was not related to how long they were exposed to North Bay water. Similar temporal patterns of mortality were noticed in most other sites as well. Generally, fish survival in the net pens was low for many groups, regardless of where a live box was located. Moreover, the factors responsible for inducing low survival were never satisfactorily deciphered.

An important exception to the latter point occurred in the Montesano groups. These groups died before the onset of a natural outbreak of *Vibrio*, which began around week ten for Series 1 and during week seven for Series 2 (Figs. 3.25 and 3.26). Autopsies

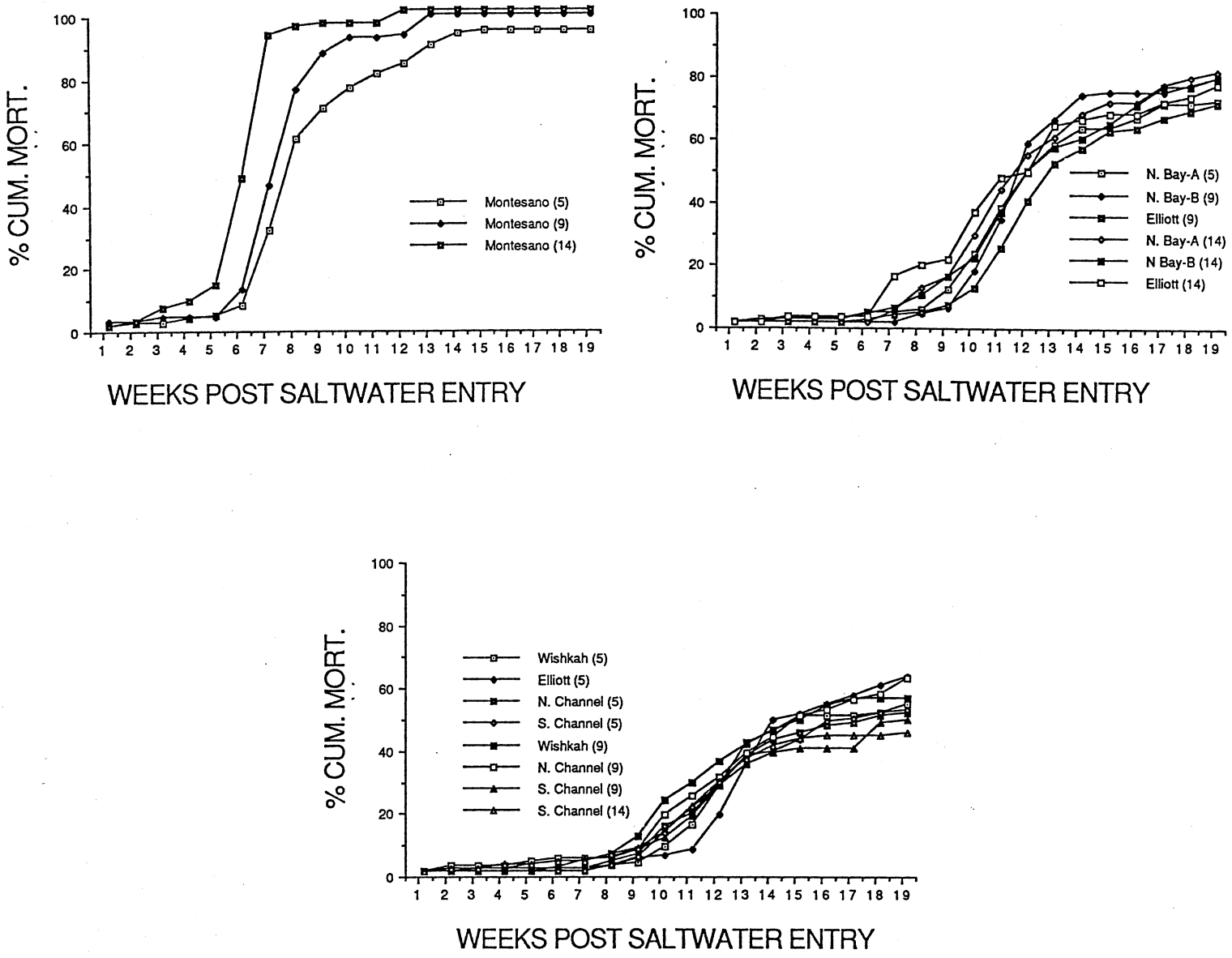


Fig. 3.25 Cumulative percent sea water mortality of Humptulips Hatchery coho held in live boxes anchored in the Chehalis River (Montesano), the inner harbor (Elliott, Wishkah, S. Channel, and N. Channel), and North Bay for varying periods of time during Series 1. After residency in their live boxes all groups were transfered to the Manchester Field Station and reared in sea water netpens. A natural out break of *Vibrio* occurred during rearing week ten.

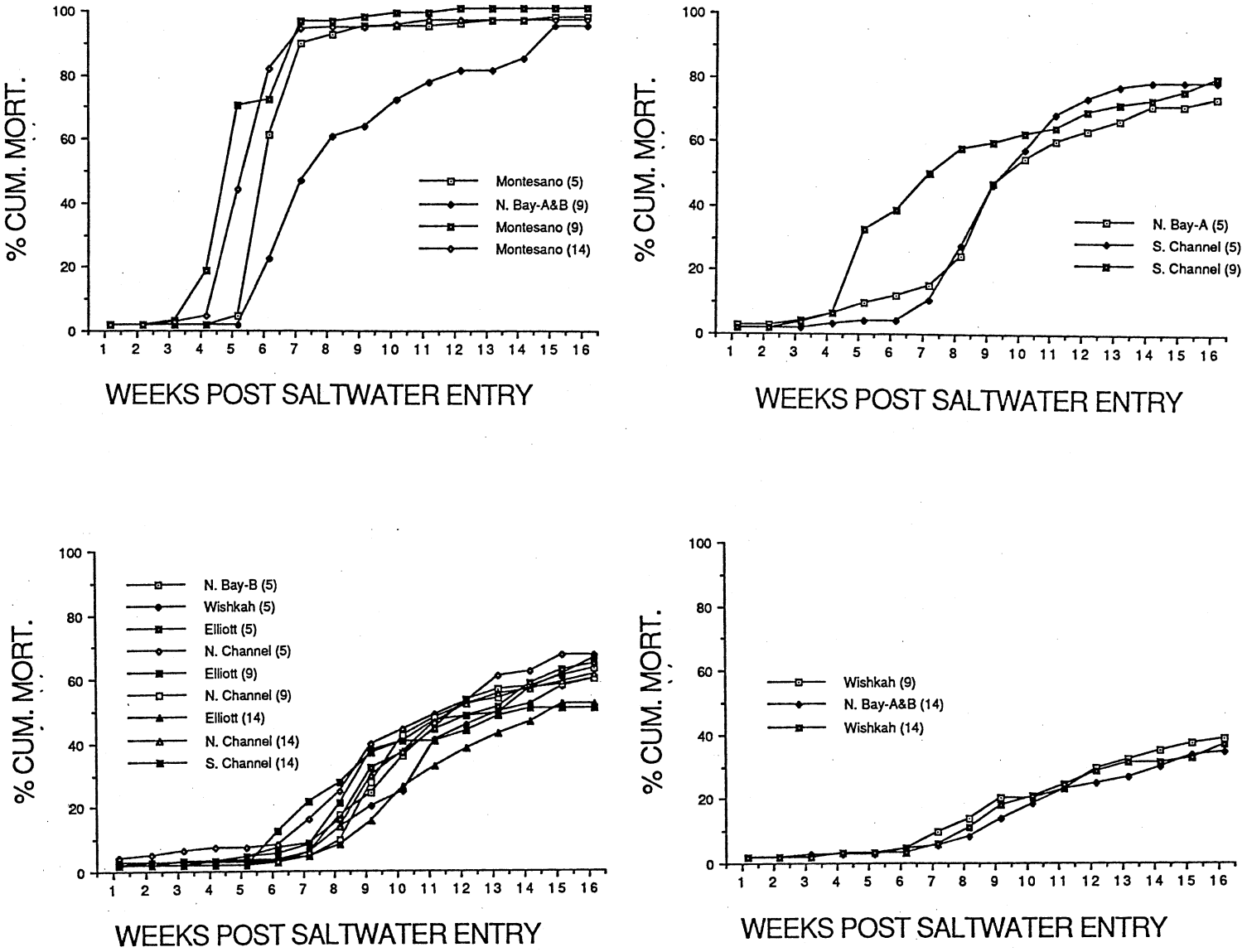


Fig. 3.26 Cumulative percent sea water mortality of Humptulips Hatchery coho held in live boxes anchored in the Chehalis River (Montesano), the inner harbor (Elliott, Wishkah, S. Channel, and N. Channel) and North Bay for varying periods of time during Series 2. After residency in their live boxes all groups were transfered to the Manchester Field Station and reared in sea water netpens. A natural out break of *Vibrio* occurred during rearing week seven.

performed on the fish disclosed that they had numerous *Nanophyetus* metacercaria in their kidneys. When the average number of kidney cysts found in mortalities from all the groups was plotted against mortality rates (Figs. 3.27 and 3.28) a positive relationship was seen. In Series 1 for instance, the mortality rate of groups that averaged less than 30 cysts in their kidneys was $\leq$ 20% for the first ten weeks of seawater residency. As cyst numbers increased, mortality rates rose until they approached 100%. The same relationship occurred in Series 2, except here a threshold of approximately 40 to 80 cysts was needed before mortalities significantly increased.

When coho used in the live boxes were collected from the Humptulips Hatchery they had few *Nanophyetus* cysts. Those placed in saltwater locations were largely protected from any additional infestations since the first intermediate host of this parasite is *Juga plicifera*, a freshwater snail. As mentioned in Part II (Hypothesis 2), infected snails release cercaria that passively move with water currents until they encounter a juvenile salmonid or other appropriate host animal (Gebhardt et al. 1966). Once contact has been made, the cercaria penetrate the integument and form metacercarial cysts in a variety of body tissues. The Montesano site was the only live box location where abundant populations of *Juga* were observed. Indeed, the longer the fish were held at this location the greater the number of metacercarial cysts were found in their kidneys.

The autopsy data made it clear that coho held at Montesano

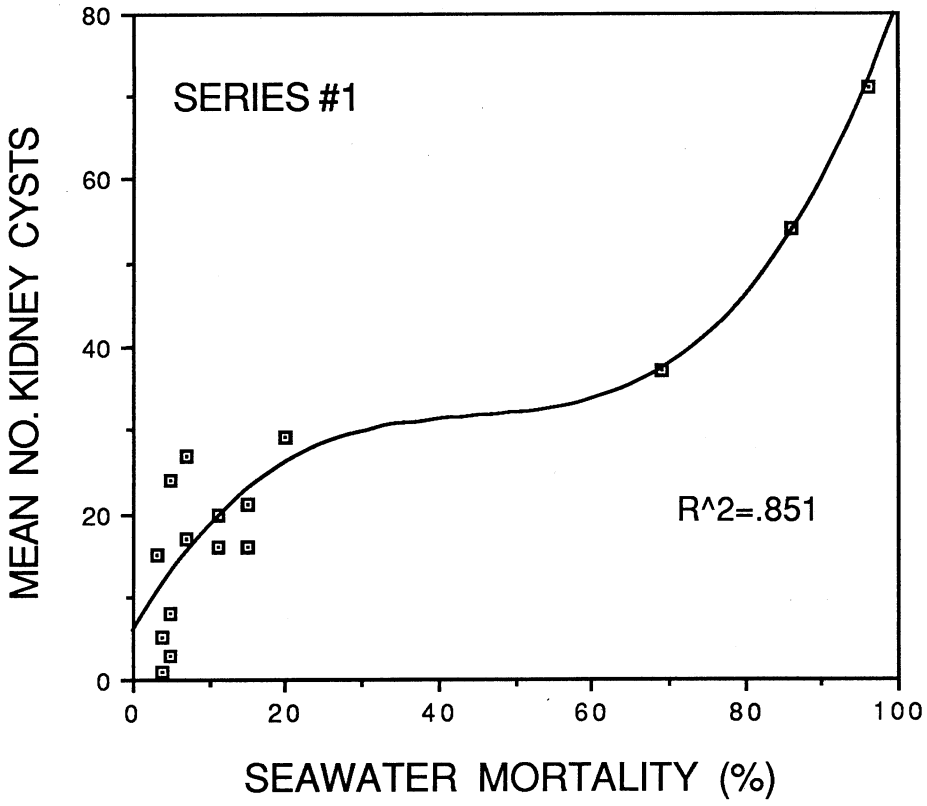


Fig. 3.27. The relationship between seawater mortality and degree of *Nanophyetus* infestation in hatchery coho held in live boxes during Series 1.

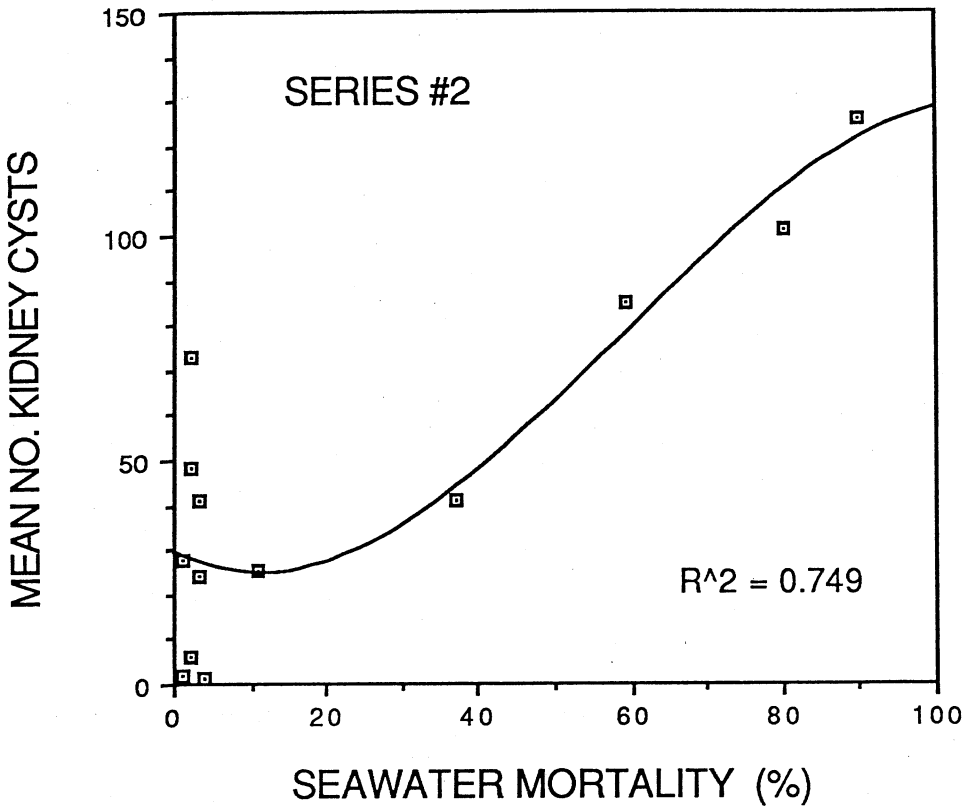


Fig. 3.28. The relationship between seawater mortality and degree of *Nanophyetus* infestation in hatchery coho held in live boxes during Series 2.

received additional *Nanophyetus* cysts and that these cysts apparently affected their survival in seawater. Circumstantial evidence from other investigators supports the notion that *Nanophyetus* infestations may affect the marine survival of salmonids. Weiseth et al. (1974) showed that only 18% of ocean-caught coho salmon had more than 24 *Nanophyetus* cysts in their kidneys. Cysts are known to survive for at least 2 years (Farrell et al. 1964) and counts averaging many hundreds per kidney have been found in out-migrating coho smolts (Bennington and Pratt 1960). However, *Nanophyetus* cysts have rarely been looked for in adult coho kidneys, and therefore it is not possible to state whether there is an upper limit to the number of metacercaria that coho can successfully tolerate. Moreover, other evidence suggests that the number of cysts a fish possesses may not always influence its survival. For example, wild coho smolts sampled from the Deschutes River, a southern Puget Sound stream, commonly had over 300 cysts/kidney (NMFS unpubl. data). Yet, for the past ten years the marine survival of these fish has ranged between 18 and 22%, a rate comparable to Puget Sound populations that are not parasitized by this fluke (Seiler per. comm.).

These observations can be reconciled if the affects of multiple stressors on marine survival are considered. It has long been known that the cumulative effects of sublethal stressors can lead to individual deaths and cause population declines (Vaughn et al. 1984; Adams et al. 1985; Wedemeyer et al. 1990). Plainly, being infested with high levels of *Nanophyetus* metacercaria must induce

stress. Coho held in the Montesano live boxes probably experienced additional stress because they were prevented from entering seawater (this was the only entirely freshwater site) and thus were liberated into the net pens at Manchester in a less acclimated state than any of the other groups. The collective effects of heavy parasitism and reduced pre-adaptation to seawater undoubtedly influenced the survival rates of these groups.

This reduced survival, prompted the development of a hypothesis that explains why fish leaving the Chehalis system survive at relatively low rates. First, we believe that coho from this system often enter the inner harbor estuary with high infestations of *Nanophyetus* cysts. The data presented in Fig. 3.29 show that the mean numbers of cysts found in coho seined in the inner harbor generally exceeded 50, a level high enough to cause mortalities in the Montesano net pens when accompanied by additional stress. Second, the two *in situ* bioassays reported here, demonstrated that fish held in the inner harbor were often stressed by conditions they experienced. Thus, it appears likely that many coho are entering the inner harbor stressed by nanophyetiasis only to encounter additional stressors that cumulatively may cause death. Currently, portions of this hypothesis and the role that *Nanophyetus* infestation may play in the early marine survival of Grays Harbor coho salmon are being tested by NMFS and WDF researchers. The results of this work will be reported elsewhere.

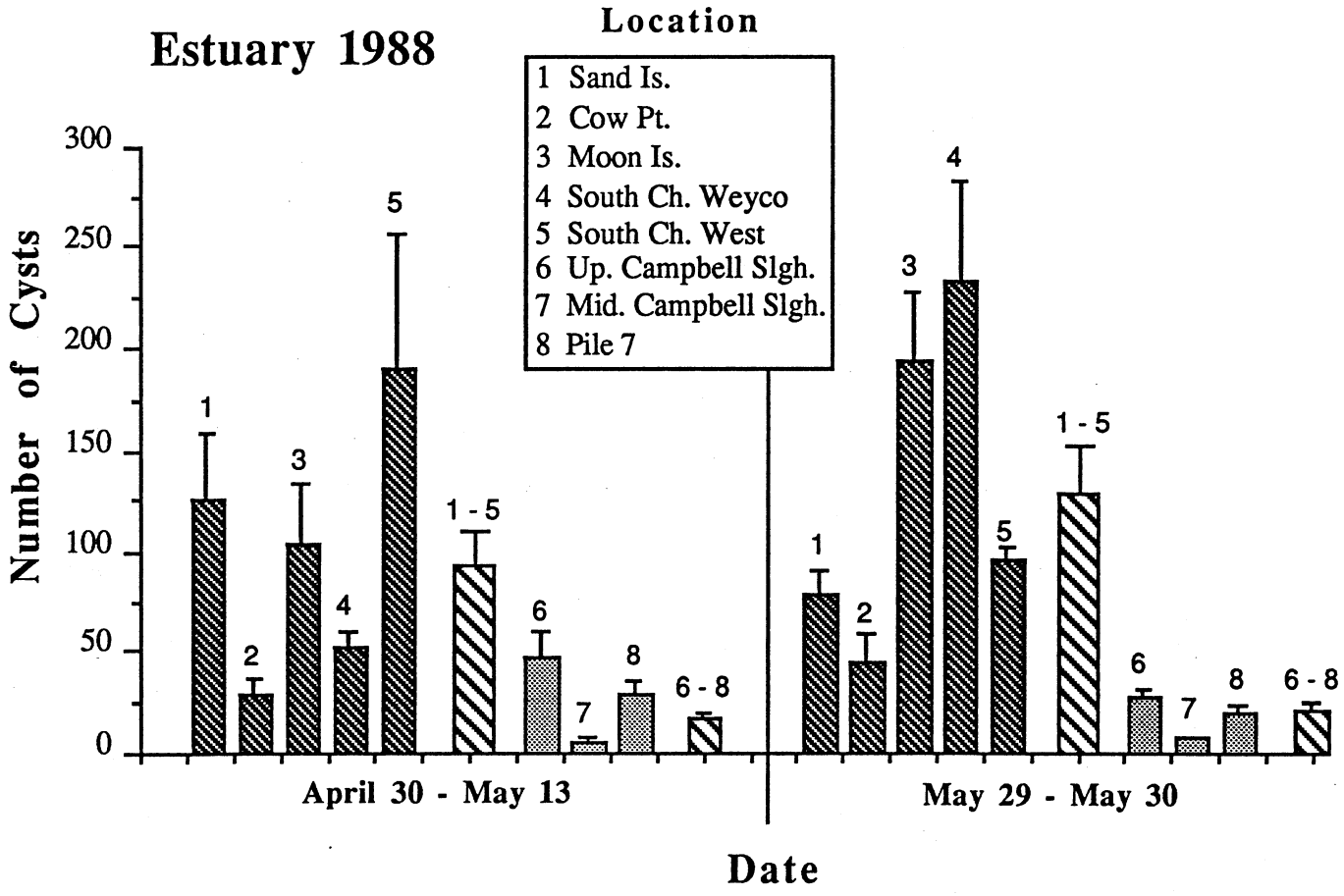


Fig. 3.29. Mean number of metacercarial cysts of *Nanophyetus salmincola* in the posterior kidney of migrant coho salmon juveniles captured by beach seine in the Chehalis estuary (fine-hatched bars) and in North Bay (shaded bars) from 4/30-5/30/88. The thick hatched bars represent area averages.

Continuous-Flow Bioassays

Introduction and Background

The *in situ* assays described above assessed how environmental conditions in the inner harbor affected smolting coho. These studies were not designed to test the effects of individual effluents that enter the inner harbor. To determine whether specific effluents have potentially toxic effects on juvenile coho, either static or continuous-flow bioassays can be used. In a static assay, fish are held in tanks with a known concentration of effluent for a set period of time. Depending on how long the assay lasts, static effluent baths may be periodically replaced. In continuous-flow bioassays, test organisms are constantly being exposed to new effluent at appropriate concentrations. Because the chemical constituents of the effluents we wished to appraise appeared to be dynamic, we decided to use continuous-flow bioassays. In these experiments, which were conducted in 1988 and 1989, smolting coho salmon were exposed to different concentrations of effluents for variable lengths of time.

Obviously, a wide variety of effluents from point and non-point sources enter the inner harbor. However, the major contributors to the inner harbor include the Chehalis River, the waste water streams from the pulp mills, and the discharge waters originating from the Aberdeen and Hoquiam sewage treatment plants (STP). Thus, these were the effluents or waters tested in the bioassays. After the fish had been exposed to these materials, they were transported to seawater rearing locations, subjected to

a variety of tests, and reared for up to nine months to assess the effects of effluent exposure.

Exposing Coho to Effluents

To continuously expose the fish to effluents, a tank farm complex was built at the City of Aberdeen's Sewage Treatment Plant. The complex possessed large storage tanks (four 22,800, two 11,400, and four 4,180 liter tanks) that delivered effluents and dilution water to 30 smaller (285 liter) holding vessels that contained fish. Since the delivery lines were equipped with flow valves and pressurized it was possible to deliver precise concentrations of effluent into each tank. Fresh loads of effluent were transported to the tank farm once or twice per day depending on the quantity needed. Moreover, effluents were collected as close as possible to where they normally would be discharged into the environment.

Effluent collected from the Weyerhaeuser pulp mill had to be neutralized with concentrated NaOH before it was introduced into the holding vessels since at the time of our investigation it was discharged with a pH of around 3. While the fish were being exposed to effluents, dissolved oxygen readings were routinely made in each holding vessel. In several instances, air was slowly bubbled through the fish holding tanks to maintain at least 6 ppm of dissolved oxygen throughout the exposure period.

The effluents, concentrations, and exposure times used in the 1988 and 1989 experiments are shown in Table 3.6. In 1988, effluents from the Weyerhaeuser and ITT Rayonier pulp mills (30%, 10%, and 3%), the Aberdeen and Hoquiam STPs (3%), and Chehalis

Table 3.6 The effluents tested and periods of exposure used during the continuous-flow bioassays conducted in 1988 and 1989. Equal proportions of effluents were combined and diluted to create the mixed effluent treatments.

1988 Treatments	Length of Exposure Day 5	Day 14
Dilution Water (controls)	x	-
12 µg/L, Cu^{++}	x	-
Weyerhaeuser Effluent		
3%	x	-
10%	x	-
30%	x	-
ITT Effluent		
3%	x	-
10%	x	-
30%	x	-
Aberdeen STP - 3%	x	-
Hoquiam STP - 3%	x	-
Chehalis River Water	x	-

1989 Treatments	Length of Exposure Day 5	Day 14
Dilution Water (controls)	x	-
Weyerhaeuser Effluent		
5%	x	x
10%	x	-
30%	x	-
ITT Effluent		
5%	x	x
10%	x	-
30%	x	-
Dilution Water (controls)	x	x
Weyerhaeuser/ITT Mixed Effluent		
5%	x	x
10%	x	-
30%	x	-

River water (100%) obtained about 3.5 km upriver of Montesano, were tested. Some groups of fish were also exposed to a reference toxicant ($\simeq$12 $\mu g/L$ Cu^{++}) and pure dilution water which was dechlorinated Wishkah River water. All fish were exposed for a period of five days which appears to be the average period of time coho spend in the inner harbor.

In 1989, only effluents from the two pulp mills were tested because neither Chehalis River water or waste waters from the two STPs had any detectable affect on the fish. Conversely, some effects due to pulp mill wastes were observed. As Table 3.6 shows, many treatments used in the 1989 assay repeated those that had been employed before. However, additional treatments were also included to determine how longer exposure times and mixtures of effluents might affect the fish. In both years, duplicates of all treatments were conducted and in the case of some controls, triplicates were run.

All coho used in the bioassays were from the Humptulips Hatchery. After being transported to the tank farm and loaded into their holding vessels, the fish were acclimated for three days before being exposed to effluents. The fish used in both years were comparably sized, even though the 1989 bioassay was performed almost a full month earlier than the one conducted in 1988. The 1989 bioassay was started earlier in an effort to test fish at the peak of smoltification since those used in 1988 appeared to be reverting back to parr at the time they were tested.

Tests Used to Evaluate the Effects of Effluent Exposure

After being exposed to effluents, fish were placed into seawater rearing containers at the Marrowstone Field Station (USFWS) or saltwater net pens at Manchester (NMFS). At these locations, a number of physiological screenings, challenges and rearing studies were carried out to assess how the various treatments affected the fish (Table 3.7).

Health Assessments. Three types of fish health assessments were conducted in 1988. First, just before the fish were placed into seawater, comprehensive histological examinations were made on brain, eye, gill, pseudobranch, skin, muscle, liver, pancreas, spleen, kidney, gonad, gall bladder, and swim bladder tissues. Four additional histological examinations occurred over the next seven months while the fish were reared in seawater. Second, ten-fish samples were necropsied immediately after the bioassay and 1, 2, 4, 8, 12, and 24 weeks after conversion to seawater. Goede's (1988) necropsy-based fish health/condition methodology was used to make these appraisals. And third, each fish that died was autopsied in an effort to determine the cause of death.

Besides these tests, the U.S. Environmental Protection Agency funded the USFWS to conduct some additional tests on blood and tissue samples obtained from the necropsied fish. These analyses, which included such examinations as plasma glucose, sodium, potassium, chlorine, lactate dehydrogenase (LDH), alanine amino transferase (AAT), protein, hematocrits, hemoglobin, percent immature red cells, differential white cell count, leukocytes per

Table 3.7 The assays used to measure responses of smolting coho salmon held in effluents during the 1988 and 1989 continuous-flow bioassays.

Type of Assay Conducted	Year 1988	1989
Physiological Condition		
Sea Water Challenge	x	x
ATPase	x	x
Disease Challenge		
Natural	-	x
Induced	x	-
Health	x	x
Growth	x	x
Survival	x	x
Blood Chemistry	x	-

hundred erythrocytes, interrenal ascorbic acid, and hepatic glutathione, were conducted to detect sublethal effects caused by exposure to the tested effluents. The results of these tests are not presented here but will be included in a separate report to the EPA.

In 1989, fish health and condition were assessed by performing Goede's (1988) necropsy based evaluation procedure on the fish that survived the entire nine month seawater holding period. In addition, all dead fish were autopsied to determine their cause of death.

Osmoregulation. The osmoregulatory competence of fish exposed to different effluents in 1988 and 1989 was evaluated by performing 24-hr seawater challenge tests and by analyzing gill APTase activity levels. The seawater challenge tests occurred immediately after the fish had been transported to the Marrowstone Field Station. In these tests, ten coho from each group were placed into 29.0 ‰ seawater for 24 hrs. After which, blood plasma samples from surviving fish were analyzed to determine their Na^+ ion concentrations (Clarke and Blackburn 1977; Blackburn and Clarke 1987). Gill tissues were collected from the same fish to obtain ATPase values. In 1988, additional gill ATPase values were procured by sampling fish from each group after they had been in seawater for one month.

Vibrio Challenge. In 1988, the disease resistance of fish exposed to effluents was assessed using a *Vibrio* challenge conducted under laboratory conditions. Fish from each group were

exposed to 1.5 x 10^6 Vibrio bacteria/ml and monitored for 23 days. In 1989, the *Vibrio* challenge was accomplished at the Manchester Field Station by placing fish in seawater net pens and allowing them to experience a natural outbreak of *Vibrio*. Dead fish were removed daily and autopsied during both of these challenges.

Growth and Mortality. In both years the mortality and growth of fish held at the Marrowstone Island facility was monitored throughout the entire saltwater holding period. Fish survival was examined daily, with mortalities removed as soon as they were observed. In 1988, growth was measured monthly by weighing the fish in each tank in groups of 40, in a tared 19 *L* bucket, partially filled with water. In 1989, each surviving fish was individually measured and weighed at bimonthly intervals.

Evaluating Stress. Stress determinations were not made in 1988, however, in 1989 we measured plasma cortisol titers and the immunocompetence of fish removed from the following bioassay treatments:

Bioassay Treatment	Concentration (vol:vol)	Exposure (days)	Date Exposure Began
Wishkah R. water, control	100%	4 & 12	4/21 (both)
Weyerhaeuser Effluent (Weyco)	5%	5 & 13	4/21 (both)
ITT Rayonier Effluent (ITT)	5%	5 & 13	4/21 (both)
Weyco 2.5% + ITT 2.5% (Mix)	5%	5 & 14	4/30 & 4/21

Because imunocompetence assays are very labor intensive it was not possible to sample all the groups on days five and 14. Hence, it was necessary to sample some fish after 4, 5, 12, 13, or 14 days of exposure. For the sake of readability however, these populations

will be referred to as having had either 5 or 14 day exposure periods.

The methods used to determine cortisol levels and immunocompetence were identical to those described elsewhere. Additionally, the analytical tactic of transforming the resulting cortisol titer and immune response data into natural logarithms to improve their homoscedasticity was followed. And as before, ANOVA's and multiple range tests were used to determine whether differences existed between: 1) pre-treatment fish (Humptulips Hatchery coho that had been sampled one day prior to the onset of the continuous-flow bioassay) and treated individuals, and 2) control populations and fish that had been exposed to effluents.

Mixed Function Oxidase. Mixed function oxidase tests were conducted during 1988 and 1989. As discussed previously, this assay is used to assess cytochrome P-450 activity which increases when an animal is exposed to a variety of chemical contaminants.

In 1988, fish exposed to 30% Weyerhaeuser and 30% ITT Rayonier pulp mill effluents, 3% Hoquiam STP, 3% Aberdeen STP waters, Chehalis River and dechlorinated Wishkah River waters were examined. Eight fish from each of these treatments were collected after five days of exposure. Because livers from smolting coho are small, two fish were pooled to create one sample. The hepatic aryl hydrocarbon hydroxylase (AHH) activity in each sample was determined using methods described by Collier et al. (1986). Tests were initially conducted to confirm that under standard assay conditions AHH activity was linear with time and increased with the

protein concentration in a microsomal suspension. Both of these conditions were met, and it was also determined that 25° C was the optimal assay temperature for this species.

The numbers obtained from each sample represented the picomoles of benzo[a]pyrene (BaP) metabolized per milligram of microsomal protein per minute. A one-way ANOVA was performed on these data to determine if any of the treatments elicited higher AHH activities than control populations.

During the 1989 assay, measurements of mixed function oxidase activities (AHH and EROD) were made on coho exposed for five days to 5%, 10%, and 30% concentrations of pulp mill effluents from the Weyerhaeuser and ITT plants. Fish exposed to Weyerhaeuser and ITT effluents that had been mixed in equal volumes to give 5%, 10%, and 30% effluent treatments were also tested after five days of exposure. Finally, coho held for 14 days in 5% Weyerhaeuser, 5% ITT, and 2.5% Weyerhaeuser/2.5% ITT effluent were assayed along with suitable control populations which were held in dechlorinated Wishkah River water.

At each sampling period, livers from 16 coho were collected and placed into four composite samples. The AHH response in the sampled livers was ascertained as in 1988. In addition, hepatic EROD activity was evaluated by using the fluorometric method of Prough et al. (1978). Results obtained from this latter assay showed that there was a dose-response for the induction of EROD activity in fish exposed to Weyerhaeuser effluent. A similar response did not occur in the AHH assays which suggests that the

EROD test is a more responsive and sensitive measure of cytochrome P-450 induction than the AHH assay in coho smolts exposed to pulp mill effluents. Consequently, only the results of the EROD assays will be used to characterize the outcomes of the mixed function oxidase tests performed in 1989.

As in 1988, one-way ANOVAs were used to test for differences among treatment groups and between these groups and their control populations. When these analyses rejected the null hypothesis, Fisher's protected least significant difference test (Dowdy and Wearden 1983) was used to determine which populations differed from one another.

Water Quality Assessments. A suite of water quality assessments were made while the continuous-flow bioassays were being conducted. These tests, which are described in Part III, were carried out to discover what major classes of pollutants might be present in the tested materials, and to help explain any physiological effects exhibited by the fish.

Results of Assessments

Health Assessments. The histological examinations conducted in 1988 revealed that all treatment populations were infected with *Myxobolus* sp. in the brain, and *Nanophyetus* and *Renibacterium salmoninarum* in the kidney, both before and after being placed into seawater (Table 3.8). Fish exposed to the highest concentrations of Weyerhaeuser and ITT effluents had the highest incidences of epithelial degeneration, epitheliocystis, *Nanophyetus* infestation of the kidney, and *Myxobolus* infection of the brain immediately

Table 3.8 Results of the histopathological analyses made on juvenile coho salmon exposed to effluents for five days during the 1988 continuous-flow bioassay.

	Tests Made One Day After Effluent Exposure					
	Gill		Kidney		Eye	Brain
Treatment	ED	EC	Nano	BKD	Cor	Myxob
Weyco - 30%	8/10*	7/10	10/10	0/10	4/10	6/10
ITT - 30%	8/10	10/10	6/10	1/10	3/10	3/10
Cu^{++} - 12µg/L	7/10	0/10	7/10	4/10	1/10	6/10
Control	4/10	2/10	5/10	1/10	3/10	1/10

	Tests Made Thirty Days After Effluent Exposure					
	Gill		Kidney		Eye	Brain
Treatment	ED	EC	Nano	BKD	Cor	Myxob
Weyco - 30%	5/10	0/10	7/10	5/10	6/10	2/10
ITT - 30%	0/10	0/10	6/10	1/10	3/10	5/10
Cu^{++} - 12µg/L	3/10	0/10	10/10	3/10	4/10	3/10
Control	0/10	0/10	6/10	2/10	2/10	5/10

* = Number of fish testing positive/number examined
ED = Epithelial degeneration
EC = Epitheliolcystis
Nano = *Nanophyetus salminocola*
Cor = Pathological changes in the cornea or corneal epithelium
Myxob = *Myxobolus kisutchi*

after the bioassay. However, only epithelial degeneration in fish exposed to 30% Weyerhaeuser effluent distinguished the effluent-exposed fish from the controls after one month of seawater residence.

In 1988, many of the fish that were necropsied after seven months in seawater had enlarged spleens and swollen kidneys, symptoms that are commonly, but not exclusively, associated with bacterial kidney disease (BKD). Additionally, those that had been exposed to 30% Weyerhaeuser effluent commonly had the lowest percent normal pseudobranchs, thymus glands, spleens, and kidneys. These findings suggest that the fish were in especially poor health, possibly as a result of their effluent exposures (Table 3.9). Similar results were not observed in fish exposed to 30% Weyerhaeuser effluent in 1989 (Table 3.10). This lack of consistency isn't surprising since the extent to which the physical appearance of an organ after nine months can be attributed to effluent exposure is unknown. Moreover, because the chemical composition of the effluents are known to be variable the fish may have been responding to disparate effluent mixtures.

Histological examinations conducted on live fish and autopsies of dead fish indicated that the coho used in 1988 and 1989 were in generally poor health throughout the bioassays. It is possible that the fish were in poor health at the time they were collected from the Humptulips Hatchery. However, this seems unlikely because the health evaluations performed in 1987 and 1988 (see Part II, Hypothesis 2) suggested that hatchery fish were in reasonably good

Table 3.9 Necropsy data collected from juvenile coho salmon exposed to effluents for 5 days in May 1988, transferred to seawater, and reared for an additional seven months. Condition factor (Ktl) = wt(g) x 10^5/l(mm)^3. CV = coefficent of variation. N = the number of fish examined.

Treatment	N	$\bar{L}$(mm)	L_{cv}	$\bar{W}$(g)	Wt_{cv}	$\bar{K}tl$	Ktl_{cv}	Percent Normal							Means		
								Eyes	Gills	Pseudo-branchs	Thymus	Spleen	Hind Gut	Kidney	Liver	Thymus	Mesentery Fat	Hind Gut
Control	83	184	15.3	77.7	56.8	1.116	14.3	86	100	98	95	65	46	93	94	0.05	1.4	0.54
Chehalis River (100%)	65	192	16.5	92.6	53.5	1.171	11.9	85	100	99	91	52	42	95	97	0.12	1.7	0.58
Cu^{++} (12 µg/L)	60	185	15.8	80.8	58.3	1.145	14.7	85	100	97	83	60	65	87	93	0.25	1.5	0.35
Weyco - 30%	19	200	11.7	95.2	42.3	1.132	14.2	84	100	90	79	53	58	84	95	0.26	1.6	0.42
- 10%	53	180	14.4	73.7	52.2	1.157	13.8	77	98	94	92	60	83	89	94	0.08	1.6	0.17
- 3%	54	197	18.9	102.5	65.3	1.183	12.3	83	100	96	87	48	52	93	94	0.15	1.8	0.48
ITT - 30%	33	182	17.0	79.8	69.9	1.164	14.3	79	100	94	94	55	45	85	88	0.09	1.7	0.54
- 10%	65	188	15.4	86.1	58.1	1.174	13.9	82	100	94	88	40	35	88	94	0.14	1.8	0.49
- 3%	63	184	18.1	81.1	73.9	1.115	15.5	75	98	97	83	60	60	95	95	0.17	1.5	0.40
Aberdeen STP (3%)	38	195	18.2	93.6	58.2	1.123	11.3	79	100	100	79	50	47	92	95	0.29	1.4	0.53
Hoquiam STP (3%)	78	184	14.0	77.3	49.3	1.134	13.7	77	99	96	89	68	53	89	92	0.13	1.4	0.47

Table 3.10 Necropsy data collected from juvenile coho salmon exposed to effluents for 5 days or 14 days in April 1989, transferred to seawater, and reared for an additional nine months. Condition factor (Ktl) = wt(g) x 10^5/l(mm)^3. CV = coefficent of variation. N = the number of fish examined.

								Percent Normal								Means		
Treatment	N	$\bar{L}$(mm)	L_{cv}	$\bar{W}$(g)	Wt_{cv}	$\bar{K}tl$	Ktl_{cv}	Eyes	Gills	Pseudo-branchs	Thymus	Spleen	Hind Gut	Kidney	Liver	Thymus	Mesen-tery Fat	Bile
Five-Day Control	37	218	15.7	125.4	62.7	1.100	16.1	89	100	100	84	92	100	89	100	0.22	1.3	1.6
Weyco - 30%	47	201	15.5	98.9	53.3	1.096	12.3	87	100	100	94	89	100	94	92	0.06	1.3	1.8
- 5%	40	207	17.3	110.2	66.1	1.094	13.8	90	100	100	92	95	100	98	95	0.08	1.3	1.9
ITT - 30%	59	201	14.7	96.9	53.9	1.104	10.2	85	100	97	92	83	100	95	86	0.12	1.6	1.6
- 5%	43	209	15.2	111.4	49.7	1.285	14.7	81	100	98	86	88	100	91	93	0.16	1.6	1.7
Mixed Effluent Control	36	213	17.0	112.6	65.3	1.019	12.6	83	100	89	86	83	100	94	100	0.19	1.1	2.1
Mix - 30%	51	212	17.5	109.6	54.6	1.028	13.6	80	100	90	82	82	100	96	98	0.20	1.0	2.0
- 5%	44	212	16.2	109.5	56.4	1.040	10.5	86	100	98	95	86	100	98	100	0.04	1.1	1.9
Fourteen-Day Control	49	218	10.9	117.1	53.2	1.043	10.9	74	100	94	86	80	100	89	94	0.18	1.1	2.1
Weyco - 5%	31	211	17.6	109.6	63.2	1.030	16.1	68	100	94	84	81	100	90	97	0.13	0.8	1.9
ITT - 5%	60	211	14.3	104.5	48.4	1.032	9.6	75	97	95	87	95	100	95	98	0.15	1.3	2.1
Mix - 5%	46	211	14.2	103.2	48.8	1.026	12.9	85	100	89	91	76	100	96	98	0.11	1.2	2.0

health. On the other hand, it is certainly conceivable that being exposed to effluents contributed to the poor condition of the fish. This notion is consistent with the observation that a number of effluent treated populations, but not controls, were ailing when they arrived at the Marrowstone Field Station. Yet, after one month of residency at the field station the health of control fish also began to deteriorate, suggesting that unknown factors in the experimental protocol (especially over the long term) had a greater influence on the populations than any of the effluents. The validity of this idea is supported by the generally high mortality observed in all groups, especially in 1989.

Osmoregulation. Coho salmon that can maintain their blood Na^+ concentration at 165-170 meq/*L* or less after experiencing a 24-hr seawater challenge are considered to be fully smolted (Clarke and Blackburn 1977; Blackburn and Clarke 1987). None of the fish experiencing the seawater challenge test in 1988 achieved this level of osmoregulatory competence, even though every group exposed to effluents survived the test at or near 100%. Three weeks prior to this test, samples of coho from the Humptulips Hatchery were tested in a similar manner and these fish clearly were able to achieve desired Na^+ concentrations in their blood (Table 3.11). Thus, this test indicates that fish used in the bioassay were probably reverting back to parr at the time of the 1988 treatments.

In 1989, the bioassay was performed about one month earlier in an attempt to use fish at the peak of their osmoregulatory ability. The results of the seawater challenges, however, were similar to

Table 3.11 Mean plasma Na^+ concentrations and percent survival data collected on Humptulips Hatchery coho salmon smolts exposed to effluents in a bioassay and subjected to a 24-h seawater challenge in 1988. Fish from the Humptulips Hatchery were transported to the Marrowstone Island Field Station and challenged on 28 April. The bioassay fish were transported to the field station and challenged on 2 June. N = the number of fish examined.

Treatments	N	Survival (%)	Mean Na^+ (meq/L)	S.D.
Humptulips Hatchery	20	100	164.7	8.73
Bioassay fish				
Control	30	100	184.1	8.97
Chehalis River-100%	19	100	182.4	7.72
Cu^{++} (12 µg/L)	20	100	195.5*	8.70
Weyco - 30%	20	100	175.7	11.61
- 10%	19	95	177.0	6.49
- 3%	19	100	184.2	6.66
ITT - 30%	20	100	176.9	8.51
- 10%	20	100	177.6	9.00
- 3%	20	100	183.8	5.98
Aberdeen STP-3%	20	100	185.2	7.65
Hoquiam STP-3%	20	100	186.4	9.63

* Significantly different than the Humptulips Hatchery fish ($\alpha = 0.05$, Dunnett's test).

those obtained in 1988. Fish exposed to increasing effluent concentrations, prolonged exposure periods, or combinations of effluents regulated their plasma Na^+ to a lower level than did control populations (Table 3.12). Clearly, the fish exhibited a physiological response to the effluents; one that apparently did not impair their hypoosmoregulatory capacity.

Another way of assessing osmoregulatory competence in salmonids is to measure gill ATPase activity. As mentioned previously, the activity of this enzyme is expected to increase in the spring as the fish move through the smoltification process, eventually reaching a maximum once seawater adaptation has taken place. If a smolting fish is denied access to saltwater, its ATPase levels should decline as it reverts back to the parr stage. The ATPase values collected on coho sampled immediately after the seawater challenge tests in 1988 were low (Table 3.13) and indicated that both control and treated groups had apparently begun to regress back to the parr stage. However, once these fish had resided in seawater for a month, all groups had ATPase values that averaged around 60, which are typical of fully smolted salmon (Table 3.13). The ATPase values obtained from fish sampled from each treatment group in 1989 were slightly higher ranging from 7.9 to 11.6 (Table 3.14). Furthermore, as in 1988, none of the treatment groups differed from the control populations. No ATPase samples were collected after seawater residency had begun in 1989.

Vibrio Challenge. The results of the 1988 laboratory *Vibrio* challenge are presented in Table (Table 3.15). Some fish died in

Table 3.12 Percent survival and mean plasma Na^+ concentrations of Humptulips Hatchery coho salmon smolts exposed to pulp-mill effluents and subjected to a 24-h seawater challenge in 1989. Hatchery fish were transported to Marrowstone Island Field Station and tested on the dates indicated. The bioassay fish were tested on 28 April (Group 1) and 7 May (Groups 2 and 3). To create the mixed treatments, Weyerhaeuser and ITT pulp-mill effluents were combined in equal amounts and diluted as indicated. N = the number of fish challenged.

Treatment	N	Survival (%)	Na^+ (meq/L)	S.D.
Humptulips Hatchery Stocks				
Date tested				
3/29	10	100	171.2	13.092
4/04	10	100	175.3	11.023
4/11	10	100	165.1	5.493
4/19	10	100	169.5	10.757
4/25	10	100	168.0	7.788
Bioassay				
Group 1 (5 d)				
Control	30	100	178.8	7.024
Weyco - 30%	20	100	162.8	6.234
- 10%	20	100	168.4	5.767
- 5%	20	100	171.7	6.859
ITT - 30%	20	100	171.2	6.333
- 10%	20	100	172.8	8.462
- 5%	20	100	172.9	10.780
Group 2 (5 d)				
Control	20	100	171.7	7.589
Mix - 30%	20	100	159.6	7.833
- 10%	20	100	164.6	7.229
- 5%	20	100	168.8	9.028
Group 3 (14 d)				
Control	20	100	176.4	9.147
Weyco - 5%	20	100	164.8	8.424
ITT - 5%	20	100	165.4	7.601
Mix - 5%	20	100	160.4	8.000

Table 3.13 Mean gill ATPase activities of smolting coho salmon exposed to effluents for 5 days in May 1988. Some fish were assayed immediately after the completion of their bioassay while others were reared in seawater for 4 weeks at the Marrowstone Island Field Station and then tested. N = the number of fish tested.

	Immediately After The Bioassay			Four Weeks After The Bioassay		
Treatments	N	ATPase	S.D.	N	ATPase	S.D.
Control	30	5.6	1.0128	30	56.2	10.3911
Chehalis R. 100%	20	5.7	1.1691	20	57.6	8.8028
Cu^{++} (12 µg/L)	20	5.9	0.2666	20	53.3	6.4910
Weyco - 30%	20	6.4	1.5694	20	58.0	7.6665
- 10%	20	5.9	2.4947	20	59.0	7.8109
- 3%	20	7.1	1.6100	20	61.7	7.5119
ITT - 30%	20	5.7	1.9947	20	58.1	5.2257
- 10%	20	6.3	1.5129	20	59.1	7.1329
- 3%	20	5.9	1.4198	20	61.7	4.8885
Aberdeen STP - 3%	20	5.6	1.0375	20	61.7	9.4366
Hoquiam STP - 3%	20	4.4	1.4054	20	60.4	8.4693

Table 3.14 Mean ATPase activities of smolting coho salmon exposed to effluents in 1989. After their exposure period was completed the fish were transported to Marrowstone Field Station, where gill samples were taken within 2 days. Fish in group 1 were sampled on 4/28 while fish in groups 2 and 3 were sampled on 5/7. To create the mixed treatments, Weyerhaeuser and ITT pulp mill effluents were combined in equal amounts and diluted as indicated. N = the number of fish sampled.

Treatment	N	ATPase	S.D.
Group 1 (5 d)			
Control	30	10.3	3.067
Weyco - 30%	20	11.4	3.197
- 10%	20	9.1	2.604
- 5%	20	10.5	1.943
ITT - 30%	20	10.1	2.106
- 10%	20	10.5	2.839
- 5%	20	9.8	1.656
Group 2 (5 d)			
Control	20	9.5	1.836
Mix - 30%	20	11.6	3.217
- 10%	20	9.5	2.288
- 5%	20	10.3	2.591
Group 3 (14 d)			
Control	20	7.9	1.080
Weyco - 5%	20	9.3	2.772
ITT - 5%	20	8.6	2.129
Mix - 5%	20	11.1	3.851

Table 3.15 Disease resistance of smolting coho salmon juveniles exposed to effluents for 5 days in May 1988. After effluent exposure, the fish were transported to Marrowstone Island Field Station, and challenged with a high titer (1.5 x 10^6 bacteria per ml) of *Vibrio anguillarum*. Dead fish were counted daily for 23 d, and autopsied to determine the cause of death. N is the number of replicate groups (10 fish/replicate) tested.

Treatments	N	Total Mortality (%)	Total Mortality Attributed to BKD (%)
Control	3	17	13
Chehalis River			
- 100%	2	0	0
Cu^{++} (12 µg/L)	2	15	15
Weyco - 30%	2	5	5
- 10%	2	15	10
- 3%	2	0	0
ITT - 30%	2	10	5
- 10%	2	5	5
- 3%	2	30	30
Aberdeen STP			
- 3%	2	5	5
Hoquiam STP			
- 3%	2	5	5

most of the groups but none of the deaths could be attributed to *Vibrio*; instead autopsies showed that the fish had died from BKD. Apparently the virulence of the *Vibrio* culture used or other factors occurring at the time of the challenge interfered with this test. Because of the uncertainty of obtaining virulent cultures of *Vibrio*, 50 fish from each of the 1989 groups were exposed to a natural *Vibrio* outbreak by holding them in seawater net pens located at the Manchester Field Station. A *Vibrio* outbreak occurred about three months after the fish were transferred and as Table 3.16 indicates, the control populations suffered mortality rates that were either higher or comparable to the groups that had been exposed to effluents. The high mortality, over 70% in some groups, suggests that all generally had poor disease resistance.

Growth and Mortality. Monthly changes in mean body weight experienced by each of the 1988 treatment groups are presented in Table 3.17. After three months of seawater residency, the mean weights of control fish were the smallest of any of the groups. This trend persisted until the seventh or last month of the rearing period when they became slightly larger than one of the effluent-exposed groups. Conversely in 1989, fish held in control tanks ended up being the largest at the end of the nine month seawater rearing period (Table 3.18). In general, the growth data are difficult to interpret because all populations held at Marrowstone survived at such low rates. Perceived changes in mean weights may merely reflect differential mortality on variously-sized fish and not true differences in weight gain. Furthermore, the numbers of

Table 3.16 Disease resistance of smolting coho salmon exposed to effluents for 5 or 14 days in April, 1989. After effluent exposure, fish were transported to Manchester Field Station and held in seawater net pens for 5 months. During this period, they experienced a natural outbreak of *Vibrio*. Mortalities were counted daily and are shown as percentages. Fish in group 1 were transferred on 4/28 while those in groups 2 and 3 were transferred on 5/7. The mixed treatments were created by combining equal amounts of Weyerhaeuser and ITT effluent and diluted as indicated. Each replicate group (N) had 50 fish.

		Cumulative Percent Mortality				
Treatment	N	1-Mo (%)	2-Mo (%)	3-Mo (%)	4-Mo (%)	5-Mo (%)
Group 1 (5 d)						
Control	3	6	13	60	71	73
Weyco - 30%	2	1	3	42	54	60
- 10%	2	0	6	44	57	68
- 5%	2	2	6	42	54	62
ITT - 30%	2	2	5	39	50	59
- 10%	2	4	8	46	57	67
- 5%	2	3	10	53	65	72
Group 2 (5 d)						
Control	2	13	15	55	71	78
Mix - 30%	2	5	9	42	60	65
- 10%	2	2	3	44	58	65
- 5%	2	7	9	43	55	59
Group 3 (14 d)						
Control	2	2	18	57	67	72
Weyco - 5%	2	3	8	36	49	52
ITT - 5%	2	4	9	43	56	57
Mix - 5%	2	2	11	44	53	60

Table 3.17 Temporal changes in the mean weights of juvenile coho that had been exposed to pulp-mill or sewage-treatment effluents for 5 days in May 1988. After exposure to the effluents the fish were reared for seven months at Marrowstone Island in full strength seawater. Standard deviations are in parentheses beneath each weight value. N is the number of replicate populations.

		Sampling Dates							
Treatment*	N	6/13 Wt(g)	7/13 Wt(g)	8/14 Wt(g)	9/13 Wt(g)	10/13 Wt(g)	11/15 Wt(g)	12/14 Wt(g)	12/28 Wt(g)
Control	3	29.2	34.0	38.6	46.5	56.8	65.8	73.6	77.7
		(0.61)	(0.25)	(1.77)	(2.93)	(3.82)	(5.90)	(3.11)	(4.30)
Chehalis River -100%	2	28.6	34.0	38.2	49.3	63.4	76.4	88.7	93.6
		(0.28)	(0.42)	(0.99)	(2.05)	(3.11)	(3.46)	(14.57)	(18.95)
Cu^{++} Control (12 µg/L)	2	28.9	33.8	38.4	49.7	62.0	72.7	77.6	82.2
		(0.85)	(0.71)	(2.83)	(1.84)	(2.76)	(7.50)	(7.07)	(9.76)
Weyco - 30%	2	30.0	35.0	36.9	51.3	63.2	76.0	92.5	99.2
		(1.20)	(0.99)	(1.41)	(0.50)	(2.62)	(0.78)	(16.12)	(21.21)
- 10%	2	28.9	33.9	38.5	49.4	60.6	70.8	74.8	75.4
		(1.06)	(1.27)	(0.35)	(0.14)	(1.06)	(4.31)	(11.81)	(14.42)
- 3%	2	29.6	35.5	40.9	51.2	65.4	80.3	94.4	101.8
		(1.41)	(2.12)	(1.27)	(5.09)	(6.36)	(5.94)	(10.68)	(6.01)
ITT - 30%	2	28.5	34.1	38.4	47.8	62.2	71.1	80.2	90.4
		(0.35)	(0.07)	(0.71)	(2.33)	(7.92)	(7.57)	(18.60)	(29.20)
- 10%	2	29.2	34.8	41.7	52.0	65.8	75.1	81.4	85.7
		(1.27)	(0.64)	(2.33)	(2.90)	(4.45)	(2.05)	(6.29	(5.52)
- 3%	2	28.8	34.4	39.6	50.4	63.8	75.0	76.2	79.8
		(0.07)	(0.35)	(0.64)	(0.57)	(2.33)	(3.11)	(13.44)	(12.23)
Aberdeen STP	2	28.5	33.9	39.3	48.6	61.5	71.8	88.9	93.6
		(1.91)	(0.92)	(3.39)	(6.29)	(11.03)	(11.81)	----	----
Hoquiam STP	2	28.1	33.6	39.5	49.7	61.3	68.5	76.8	84.3
		(0.07)	(0.85)	(2.40)	(3.75)	(4.67)	(5.09)	(6.51)	(9.26)

* Both Sewage Treatment Plant effluents were diluted to 3%

Table 3.18 Temporal changes in the mean weights of juvenile coho exposed to pulp-mill effluents for 5 days (Groups 1 and 2) or 14 days (Group 3) in April 1989. The fish were reared in full-strength seawater for nine months at Marrowstone Island. Standard deviation values are in parentheses beneath each weight value and N is the number of replicate populations.

		Sampling Date					
Treatments	N	5/9 Wt(g)	6/8 Wt(g)	8/15 Wt(g)	10/12 Wt(g)	12/4 Wt(g)	1/17 Wt(g)
Group 1 (5 d)							
Control	3	27.8	35.8	58.8	76.6	99.3	126.2
		(0.45)	(0.28)	(7.28)	(15.6)	(2.67)	(8.88)
Weyco - 30%	2	27.1	36.4	55.9	73.0	86.4	98.9
		(0.11)	(1.09)	(0.42)	(4.59)	(9.45)	(5.70)
- 5%	2	27.8	36.5	56.2	68.7	89.0	110.2
		(0.12)	(0.28)	(5.28)	(3.03)	(2.38)	(5.72)
ITT - 30%	2	28.4	36.4	54.4	68.2	85.0	96.9
		(0.37)	(0.51)	(2.92)	(6.40)	(17.3)	(20.4)
- 5%	2	28.8	36.5	55.8	70.6	90.8	111.4
		(1.46)	(1.28)	(0.60)	(7.90)	(9.42)	(17.3)
Group 2 (5 d)							
Control	2	28.7	39.0	55.6	82.8	105.6	109.8
		(0.69)	(1.36)	(2.29)	(11.4)	(24.8)	(24.6)
Mix - 30%	2	28.6	38.5	56.9	79.7	99.4	109.3
		(0.58)	(0.04)	(3.49)	(7.30)	(10.2)	(6.97)
- 5%	2	29.0	39.1	58.2	80.2	99.4	112.6
		(0.32)	(0.10)	(0.93)	(1.21)	(0.58)	(14.0)
Group 3 (14 d)							
Control	2	27.4	38.1	61.2	86.4	112.2	119.9
		(0.08)	(0.28)	(2.82)	(18.5)	(5.67)	(7.35)
Weyco - 5%	2	27.0	36.6	55.6	78.4	86.9	94.8
		(0.17)	(1.06)	(5.52)	(17.0)	(29.2)	(28.1)
ITT - 5%	2	27.6	37.1	60.6	84.6	102.5	105.7
		(0.74)	(1.09)	(0.78)	(3.45)	(1.96)	(16.8)
Mix - 5%	2	27.1	37.2	56.8	78.0	97.5	96.7
		(1.21)	(3.43)	(4.74)	(9.75)	(15.3)	(21.2)

fish reared in a tank can influence how rapidly they grow. Unless fish densities are regularly adjusted, populations with initially high mortalities may actually grow more rapidly because of size-selective mortality and decreasing competition. In our assays, however, it was not possible to adjust population numbers because of the need to acquire mortality information.

In the 1988 assay, fish exposed to 30% Weyerhaeuser effluent had the highest, or close to the highest cumulative mortality throughout the entire seven month rearing period. Moreover, during the first month of the rearing period, fish exposed to 30% ITT Rayonier effluent and 12 $\mu g/L$ Cu^{++} died at higher rates than fish in their control populations. Unlike the Weyerhaeuser group, however, these trends were not statistically significant nor did they persist throughout the entire bioassay (Table 3.19).

Significant differences in mortality did not occur during the 1989 bioassay. For example, none of the mixed effluent groups, or populations held for 14 days had greater mortality rates than their control populations. Moreover, even though fish held for five days in 30% Weyerhaeuser effluent had slightly higher mortalities at the beginning of their seawater residence, their nine-month survival rate was not statistically different from any of the other populations. (Table 3.20).

As stated above the mortality rates of the groups tested in both years were high. In 1988, it ranged from 45% to 70% while in 1989 it varied between 70% and 84%. Not only do such high mortality rates complicate the interpretation of growth comparisons

Table 3.19 Mortality rates of juvenile coho salmon exposed to effluents for 5 days in May 1988 and reared in seawater for seven months at Marrowstone Island. N is the number of replicate populations.

		Cumulative Mortality			
Treatment	N	In the bioassay	Through SW conversion	Through 1 Mo in SW	Through 7 Mo in SW
Control	3	0.0	0.9	3.0	58.0
Chehalis River-100%	2	0.0	0.3	3.0	52.0
Cu^{++} (12 µg/L)	2	2.0	5.0	8.0	51.0
Weyco - 30%	2	2.0	8.0	14.0	70.0*
- 10%	2	0.5	2.0	4.0	54.0
- 3%	2	0.5	1.0	5.0	61.0
ITT - 30%	2	0.5	2.0	6.0	62.0
- 10%	2	0.7	1.0	4.0	54.0
- 3%	2	0.3	0.9	4.0	54.0
Aberdeen STP-3%	2	0.7	2.0	5.0	54.0
Hoquiam STP-3%	2	0.3	1.0	3.0	45.0

* Significantly different from the control fish (α = 0.05, Dunnett's test).

Table 3.20 Mortality rates of juvenile coho salmon exposed to effluents for 5 days or 14 days in April 1989 and reared in seawater for nine months at Marrowstone Island. N is the number of replicate populations.

Test group	N	Cumulative Mortality In the bioassay	Through SW conversion	Through 1 mo in SW	Through 9 mo in SW
Group 1 (5 d)					
Control	3	0.3	0.6	2.0	79.0
Weyco - 30%	2	2.0	3.0	5.0	79.0
- 10%	2	0.8	0.8	---	---
- 5%	2	0.0	0.5	3.0	78.0
ITT - 30%	2	0.4	0.4	0.9	73.0
- 10%	2	0.4	1.0	---	---
- 5%	2	0.5	0.5	2.0	77.0
Group 2 (5 d)					
Control	2	3.0	4.0	6.0	82.0
Mix - 30%	2	2.0	2.0	3.0	79.0
- 10%	2	0.4	0.8	---	---
- 5%	2	2.0	4.0	5.0	78.0
Group 3 (14 d)					
Control	2	2.0	2.0	4.0	76.0
Weyco - 5%	2	0.3	0.8	2.0	84.0
ITT - 5%	2	1.0	1.0	3.0	70.0
Mix - 5%	2	0.6	0.6	2.0	76.0

but they also may distort measurements of health and other parameters. Our attempt to evaluate fish health by necropsy, for instance, only reflects the physiological condition of the survivors. Such fish probably do not represent the physiological condition of those that died, i.e. dead fish may have had even lower incidences of normal pseudobranchs, thymus glands, spleens, and kidneys. Consequently, data gathered to measure long-term effects of exposure most likely reflect minimal impacts.

Cortisol and Immunocompetence. Plasma cortisol levels in coho sampled at the Humptulips Hatchery (pre-treatment specimens) and at the bioassay tank complex are shown in Fig. 3.30. Cortisol titers from all five-day effluent groups were not significantly different than those collected from Humptulips Hatchery coho 24 hrs prior to the onset of the assays. Furthermore, after being exposed to effluents for 14 days, the fish had significantly lower cortisol levels than they did on day five. Surprisingly, cortisol titers obtained from control fish sampled on day five were appreciably greater than those obtained on fish exposed to effluents. Indeed control fish held for five days had cortisol titers (85.2 ng/mL) that were nearly three times the level of pre-treatment fish (30.5 ng/mL). By day 14, however, the control fish had reduced their cortisol titers (14.4 ng/mL) to a level that was comparable to those observed on the other 14-day groups.

The immune responses of coho sampled at Humptulips Hatchery and the tank complex are presented in Fig. 3.31. As can be seen, all five day groups (except for the 5% mix group which lacked an

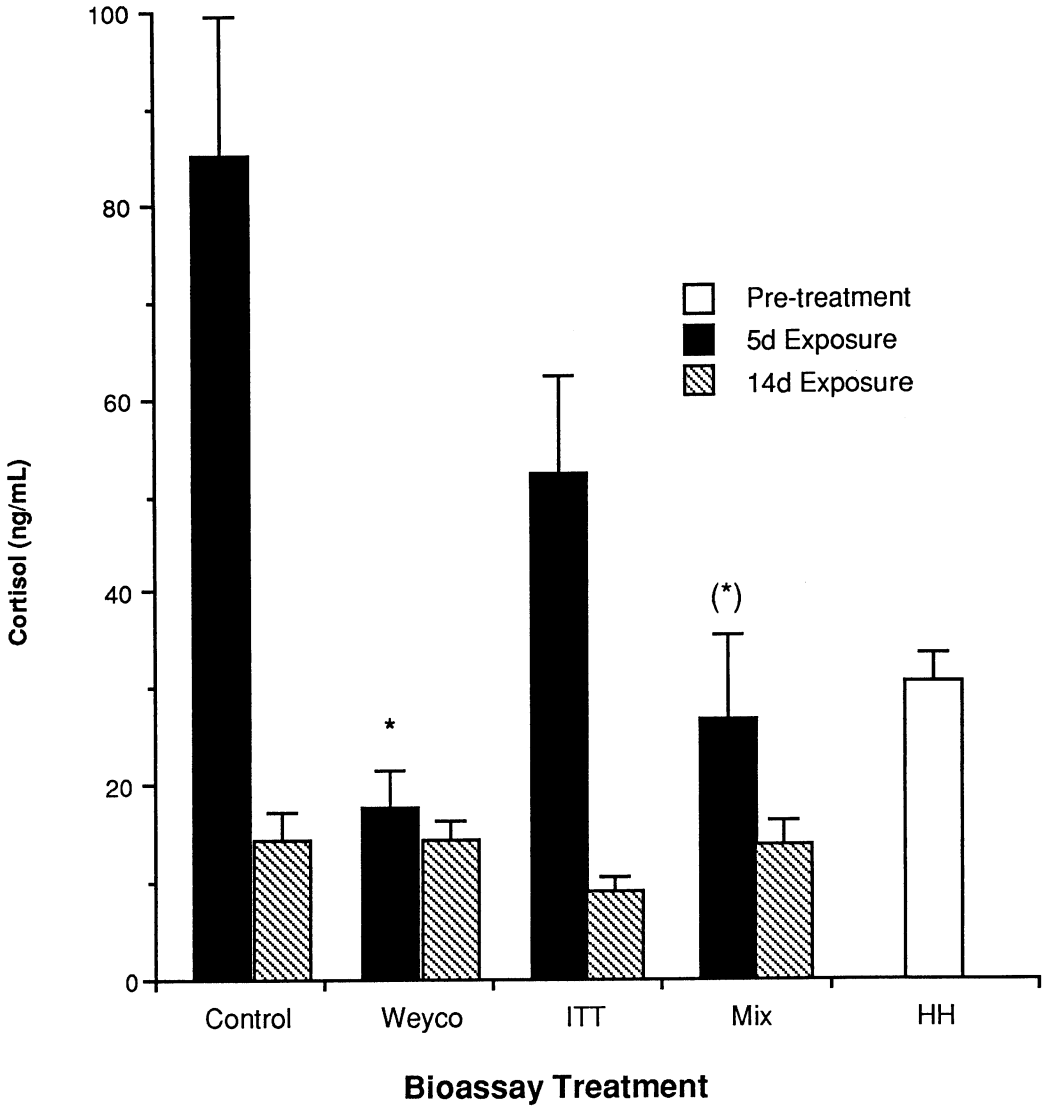


Fig. 3.30 Plasma cortisol concentrations (mean + S.E., n = 15) in juvenile coho salmon sampled before and after either a 5 or 14 day exposure to various effluents. Control = 100% Wishkah River water, Weyco = 5% Weyerhaeuser effluent, ITT = 5% ITT Rayonier effluent, Mix = 2.5% Weyerhaeuser + 2.5% ITT Rayonier, and HH = Humptulips Hatchery. Bars with an * had significantly different cortisol titers than their control population (the Mix treatment lacked an exact control).

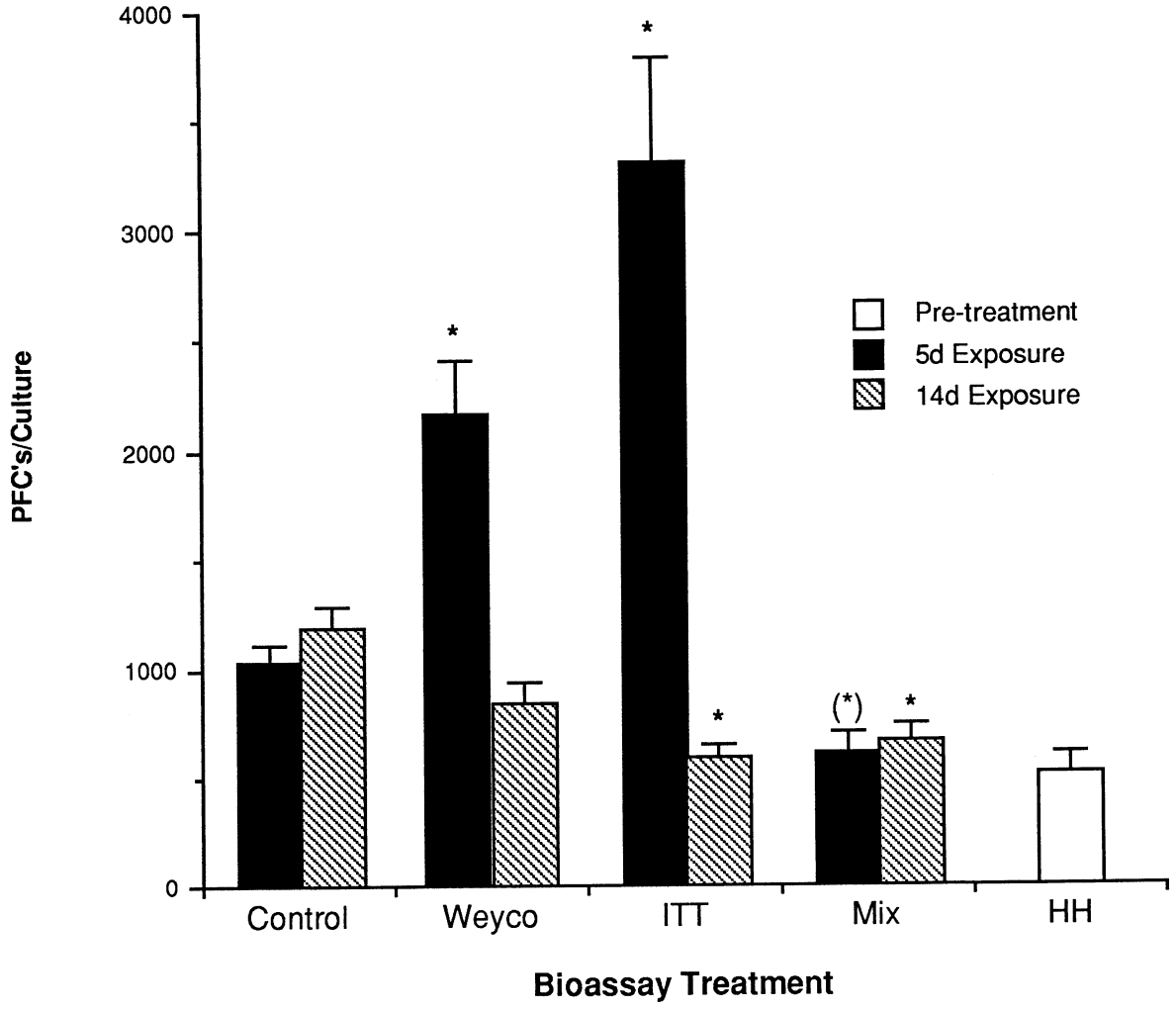


Fig. 3.31. Plaque-forming cell (PFC) response (mean + S.E., n = 15) in juvenile coho salmon sampled before and after either a 5 or 14 day exposure to various effluents. Control = 100% Wishkah River water, Weyco = 5% Weyerhaeuser effluent, ITT = 5% ITT Rayonier effluent, Mix = 2.5% Weyerhaeuser + 2.5% ITT Rayonier, and HH = Humptulips Hatchery. Bars with an * had significantly different PFC responses than their control population (the Mix treatment lacked an exact control).

appropriate control) had significantly more PFC's /culture than pre-treatment fish (516 PFCs/culture). Like cortisol, these responses dropped dramatically; in fact only the control population and fish exposed to 5% Weyerhaeuser effluent had significantly higher PFCs than the pre-treatment fish after 14 days of exposure. The most dramatic decreases in these values occurred in the 5% Weyerhaeuser and ITT populations. On day five these populations had significantly higher PFC counts (2158 and 3319 PFCs/culture, respectively) than the control population (1037 PFCs/culture) but on day 14 their PFC counts (Weyerhaeuser = 835 and ITT = 581 PFCs/culture) had fallen below those observed on control fish (1180 PFCs/culture). Fish held in the 5% mixture had PFC responses (620 and 671 PFCs/culture for 5 and 14 days respectively) that were consistently lower than those observed in the control population.

Collectively, these data suggest that a five-day exposure to Weyerhaeuser and ITT effluents elicits an enhanced immune response. However, the anomolously low PFC values in the pre-treatment fish and their correspondingly high cortisol titers on day five, make the data suspect. We believe the elevated cortisol values found in the control fish were caused by an inadequate post-transport adjustment period (3 days) and a lack of cover. The tanks used to hold the fish during the bioassays did not have solid covers. Hence, control fish, which resided in transparent rearing water could perceive routine movements made by staff while the bioassay was being conducted. We feel these movements initially stressed the control fish until they eventually became habituated to them.

Coho held in tanks supplied with pulp effluent were not stressed in this manner because the effluents stained the water in their tanks a dark, opaque brown.

In conclusion, a 14 day exposure to 5% Weyerhaeuser, 5% ITT or a 2.5%/2.5% mixture of these effluents does not appear to substantially change the levels of plasma cortisol or immunocompetence of Humptulips Hatchery coho. However, at the onset of the bioassay, control fish possessed high cortisol titers and depressed PFC values. Thus it is notable, that the PFC values in fish exposed to effluents declined over time, eventually becoming lower than those found in control fish.

Mixed Function Oxidase. In 1988, hepatic AHH activity was determined on coho exposed to 30% concentrations of Weyerhaeuser and ITT pulp mill effluents, 3% sewage treatment waste waters, Chehalis River water and dechlorinated Wishkah River water. The numbers obtained from these assessments are shown in Table 3.21 and are expressed in picomoles of benzo[a]pyrene (BaP) metabolized per milligram of microsomal protein per minute. A one-way ANOVA performed on the data demonstrated that fish exposed to 30% Weyerhaeuser effluent had higher levels of AHH than any of the other groups.

Generally, induction of AHH activity in fish is considered to be evidence of exposure to a variety of environmental contaminants, including compounds present in pulp mill effluents (Andersson et al. 1988; Lindstrom-Seppa and Oikari 1990). At this time, it is not possible to say which chemicals might have been responsible for

Table 3.21 Aryl hydrocarbon hydroxylase (AHH) activities in liver tissue of coho salmon exposed to pulp mill effluents for 5 d in May 1988. The fish were sampled immediately after their exposure period had been completed. AHH is measured in picomoles of benzo[a]pyrene metabolized per mg of microsomal protein per minute.

Treatments	AHH (pmol/mg protein/minute)	Standard Error
Control	12	3.6
Chehalis River water - 100%	25	1.2
Weyerhaeuser Effluent - 30%	45*	9.4
ITT Effluent - 30%	13	4.7
Hoquiam STP - 3%	16	4.4
Aberdeen STP - 3%	15	2.0

* Significantly different from the control (α=0.05).

the inductive effect of the Weyco effluent. Additionally, because not all potentially toxic contaminants will induce AHH activity, the lack of induction by the other effluents cannot be taken as evidence for their lack of toxicity. Some toxic compounds can actually decrease AHH activity, through such mechanisms as general hepatotoxicity.

Hepatic EROD and AHH levels were measured on all samples of fish collected during the 1989 continuous-flow bioassay. As indicated earlier, only the results obtained from analyzing EROD values will be described here. Table 3.22 presents the EROD activities in coho salmon exposed to serial dilutions of Weyerhaeuser and ITT effluents. The major effect observed was the significant induction of EROD activity by Weyerhaeuser effluent while effluent from ITT did not induce EROD activity after five days of exposure at any dose tested. Indeed, as Fig. 3.32 illustrates, coho smolts exposed to Weyerhaeuser effluent increase their EROD activity in a significant dose-response fashion.

When coho salmon were exposed to 5% effluents for 14 days, a significant increase in EROD activity was observed for fish exposed to Weyerhaeuser effluent while no increase was observed in fish exposed to 5% ITT effluent (Table 3.22). Thus, the results of both dose- and time-response experiments showed that Weyerhaeuser effluent is more potent than ITT effluent in the induction of cytochrome P-450 in coho salmon smolts.

No induction of hepatic EROD activity was observed in coho held for five days in various mixtures of Weyerhaeuser and ITT

Table 3.22 EROD (7-ethoxyresorufin O-deethylase) activities in hepatic microsomes of coho salmon exposed to pulp mill effluent in the 1989 continuous-flow bioassay.

Treatment	Length of Exposure	Number of Composites	EROD[a] (pmol/mg protein/minute)
Control	5 days	8	1.6 ± 0.5
Weyerhaeuser			
- 5%	5 days	4	4.9 ± 1.1
- 10%	5 days	4	9.5 ± 1.3*
- 30%	5 days	4	28.0 ± 7.2*,b
ITT Rayonier			
- 5%	5 days	4	2.6 ± 0.2
- 10%	5 days	4	2.0 ± 0.3
- 30%	5 days	4	5.1 ± 1.0
Mixed Effluents (Weyco + ITT)			
- 5%	5 days	4	2.3 ± 0.3
- 10%	5 days	4	3.5 ± 0.4
- 30%	5 days	4	5.5 ± 2.1
Control	14 days	4	2.5 ± 1.0
Weyerhaeuser			
- 5%	14 days	4	25.0 ± 7.0*
ITT Rayonier			
- 5%	14 days	4	1.6 ± 0.7
Mixed Effluents (Weyco + ITT)			
- 5%	14 days	4	25.0 ± 5.0*

[a] = Values given as mean ± sem.

[b] = Induction of AHH (aryl hydrocarbon hydroxylase) activity was observed only in coho salmon exposed to 30% Weyerhaeuser effluent.

* = Significantly different from corresponding controls

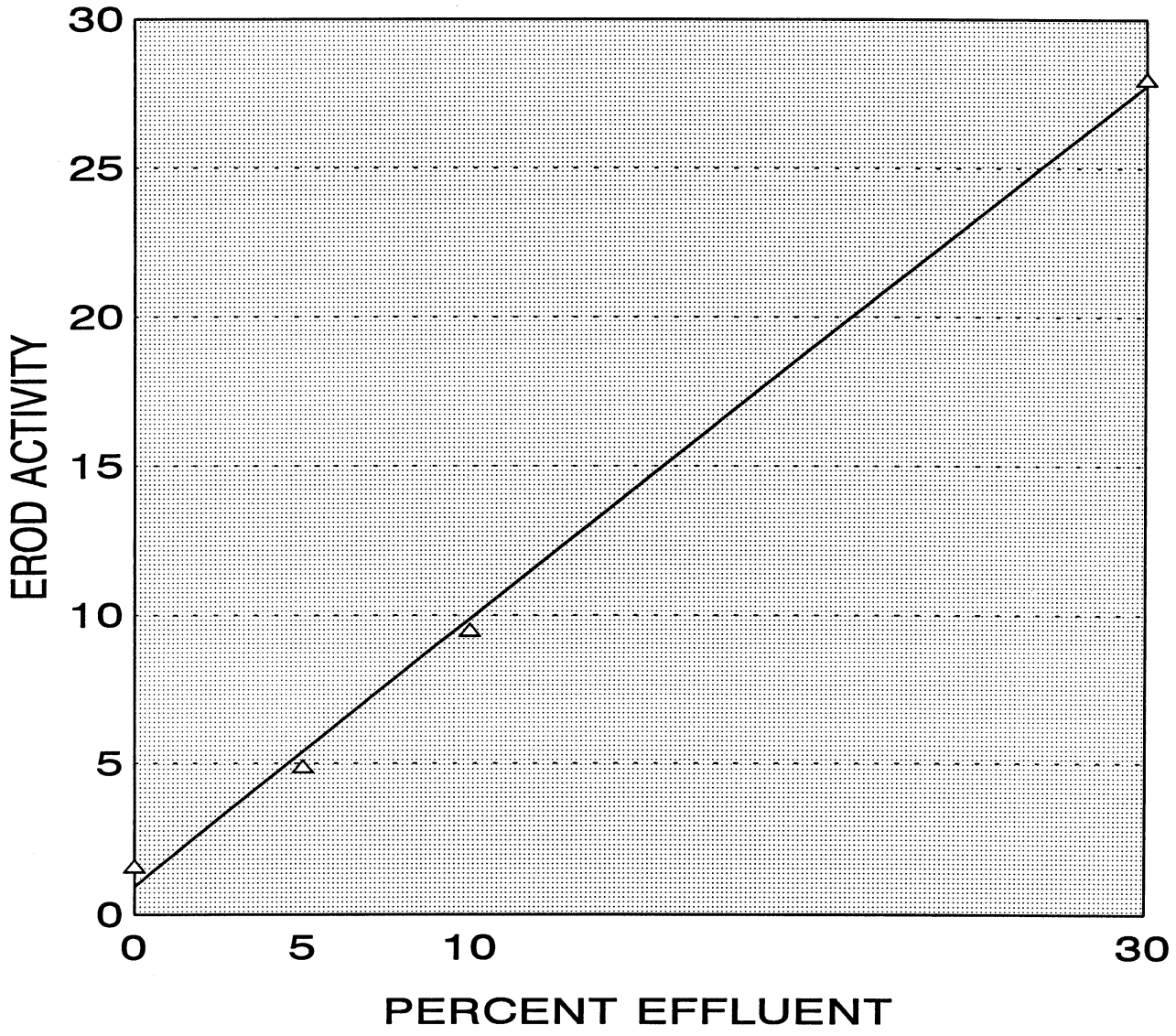


Fig. 3.32. Dose-response of hepatic 7-ethoxyresorufin-O-deethylase (EROD) activity in microsomes of coho salmon exposed to various concentrations of Weyerhaeuser pulp effluent for five days. The correlation coefficient (r) for this dose-response relationship equals 0.999.

effluents. These results indicate that ITT effluent delays the ability of Weyerhaeuser effluent to induce EROD production. Interestingly though, EROD activity was induced in fish exposed for 14 days to the 2.5%/2.5% mixture of the two effluents. In fact, the EROD activity of these fish was comparable to those exposed to 5% Weyerhaeuser effluent alone for 14 days and to fish exposed to 30% Weyerhaeuser effluent alone for five days (Table 3.22). These results cannot be readily explained without further information on the time course of EROD induction in coho exposed to higher concentrations of ITT and Weyerhaeuser effluent mixtures; however, the results do disclose that EROD activity is induced in animals placed into mixtures of pulp mill effluents.

In summary, the results of the mixed function oxidase tests performed in 1989 showed a significant dose-response induction of cytochrome P-450 activity in coho exposed to Weyerhaeuser effluents. Fish held in ITT effluents, however did not exhibit a similar dose-response. These results parallel those obtained in 1988, which revealed that AHH induction occurred in coho smolts exposed to 30% Weyerhaeuser but not 30% ITT effluent. Collectively, these findings demonstrate that the Weyerhaeuser effluent contained chemicals that induced hepatic EROD activity in coho salmon smolts, whereas the ITT effluent tested did not contain such compounds at biochemically significant concentrations.

Some Additional Considerations

As a juvenile salmon migrates through the Chehalis-Grays Harbor estuary it is likely to encounter a broad range of

acidities, salinities, temperatures, dissolved oxygen levels, and effluents in varying concentrations. All of these conditions are affected by river flows, tidal conditions and other factors and are thus difficult to recreate in an experimental setting.

Perhaps more importantly, some of these conditions may influence the toxicity of waste waters. Thus, controlled laboratory evaluations of effluents may over- or underestimate the effects of specific effluents when they are present in dynamic environments. Moreover, the assessments described above measured physiological responses to effluents. Such changes can clearly influence survival but behavioral changes are equally important. For example, does exposure to various effluents interfere with predator avoidance, swimming performance, habitat preferences and so on?

While the fish were held at the tank complex and continuously exposed to freshly obtained effluents, two ancillary experiments were performed to assess behavioral changes. In one, the short-term swimming stamina of coho smolts exposed to pulp mill effluent was examined. The primary objective of the other study, was to ascertain whether coho smolts would avoid waters containing pulp mill effluent. Additionally, tests were conducted to see if exposure to effluents interfered with the olfactory acuity of these fish. Background information and results of these studies are presented below.

Swimming Stamina Tests

Background and Introduction

Swimming stamina tests have long been used to evaluate the

overall health and vigor of fish. Commonly, fish are placed into swimming chambers and exposed to a stepwise progression of increasing velocities that are usually instituted on an hourly basis (Brett 1964). Eventually the fish become fatigued and the length of time and velocities they have experienced before the onset of exhaustion are used to estimate their sustainable swimming capacities.

Recently, Smith and Carpenter (1987) modified this procedure by exposing fish to short periods of high water velocities. In traditional tests, fish predominately use their aerobic (red) muscles in ways that are comparable to marathon runners. During a Smith and Carpenter test (sometimes called a burst swimming test) fish primarily use their white (mostly anaerobic) muscles much like a runner engaged in a sprint. Although these swimming stamina tests are not directly comparable, both measure cardiovascular, respiratory and muscular system competence.

We used burst tests to determine if effluents from the ITT Rayonier and Weyerhaeuser pulp mills affected the short-term stamina of coho smolts because more fish could be examined than if Brett's protocols had been followed. At the completion of these tests, the fish used were immersed in a saltwater bath and held for varying periods of time to see if exercising in different effluents influenced their osmoregulatory capacity. This was determined by assessing changes in body weight and length before and after exposure to the salt bath.

Experimental Conditions

The swimming stamina and salt bath tests took place on 5/28-30/88 and 6/4-5/88 and were performed in a mobile trailer located next to the continuous-flow tank farm. Smolting coho salmon from Humptulips Hatchery were used as test animals. During a test, these fish were exposed to either 100% dechlorinated Wishkah River water (hereafter referred to as Wishkah River water) or 30% Weyerhaeuser or 30% ITT Rayonier pulp effluents mixed with Wishkah River water.

Four swimming tubes were used. Each one had a clear vinyl swimming area that was 7.6 cm in diameter by 1.7 m long. Effluents were fed into the swimming areas via a 3.2 cm white PVC pipe that was equipped with a ball check valve and flow meter so that flows could be adjusted to desired amounts. At the upper end of the swimming area a flow-straightening screen was inserted and at its lower end a rotatable screen was fitted inside a 7.6 cm tee. By rotating the lower screen, fatigued fish could be passed through the outlet pipe into a holding box. A 1-hp Jacuzzi pump took tailbox waters and recirculated them back through the chambers. At the end of every other test, or when new effluents were run, the system was flushed and refilled with fresh effluent.

The Weyerhaeuser effluent tests and their associated controls were done without temperature control. Water temperatures were initially 12-13° C but as a test progressed temperatures rose to 18° C because of the heat generated from the Jacuzzi pump. A frigid cooling unit was used during the ITT tests and water temperatures

remained within 2° C of ambient.

To perform a swimming stamina test, the pump was briefly used to fill each tube. The downstream screens were then removed and three or four coho were individually inserted into a tube. After these screens had been replaced, the pump was turned back on and the fish were subjected to very low flows (≃15 cm/sec). After acclimating for an hour, flows were increased to 22 cm/sec which caused the fish to swim slowly and steadily. At the beginning of the third hour, flows were increased to 70 cm/sec (for ITT effluents and controls) or 73.4 cm/sec (for Weyerhaeuser effluents and controls). These high velocities were maintained until all fish in a tube were fatigued. Once a fish became impinged on the lower screen for more than 30 secs it was removed from its tube, anesthetized with MS222, weighed and measured.

After being weighed and measured, fatigued fish were placed into twenty gallons of sea water made from Instant Ocean salts and Wishkah River water and held for variable periods of time. Fish were weighed and measured again as soon as they died or under anesthesia at the end of an observation period. The seawater bath was static without temperature control (except shading from insolation) and it was not aerated, so dissolved oxygen levels equal to 1/3 saturation were sometimes observed. Thus, fish were subjected to severe hypoxia in addition to increased salinity.

Results of the Swimming Stamina Tests

The results of the swimming stamina tests appear in Table 3.23. Before interpreting these results two factors must be

Table 3.23 Swimming endurance data collected from juvenile coho salmon which swam in 30% ITT Rayonier or 30% Weyerhaeuser pulp-mill effluent.

Treatment	N	Fork Length X cm	Std. Dev.	Range	Body Lengths/Sec X	Std. Dev.	Range	Endurance (min) X	Std. Dev.	Range
ITT Rayonier - 30%	21	14.2	± 1.1	11.9-16.3	5.0	± 0.4	4.3-5.9	2.2	± 1.2	0.3-4.5
ITT Control	13	14.3	± 1.2	12.9-17.6	4.9	± 0.4	4.0-5.3	3.9	± 2.9	0.8-11.0
Weyerhaeuser - 30%	19	14.2	± 1.0	13.0-17.1	5.2	± 0.4	4.3-5.6	1.5*	± 1.0	0.1-4.0
Weyerhaeuser Control	21	14.2	± 0.7	12.5-25.2	5.2	± 0.3	4.9-5.9	2.8	± 1.4	0.8-7.0

* = Significantly different from the corresponding control (α = .05)

considered. First, during the ITT tests, the temperature of the recirculating effluent was relatively constant (± 2° C) while in the Weyerhaeuser tests, temperatures[1] rose 6 to 7° C. Second, the water velocities used in the ITT tests were 4.6% slower than those experienced by coho during the Weyerhaeuser trials (70 vs 73.4 cm/sec). Velocities were decreased during the ITT tests to spread out any observed differences in swimming endurance. Because the conditions experienced by both of these groups were dissimilar, comparisons between the two effluents cannot be made, but differences between an effluent treatment and its control can be examined.

As Table 3.23 illustrates, the average swimming endurance times of fish exposed to Weyerhaeuser effluent were statistically different from their controls. A similar, but statistically nonsignificant trend occurred in the ITT tests. In general, fish subjected to effluent laden waters tended to have less endurance than their controls.

Results of the Saltwater Exposure Tests

Results of the seawater exposure tests are presented in Table 3.24. These tests were designed to see if any noticeable

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[1] The physiological and performance effects of such a temperature change may be less than expected. First, the change was a gradual one that took place over a fairly long period and is thus analogous to the procedure used to move salmonid fishes into waters having disparate temperatures. Second, Griffiths and Alderdice (1972, as cited by Smith and Carpenter 1987) found that maximum swimming performance in coho juveniles occurs at 20° C and that these fish swam better at test temperatures that were slightly higher than those they had been previously acclimated to.

Table 3.24 Changes in weight and length in smolting coho that swam until fatigued in pulp-mill effluent or control waters and then were put into 30 ‰ artificial seawater for varying periods of time.

Treatment	N	% Weight Change %	Std. Dev.	Range	% Length Change %	Std. Dev.	Range	Exposure Time Hours
ITT Rayonier - 30%	23	-8.3	± 5.5	+3.0 to -17.9	-2.2	±1.1	+0.0 to -4.3	48-50
Weyerhaeuser - 30%	20	-5.1	± 6.6	+9.4 to -13.2	-0.4	±0.8	+1.4 to -6.0	13-18
Controls	49	-6.5	± 4.1	-0.4 to -21.0	-0.5	±1.5	+1.4 to -6.0	17-25[a]

[a] = One fish died after only 4 hours

differences in weight gain or loss would occur to fish that had swum to exhaustion in different waters. The outcomes of these tests are very difficult to interpret because the salt bath was not aerated and the fish were held for varying periods of time. In spite of these experimental difficulties, it is noteworthy that none of the control fish gained weight after being exposed to sea water, whereas a number of fish exposed to effluent did.

Conclusions

Although a number of factors prevented some comparisons from being made, these tests showed that pulp mill effluent can affect swimming stamina. Additionally, the seawater bath test suggested that exposure to these materials may influence the absorption of water into a smolting coho while it resides in saline waters. Obviously, to refine these findings, additional swimming chamber and salt bath studies would have to be conducted.

Olfactory Detection of Effluents by Coho Smolts

Introduction and Background

Olfaction in fishes can be impaired by several common waterborne pollutants (Brown et al. 1982). Since fish leaving the Chehalis watershed encounter a variety of effluents as they migrate through the inner harbor we assessed how several of these may affect the olfactory acuity of smolting coho. Consequently, the primary objective of the olfaction detection experiments was to determine what concentrations of pulp mill effluent, if any, coho smolts would perceive as noxious. Paradoxically perhaps, discovering which concentration of an effluent did not elicit

significant avoidance/preference behavior was also an important objective of this study because we wanted to see if this neutral concentration would be avoided if mixed with L-serine. L-serine, an amino acid found in mammal skin, is known to elicit strong avoidance behavior in salmonids. Thus, the second objective of this study was aimed at evaluating whether an effluent could confound recognition of other biologically significant odorant cues. For example, Rehnberg and Schreck (1986) found that toxicants such as heavy metals could eliminate recognition of other odorants. Such an effect can have obvious consequences for processes such as predator avoidance, foraging, and imprinting that rely on olfactory inputs.

Determining Behavioral Responses to Effluents

Smolting coho salmon from the Humptulips Hatchery were transported to the continuous-flow bioassay site (Aberdeen Sewage Treatment Plant) and held in 750 *L* flow-through circular tanks supplied with dechlorinated Wishkah River water (hereafter referred to as Wishkah River water). Fish were given at least six days to adjust to these conditions before being tested.

Avoidance/preference behavior to effluent routinely discharged by the Weyerhaeuser and ITT Rayonier pulp mills was assessed by placing coho into two-choice Y mazes (see Rehnberg et al. 1985). Three effluent dilutions were tested (ranging from 0.03% to 30% (v:v)) using Wishkah River water as diluent. Since a 10^{-4} M solution of L-serine was found to be avoided by coho in preliminary trials, this concentration was tested using 0.03% Weyco or 0.3% ITT

effluents as the diluent. These effluent concentrations were used because they were the highest ones tested that did not elicit significant avoidance.

Test dilutions were supplied via a constant-head delivery system capable of dispensing effluent to one arm of a maze and control water (Wishkah River water) to the other arm. Flow rates in each arm of the maze were 3.6 *L*/min. To prevent cross contamination, the head tanks used in the delivery system always received the same type of effluent.

Trials were conducted in two identical Y mazes as follows: A single, naive fish was placed in the fork area of each maze and allowed to adjust for 12 minutes. A gate was lifted and the fish was then given five minutes to choose between entering an arm possessing either control or effluent waters or remaining in the fork area. Following the choice period, arm gates were dropped and the fish's position was recorded. Thirty such trials were run on each effluent concentration tested. After each trial was completed, the mazes and delivery systems were drained and thoroughly rinsed with Wishkah River water. In addition, to avoid bias, test solutions were alternated between the two arms of each maze.

Arm selection behavior was analyzed by a G test with a correction for continuity (Zar 1984). Data from both control (Wishkah River water in both arms) and effluent trials were compared to a 1:1 ratio (i.e. random arm choice). Only fish entering an arm were included in the analyses.

Results of the Maze Trials

The results of the maze trials and dates that tests were conducted are given in Table 3.25. While fish exposed to control water selected each arm equally (i.e. selection did not differ from the expected 1:1 ratio), 30% concentrations of both Weyerhaeuser and ITT effluents were significantly avoided (Table 3.25). Effluent concentrations had to be reduced to 0.03% for Weyerhaeuser and 0.3% for ITT before they were no longer avoided.

In the trials where an avoided concentration of L-serine (10^{-4} M) was mixed with either 0.03% Weyerhaeuser or 0.3% ITT (unavoided concentrations of effluent), the Weyerhaeuser/serine mixture was significantly avoided (83%) but not the ITT/serine mixture (61%).

In conclusion, these results indicate that coho smolts are sensitive to relatively minute amounts of both pulp mill effluents, and given the choice, generally prefer to avoid such waters. Ancillary experiments (Stone and Schreck unpubl.) revealed that acute exposure (3 to 4 hr) to 30% Weyerhaeuser effluent elicited an increase in plasma cortisol titers. This physiological response parallels the behavioral reactions of coho placed into effluent laden waters. Thus, these results show that juvenile coho perceive at least higher effluent concentrations as noxious and respond accordingly.

A previously unavoided concentration of Weyerhaeuser (0.03%) was generally avoided by smolts when a noxious concentration of L-serine (10^{-4} M) was added. However, similar tests using ITT (0.03%) effluent demonstrated that low concentrations of this effluent may

Table 3.25 Results of the Y-maze trials performed in 1989 to determine: 1) whether smolting coho salmon would avoid various concentrations of pulp-mill effluents, and 2) if these effluents would interfere with normal avoidance of L-serine.

Treatments	Dates	# Avoiding	# Preferring	# No Choice
Wishkah River Water	4/19	11	12	7
Wishkah River water + 10^{-4} M L-Serine	4/20&29	15*	5	10
Weyerhaeuser				
- 30%	4/21-22	18*	4	8
- 0.3%	4/22-23	19*	4	7
- 0.03%	4/27-28	17	11	2
0.03 % + 10^{-4} M L-Serine	4/30-5/1	19*	4	7
ITT Rayonier				
- 30%	4/23-24	17*	5	8
- 3%	4/26-27	23*	4	3
- 0.3%	4/25	17	8	5
0.3% + 10^{-4} M L-Serine	4/30-5/1	17	11	2

* = Significantly different (α = .05) than the expected 1:1 ratio

have a masking effect that could impair a young salmon's perception of other biologically important odorants.

Summary of Hypothesis Three

Both *in situ* and laboratory studies were performed to test whether environmental conditions in the inner harbor were deleteriously affecting smolting coho salmon. The *in situ* investigations appraised how fish migrated and physiologically responded to conditions in the inner harbor, while laboratory work evaluated how various effluents entering the inner harbor affected coho both physiologically and behaviorally.

The *in situ* studies provided several important results. First, CWT tag recoveries made on beach seined fish indicated that coho originating from the Humptulips and Chehalis basins do not occupy the same estuarine areas. Thus, juvenile coho from these two systems experience different estuarine conditions while making their final adjustments to seawater. Second, the acoustic tracking work illustrated that coho emigrating through the inner harbor generally moved back and forth with tidal currents and that this movement was often interspersed with prolonged holding periods. The tracking data also indicated that residency in the inner harbor ranged from less than one to over 12 days.

What induces such variation in inner harbor residency is unknown, but those fish that rapidly move through the inner harbor probably survive at higher rates than individuals that remain for extended periods of time. For instance, measurements made on fish held in barges, live boxes, or beach seined indicated that

conditions in the inner harbor generally reduced immunocompetence and often elevated cortisol titers. Moreover, the mixed function oxidase tests performed on fish held in live boxes demonstrated that xenobiotics were present in the inner harbor and that the fish were using cytochrome P-450 enzymes to detoxify themselves.

The seawater rearing tests performed as part of the barging and live box bioassays were also quite revealing. Fish removed from the inner harbor barge and reared in seawater died at four times the rate as comparable fish taken from the North Bay barge when both groups were simultaneously exposed to a natural outbreak of *Vibrio*. Physiological data collected on the barged fish indicated that those held in the inner harbor were less healthy than coho moved through North Bay. Similar seawater rearing studies performed on fish taken from the live boxes showed that all groups died at high rates regardless of where the boxes had been berthed. Interestingly, most coho held at the Montesano site perished before the groups were exposed to an outbreak of *Vibrio*. This mortality was later linked to high infestations of the parasite *Nanophyetus salmincola*.

The above results have led us to hypothesize that stressors in the inner harbor combined with *Nanophyetus* are largely responsible for the poor survival of coho smolts exiting the Chehalis system. Generally, coho migrants leaving the Chehalis have higher infestation rates of *Nanophyetus* than those exiting the Humptulips. The levels of infestation observed do not appear to be lethal, but we believe when they are associated with additional stressors the

aggregate impact destabilizes smoltification or reduces immunocompetence to such an extent that the fish never recover. This hypothesis is currently being tested, if it cannot be rejected, then the need for good water quality in the inner harbor will be especially important since high levels of *Nanophyetus* in the Chehalis system make coho leaving this basin particularly vulnerable to additional environmental stressors.

The *in situ* assays briefly summarized above were not designed to examine effects of individual effluents on smolting coho. Four types of laboratory tests, continuous-flow bioassays, static bioassays (see Part III for the results of the static assays), swimming stamina tests, and effluent avoidance examinations were used to ascertain the toxic effects of specific effluents. For example, the continuous-flow bioassays examined how Chehalis River water, Weyerhaeuser and ITT pulp mill effluents, and Hoquiam and Aberdeen sewage treatment waste discharges affected smolting coho.

In 1988, the continuous-flow assay showed that fish exposed to the highest concentrations of pulp mill effluents (30%) had the greatest incidences of epithelial degeneration, epitheliocystis and parasite infestations after exposure to these materials. These differences tended to disappear once the fish were reared in seawater. However, at the end of seven months of seawater residency, coho exposed to 30% Weyerhaeuser effluent for five days had the lowest percentage of normal pseudobranchs, thymus glands, spleens and kidneys which suggested that these fish were in especially poor health. Comparable histological results were not

obtained in 1989. However, necropsy data were collected only after nine months and high mortalities had occurred in all groups. Therefore it was difficult to assess histological changes, since impacted fish may have died prior to being sampled. In both 1988 and 1989, fish held in effluents during the continuous-flow assay were ailing when they arrived at Marrowstone but control populations were not. Thereafter, all groups started to experience poor survival rates suggesting that laboratory conditions may have been stressful to the fish. In 1988, three treatments, 30% Weyerhaeuser, 30% ITT and the positive control all had higher mortality rates than the rest of the treatments during the first month of seawater rearing. At the end of the experiment, however, only the 30% Weyerhauser fish had a significantly higher mortality rate than the other treatments. In 1989, no mortality differences were observed, but the high mortality rates experienced by all groups during this experiment prevented these types of comparisons. In both years, differences in growth could not be associated with various treatments.

Osmoregulatory assessments made on coho used in the continuous-flow bioassays showed that fish exposed to pulp mill effluents exhibited a response, but one that apparently did not impair their hypoosmoregulatory capacity. Disease challenges conducted during these assays were inconclusive. In 1988, the *Vibrio* culture proved to be non-virulent, and therefore a valid challenge was not conducted. Conversely in 1989, when the fish were exposed to a natural outbreak of *Vibrio*, all populations

experienced high mortality rates (≃70%).

The cortisol and immunocompetence evaluations performed in 1989 showed that control fish had elevated levels of cortisol after five days of treatment but that coho held in pulp effluent maintained their cortisol titers at pre-treatment levels. We believe control fish, which were held in transparent waters, were stressed by human movement that occurred around their tanks. The fish held in pulp mill effluent escaped this stress because their treatment waters were stained a dark brown by the effluent. However, the immunocompetence of fish held in pulp effluent for 14 days was lower than the control fish in spite of the fact that these latter fish had experienced relatively elevated cortisol levels. Moreover, during the course of the bioassay, control fish increased their immunocompetence whereas treated fish decreased theirs.

Whether xenobiotics existed in the pulp effluents used in the continuous-flow tests was determined by performing mixed function oxidase appraisals that ascertained AHH and EROD values. In 1988, only AHH values were calculated and it was found that fish exposed to 30% Weyerhaeuser effluent for five days possessed elevated levels of this enzyme. In 1989, both the AHH and the more sensitive EROD assays were performed. These assessments showed Weyerhaeuser effluent contained xenobiotics that induced significant EROD activity that was both dose and time dependent. EROD levels were not increased in coho exposed to ITT effluent. However, the lack of induction of AHH or EROD activity does not by

itself imply that the ITT effluents examined were not toxic. For example, some xenobiotics cause hepatotoxicity which can suppress the production of these enzymes.

Besides the above assessments, two additional laboratory evaluations were performed. One of these consisted of placing fish in swimming tubes and examining the effects of 30% pulp mill effluent on swimming endurance. In general, fish exposed to effluent laden waters tended to have less stamina than control fish. In addition, exercising in these materials appeared to affect the absorption of water into body tissues.

The other laboratory assessment examined whether smolting coho would avoid pulp mill effluents if given a choice and whether these materials might interfere with the detection of other important odorants. These tests showed that coho preferred to avoid even low concentrations of effluent and that dilute quantities of ITT effluent prevented coho from detecting *L*-serine, a substance they would normally avoid.

The continuous-flow bioassays and other laboratory tests are by their nature artificial and were conducted to help us understand how known quantities of effluents might impact coho juveniles. In reality the inner harbor is a complex, dynamic site where pH and salinity can rapidly change. These environmental changes can affect the toxicity of effluents and other materials that may be extant in this portion of Grays Harbor. Hence, the results of the *in situ* tests are probably more reflective of how a free-ranging fish may respond to the inner harbor but even these cannot account

for the idiosyncrasies of individual fish behavior. However, the collective results of all the studies undertaken to test hypothesis three generally show that coho moving through the inner harbor are at times stressed by the environmental regimes or major inputs present. As suggested elsewhere, this stress by itself or in combination with others probably causes the premature death of many of these fish.

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HYPOTHESIS FOUR: PREDATION ON COHO SMOLTS

Introduction

One of the hypotheses proposed to explain the difference in survival of coho originating from the Chehalis and Humptulips rivers is that predators consume a higher proportion of Chehalis River smolts than Humptulips River smolts. The predators that could most likely cause a survival difference are northern squawfish (*Ptychocheilus oregonensis*), marine mammals (e.g., harbor seals (*Phoca vitulana*), and birds (e.g., the common merganser-*Mergus merganser*). All of these predators are found in Grays Harbor or in the Grays Harbor drainage. Moreover, all are documented predators of juvenile salmonids that in certain circumstances are capable of consuming large numbers of salmonids (Foerster and Ricker 1941; Fiscus 1980; Brown and Moyle 1981; Mace 1983; Wood 1987a,b; Rieman and Beamesderfer 1988).

To evaluate the hypothesis that predators are responsible for the poor survival of Chehalis River coho, two tasks were performed. First, we reviewed published information dealing with predation on smolts by birds, marine mammals and northern squawfish to assess the likelihood that these predators could account for the survival problem. Second, we conducted studies in 1988 and 1989 to quantify the mortality of coho smolts due to squawfish predation. Attention was focused upon squawfish because we concluded from our literature reviews that squawfish was the only predator species that could potentially account for the low survival of Chehalis River coho.

One major reason for suspecting that squawfish are causing poor survival of Chehalis River fish is that there is a dramatic difference in abundance between the two river systems; squawfish are abundant in the Chehalis River but are not found in the Humptulips River. Thus, we felt that studies to quantify the extent of squawfish predation were warranted.

Literature Reviews

Marine Mammals

The most abundant marine mammal found in Grays Harbor is the harbor seal. Therefore, if marine mammal predation is affecting the survival of Chehalis River coho smolts, then harbor seals are most likely responsible. An extensive analysis of the diet of these animals was conducted in Grays Harbor and several adjacent estuaries (e.g., Columbia River) by Beach et al. (1985). Although harbor seals consumed some adult salmonids, Beach et al. (1985) found no evidence that smolts were eaten. There have also been no reports of predation on smolts in other west coast estuaries (e.g., Beach et al. 1985).

Any seal predation that does occur should be greater on the fish exiting through North Bay because most seal haul-out areas in Grays Harbor are located in North Bay and the outer estuary. Thus, because seals forage near haul out areas, smolts leaving the Humptulips River would be more accessible to seals than fish originating from the Chehalis River. In conclusion, the available literature does not support the notion that marine mammals are the source of the coho survival problem in the Chehalis River.

Avian Predators

Large numbers of migratory and resident birds utilize Grays Harbor (Herman and Bulger 1981). A number of these birds, such as common mergansers, various gull species (*Laurs* spp.) and the common murre (*Uria aalge*) (Wilson 1977; Mace 1983; Varoujean and Mathews 1983; Wood 1985; Bayer 1986; Ruggerone 1986), are potential predators of juvenile salmon migrating in freshwater or estuarine areas.

If birds account for the difference in coho smolt survival they would have to consume nearly half the smolts migrating down the Chehalis River. Information obtained from studies of bird predation on smolts in other estuaries and riverine areas indicates that avian predation on smolts is much lower than 50%, unless smolts are stressed (e.g., Ruggerone 1986; Wood 1987a,b). For example, in the Big Qualicum River and estuary, Mace (1983) found that predation on hatchery chinook was 10.4% to 31.7% while predation on hatchery coho was around 5.6%. The highest losses of chinook (i.e., 31.7%) occurred when they were sick due to a diet deficiency.

In Grays Harbor, stressors could enhance bird predation on smolts leaving the Chehalis River. For instance, poor environmental conditions in the inner harbor (see PART II, section 3) could potentially enhance their susceptability to predators. In a situation where stress affects the performance of fish, predators are not the primary cause of mortality, but rather a secondary one because their impact would be much less if they were foraging on a

non-stressed population.

Chehalis River fish could also be more susceptible to predation if birds were more abundant or foraged more in the Inner Harbor and Chehalis River. This seems unlikely, however. Smolts in both watersheds should be equally accessible to birds because many of the piscivorous birds have large foraging distances. Common murres, for example, range as far as 150 km from their breeding colony sites (unpublished data cited in Varoujean and Mathews 1983).

In summary, birds undoubtedly consume some smolts migrating from the Chehalis River. However, the available data from the literature suggests that it is unlikely that they account for the difference in survival between fish produced by the Chehalis and Humptulips rivers.

Northern Squawfish

The Chehalis River basin is largely a freeflowing system (i.e., unobstructed by dams and diversions). Squawfish predation on juvenile salmonids in this type of habitat does not appear to be significant (Falter 1969; Buchanan et al. 1981). Instead, the greatest predation by squawfish on salmonids seems to occur in lakes and riverine areas where non-natural flow patterns exist (Forester and Ricker 1941; Ricker 1941; Eggers et al. 1978; Hall 1979; Uremovich et al. 1980). Predation appears to be especially severe around dams and diversions. For example, from 1983-1986, squawfish consumed an estimated 11% of the smolts (mostly chinook) entering one reservoir on the Columbia River; predation was most

intense in the areas closest to the dams above and below the reservoir (Poe et al. 1988; Rieman and Beamesderfer 1988).

Even though studies of predation in systems similar to the Chehalis suggest predation should be low, certain conditions could create higher than expected predation rates. For instance, smolt losses could be significant if squawfish are particularly abundant, such as if they aggregate in response to increasing smolt abundance, or the abundance of juvenile salmonids is low. Certain environmental conditions (e.g., reduced flows or warmer water temperatures) could also exacerbate predation losses (Ginetz and Larkin 1976; Henchman 1986; Vigg 1988; Beyer et al. 1988; Faler et al. 1988).

Some of these conditions probably occur in the Chehalis River basin. For example, in some years, such as 1989, water flows were reduced during the spring, producing conditions that potentially enhanced the efficiency of squawfish. Also, when the number of wild outmigrants is low (e.g., 1989) high mortality rates may occur if predation is depensatory. Conversely, in years where water flows are well above average (e.g., 1990) or when the number of outmigrants is high (e.g., 1987), smolts should be less vulnerable to squawfish predation. Thus, conditions that would consistently affect the vulnerability of both hatchery and wild fish to squawfish predation do not occur.

If squawfish were largely responsible for the poor performance of Chehalis River coho salmon, then the magnitude of the difference in survival between the two river systems should vary with changes

in biotic and abiotic conditions. Instead, there is a relatively constant difference in survival between Chehalis coho and other coastal coho populations. The consistency of this difference, plus the information available in the literature, lead us to conclude that squawfish are not likely responsible for the low survival of Chehalis River coho.

Quantifying Consumption of Coho Smolts by Northern Squawfish in the Chehalis River

General Approach

To estimate the number of coho smolts eaten by squawfish, we first estimated the size of the squawfish population. We then calculated the number of smolts consumed each day by the population of squawfish and multiplied this value by the number of days smolts were available to squawfish. We felt that time of year, area in the river, and whether the fish were of wild or hatchery origin could affect how many might be consumed. Thus, separate estimates based on these factors were made. The results presented here are for 1989, although data obtained in 1988 and 1989 were used in making these estimates.

Only the impact of large squawfish (defined as $\geq$300 mm) was estimated. Available evidence (e.g., Thompson 1959; Henchman 1986) shows that squawfish >200 mm feed predominately on fish. However, we used 300 mm as a threshold because the results of our stomach content survey of 660 squawfish collected from the Chehalis River in 1988 and 1989 indicated that only fish greater than 300 mm

consumed smolts.

Three estimates of the numbers of smolts eaten and percent mortality were computed for each combination of fish type (hatchery and wild), river section (lower river: defined as R.K. 10 to R.K. 32 which is the confluence of the Satsop River; upper river: defined as R.K. 32 to R.K. 80 which is where the scoop trap was located) and time period (before and after the release of hatchery fish). The first scenario (or average case) was generated using mid-point or average estimates of parameter values, such as squawfish abundance and smolt outmigration speed. The second scenario used the upper bounds of these estimates and therefore represented a worst case (maximum number of smolts consumed) while the third represented the lower bounds of estimates. The parameters used to make these estimates are summarized in Table 4.1 and are described in more detail below.

Data Used to Estimate Squawfish Predation

Population Size of Squawfish. The number of large, predatory squawfish in the Chehalis below the scoop trap (R.K. 80) was estimated using Chapman's modification of the Petersen mark-recapture procedure (Ricker 1975). Squawfish in eight randomly selected transects (each about 1.5 km long) were collected using a Coffelt or Smith-Root boat electroshocker. Fish were placed into a live well, measured for length, jaw tagged and immediately released unless they appeared to have been injured while being captured. Five days later, transects were resampled and the numbers of tagged and non-tagged fish that were captured were

Table 4.1. Parameters used to estimate consumption and percent mortality of coho smolts due to squawfish predation. The parameters listed include abundance of large (>300 mm) squawfish, occurrence of smolts in stomachs, number of smolts eaten per squawfish, digestion time of smolts in squawfish stomachs, migration rate of smolts in the river, and exposure time. The parameters used for the lower bound (best case), average case, and upper bound (worst case) are presented. Data is partitioned on the basis of section of river, time period and when hatchery fish were released.

Parameter	Lower Bound	Average Case	Upper Bound
No. of Squawfish[1]			
Lower River	1,000	3,690	12,770
Upper River	3,200	11,805	40,864
Occurence of Smolts in Stomachs (%)[2]			
Lower River			
Before Release	0	0	0
After Release	9	17	25
Upper River			
Before Release	0	0	0
After Release	0.2	0.3	0.4
# Eaten Per Squawfish[3]			
Lower River			
Before Release	0	0	0
After Release	0.8	1.6	2.4
Upper River			
Before Release	0	0	0
After Release	1	1	1
Digestion Time of Smolts in Squawfish (a model provided in Beyer et al. 1988)[4]			
Hrs to 90% Evacuation	35	30	25
Data Used:			
Predator Wt (gr)	682	620	558

Table 4.1. Parameters used to estimate consumption and percent mortality of coho smolts due to squawfish predation. The parameters listed include abundance of large (>300 mm) squawfish, occurrence of smolts in stomachs, number of smolts eaten per squawfish, digestion time of smolts in squawfish stomachs, migration rate of smolts in the river, and exposure time. The parameters used for the lower bound (best case), average case, and upper bound (worst case) are presented. Data is partitioned on the basis of section of river, time period and when hatchery fish were released. (continued)

Meal Wt. (gr)	72	48	24
Temperature (°C)	15.1	14.1	13.1
Migration Rate[5]			
Km/hr	1.7	1.2	0.7
Time Exposed to Predators (hrs for hatchery fish, days for wild)			
Hatchery Fish			
Hatchery to Chehalis	19	27	45
Lower River	12	17	29
Wild Fish	10	15	20

1- The extremes of the 95% C.I. represent the upper and lower bounds.
2- Upper and lower bounds are arbitrarily set at 50% of mean.
3- For the lower river, ± one S.D. of the mean for upper and lower bounds. For the upper river, the value 1 was used for all three scenarios.
4- Predator weight: calculated by computing mean length and then converting this to weight. The upper and lower bounds were defined as ± 10% of the average case. Meal weight: calculated as 30 gr. smolt multiplied by the number of smolts per squawfish. Temp: upper and lower bounds are ± one S.D. as measured during our field work in spring 1989.
5- Upper and lower bounds are ± one S.D. of the average.

recorded. There are a number of assumptions associated with using the Petersen method (e.g., Ricker 1975; Beamesderfer and Rieman 1988) which were evaluated.

The numbers of squawfish per transect were converted to fish per kilometer to account for the fact that each transect was not the same length. The average of these values was then multiplied by the number of river kilometers to yield an estimate of the total number of squawfish in the lower 80 km of river. Separate estimates were also computed for the lower and upper river.

Occurrence of Smolts in Squawfish Stomachs. The occurrence of smolts in stomachs of large squawfish (>300mm) was assessed throughout the course of the coho outmigration in 1989 (4/15-5/31). Boat electroshockers were used to obtain specimens in the upper and lower river.

Overall, smolts occurred in 3.5% of the 508 squawfish stomachs examined in 1989 (only 1989 data were used since this was the only year upper and lower areas of the river were sampled). Most predation on smolts occurred in the lower river. Only one smolt was recovered from 323 squawfish stomachs examined from the upper river (above the release site of the hatchery fish) while 9% of the squawfish examined from the lower river had consumed smolts.

Prior to the time hatchery fish were present in the lower river (fish were released from Simpson Hatchery from 5/10 to 5/21), no smolts were recovered from squawfish stomachs. After hatchery fish were released, however, the occurrence of smolts in squawfish stomachs increased to 17%. A similar pattern was also observed in

1988 when 4.1% of the squawfish consumed smolts prior to the release of hatchery fish whereas after their release, this number increased to 37.5%. While there was no way to verify that any particular fish recovered from a stomach was of hatchery origin, circumstantial evidence suggests many of the fish were of hatchery origin. For example, smolt indices (e.g., ATPase activity) obtained from lower river coho caught at this time were very similar to those obtained on Simpson Hatchery coho just prior to their release. In addition, coded-wire tags found in some of the coho electroshocked from the river at this time were from Simpson Hatchery.

Number of Smolts Per Squawfish. The stomach contents data from 1989 were also used to determine the number of smolts in squawfish stomachs by area of the river and time period (before and after the release of hatchery fish). In the upper river in 1989, only one smolt was found in a squawfish stomach. In the lower river, no smolts were found in squawfish stomachs before the release of hatchery fish, however, after their release, there was an average of 1.6$\pm$ 0.8 smolts per stomach (in squawfish that had eaten coho).

Migration Rate. An estimate of the migration rate of smolts was made to determine how long they were exposed to predators in the lower river. To determine this, radio tags were inserted into wild coho smolts and the fish were subsequently followed during their downstream migration. Although hatchery fish were not tracked, we assumed that the migration rate of wild and hatchery

smolts were comparable. The results of the radio tracking studies indicated that coho moved downstream at an average rate of 1.2 km/hr.

To assess how long smolts were exposed to predators, the distance traveled from the Simpson Hatchery to the confluence with the Chehalis River (32.4 km) and the distance the fish were exposed to predators in the lower Chehalis (19.0 km) was divided by their migration rate. At an average migration rate of 1.2 km/hr, smolts would need about one day to migrate down the Satsop River to the Chehalis River. It would then take them one more day to migrate through the lower river where they are exposed to squawfish.

Gastric Evacuation Rate of Smolts. Beyer et al.'s (1988) gastric evacuation model was used to determine the time for 90% of a meal to digest, at which time squawfish were assumed to be ready to feed again. The model incorporates meal size, predator size, and temperature to estimate when 90% of a meal has been eliminated from the stomach. Depending on the parameters used, digestion ranged from 26 to 35 hrs. We thus assumed that smolt meals eaten by the squawfish would be digested within 24 hrs and therefore squawfish would be ready to eat a meal of smolts once per day.

Numbers of Outmigrants. In 1989, approximately 1.3 million hatchery-produced coho smolts were released volitionally from Simpson Hatchery between 5/10 and 5/21. An estimate of the number of wild migrants was obtained from Dave Seiler of WDF (personal communication). Since 1982, WDF has conducted a trapping and tagging program to quantify the number of wild smolts leaving the

system (Seiler 1989). Preliminary estimates for the 1987 brood are that 1,380,000 smolts were produced in the entire basin with about 300,000 of these produced in the river above RK 80 or the location of the scoop trap.

Consumption Estimates and Percent Mortality

To determine the number of smolts eaten by squawfish, we first calculated the number of smolts consumed per day in each time period and in each section of the river. This was accomplished by multiplying the abundance of squawfish in a section of the river during a specific time period by the proportion of these fish with smolts in their stomachs. This value (i.e., the number of smolt eating squawfish) was then multiplied by the number of smolts per predator stomach to yield the consumption of smolts by the squawfish population in that strata. Because the gastric evacuation analysis suggested that squawfish could digest a meal of smolts per day, we assumed that this consumption estimate represented the number of smolts eaten per day.

Multiplying the number of days the smolts were exposed to predators by the number of smolts eaten per day by the predator population yielded the total number of smolts eaten in a time period in a river section. In the lower river, we assumed hatchery smolts were exposed to predators for 12 days (average case). In the upper river, we assumed there were a certain number of days that smolts were eaten over the course of the outmigration (e.g., 15 days for the average case). Percent mortality of hatchery fish was computed by simply dividing the numbers eaten by the estimated

number of fish liberated from the hatchery. For wild fish, we summed the numbers eaten in the upper and lower river and divided them by the estimated number of outmigrants from the entire basin.

Clearly, the number of coho in the river at any one time is not the same and can affect the number of outmigrants eaten by squawfish because of the functional response of predators (e.g., Vigg 1988). Because we had no way to correct for this type of variation, we assumed that the number of smolts eaten per day was constant within a particular strata.

Table 4.2 shows the consumption of smolts and percent mortality for each of the estimates. The greatest predation occurred in the lower river on fish released from Simpson Hatchery. Our average estimate was that about 1.0% of the hatchery fish (ranging from 0.06% and 7.0%) were consumed by squawfish.

We suspect that for hatchery fish, the actual consumption of smolts is than 1.0% (i.e., the average case). This is primarily because our average case estimate of the squawfish population in the river is probably low. For one, there are undoubtedly squawfish residing in the Chehalis River below the confluence with the Wynoochee River, the lowest point in the river that we sampled. This area of the river was not sampled because of concerns over how the electroshocker would work in more progressively saline water. However, the abundance of squawfish in this portion of the river is also probably low because of the relatively high salinities. Moreover, some electroshocking that we conducted indicated that there are some squawfish present in the Satsop River that could eat

Table 4.2. Estimated numbers of coho smolts eaten and percent mortality in spring 1989. Results of three scenarios are presented: the lower bound (best case), average case, and upper bound (worst case). Data is also partitioned on the basis of section of river, time period and when hatchery fish were released.

	Lower Bound		Average Case		Upper Case	
	#	%	#	%	#	%
Hatchery Fish[1]						
Lower River						
Case 1	792	0.06	11,044	0.9	91,944	7.0
Case 2	752	0.05	10,491	0.8	87,346	6.6
Wild Fish						
Lower River						
Case 1	0	0	0	0	0	0
Case 2	40	-	552	-	4,597	-
Upper River	64	-	708	-	3,269	-
Total Wild	104	0.01	1,267	0.1	7,866	0.6

1- Case 1= 100% of the smolts eaten in the lower river are of hatchery origin.
Case 2= 95% of the smolts eaten in the lower river are of hatchery origin and 5% are wild.

hatchery smolts; however, we found very few squawfish in the Satsop, probably because of a lack of adequate habitat. Another factor that would tend to shift the numbers eaten towards the worst case estimates is that our estimates are only based on the number of squawfish $\geq$ 300mm. Small squawfish could have consumed a relatively small number of smolts.

Our analyses suggest that very few wild fish were consumed. In the worst case, much less than 1.0% of the wild run is consumed by squawfish. One important difference in the mortality rate estimates for hatchery and wild fish is that all the hatchery fish begin their outmigration from one location and thus all fish are exposed to the same predator population. However, all wild fish are not exposed to the same predator population. The number of predators they encounter depends on the distance they must migrate to reach the estuary. Fish exiting the lower river tributaries (such as the Wynoochee River) should encounter far fewer squawfish than those migrating from the upper river. Therefore, the number of smolts exposed to the predators below R.K 80 is less than the 1.3 million (the entire basin estimate) used in our estimates. This would tend to decrease the 1.3 million used the percent mortality computations which would tend to increase the estimate of mortality.

Summary

The objective of these analyses was to determine if squawfish could consume enough smolts to account for the difference in survival of coho produced by the Humptulips and Chehalis rivers.

In order for predation to be the primary factor responsible for the poor survival of Chehalis fish, squawfish would have to consume close to one-half of the hatchery and wild fish exiting the Chehalis River. Our estimates clearly show that this does not occur, particularly for wild smolts. As a result, we conclude that although squawfish eat a quantity of smolts, they do not eat enough to account for the consistent difference in juvenile coho survival that exists between the Chehalis and Humptulips watersheds.

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PART III: GRAYS HARBOR WATER QUALITY ASSESSMENTS, 1987-1989

INTRODUCTION

Poor environmental conditions in the lower Chehalis River and inner Grays Harbor may reduce the survival of juvenile coho salmon migrating through the area. If degraded environmental conditions impact survival of these fish, it is unlikely due to historical water quality problems such as acute toxicity and low dissolved oxygen that occurred in the inner harbor for much of the last 40 years. These types of problems appear to have been largely alleviated, primarily as a result of improvements made in the treatment of wastewaters from the Weyerhaeuser and ITT-Rayonier pulp mills. Of particular concern is the possibility that chemicals entering the waters of the inner harbor are adversely impacting the performance of the juvenile coho.

To determine if toxicants were affecting survival of Chehalis River coho salmon, the U. S. Environmental Protection Agency (EPA) and Washington Department of Ecology (Ecology) analyzed samples of the receiving environment and selected effluents collected from 1987-1989. Samples were screened for the presence of potentially toxic chemicals and subjected to bioassays with a variety of freshwater and marine organisms. [2]

There were three general objectives of the water quality

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[2] In this report unless otherwise noted, chemical concentrations are expressed in terms of parts per trillion (ng/L or ng/Kg), parts per billion (μg/L or μg/Kg), and parts per million (mg/L or mg/Kg). Sediment data are on a dry weight basis; tissue data are on a wet weight basis. Bioassay results are given as EC50s (the percent of effluent at which 50% of test population shows an effect), LC50s (the percent effluent lethal to 50% of test population), NOECs (no observable effect concentration), and NOEL (no-observable-effect-level).

analyses. The first was to characterize effluents discharged into the inner harbor from the two Weyerhaeuser and ITT pulp mills and Aberdeen and Hoquiam sewage treatment plants (STP). These particular effluents were selected because they are the largest point sources of potential contaminants entering the harbor. As a result, there is a high probability that any toxicants affecting the survival of coho salmon are discharged into the inner harbor as part of one or more of these effluent streams. Clearly, there are other sources of toxicants in the inner harbor, such as leachates from log storage areas, landfills, fish canneries, and runoff from urban storm drains. These, however, have low discharge volumes and thus probably do not affect the performance of coho juveniles. For example, Ecology (Pelletier and Determan 1988) found that of 90 storm drains in the area inspected in July 1987, only 29 were flowing and the maximum discharge was a fraction of a cubic foot per second. Only six exhibited anomalous water quality or suspicious visual characteristics.

The second major objective was to evaluate the chemical makeup and toxicity of Chehalis River water. There are a number of point and non-point sources of potential chemical toxicants in the Chehalis River basin, including fish farms, STPs, agricultural land use practices, and forest practices. A number of chemicals that could originate from these sources, such as some herbicides and pesticides, are known to impact smolting salmonids (e.g., Lorz et al. 1978a).

The third objective was to characterize chemical toxicants

present in the receiving environment of the inner harbor. This was addressed by analyzing samples of the water column, bottom sediments, and fish and shellfish tissues. Determining toxicants present in the water column of the Grays Harbor estuary was critical to assessing what coho might be exposed to during their transit through the inner harbor. These chemicals may be bound to suspended particulate materials (hydrophobic) in the water column or they may be dissolved (hydrophilic).

Sediments represent both a source and sink of pollutants. Many contaminants identified as concerns in urbanized estuaries have an affinity for particulates and can be deposited in bottom sediments. Deposition can occur over many years. Eventually, deposited material can be resuspended into the water column by the actions of wind, currents, or human activities (e.g., dredging) where they could impact outmigrating smolts.

Certain chemicals such as dioxins, PCB's, and chlorinated pesticides accumulate in fish and other organisms to levels that are many times higher than in the surrounding environment. Biological tissues can therefore be used to screen for these and other bioaccumulative materials that may escape detection during analyses of water and sediment.

The following is a summary of the water quality investigations. A listing of the major tasks accomplished is provided in Table 1. If more information on some element of these studies is desired, then Table 1, PART I, which identifies the investigators that performed various segments of the work, can be consulted.

Table 1. Listing of water quality studies performed during the Grays Harbor study, 1987-1989.

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1. Characterization of Effluents Discharged into Grays Harbor

   a. Chemical Characterization of ITT, Weyerhaeuser, and Aberdeen STP Effluents, Spring 1987.

   b. Analyses of Suspended Particulate Matter from Pulp Mill Effluents by EPA, 1987.

   c. Class II Inspections of Weyerhaeuser and ITT Pulp Mills, May 1988.

   d. Analyses of Pulp Mill and Sewage Treatment Plant Effluents used during the 1988 Continuous-Flow Coho Smolt Bioassay.

   e. Chemical Characterization of Grays Harbor Pulp Mill Effluents, March-June 1989.

2. Chemical Characterization of Chehalis River Water

   a. Bioassays of Chehalis River Water, 1987-88.

   b. Characterization of Chehalis River Water during the Continuous-Flow Coho Smolt Bioassay, May 1988.

   c. Characterization of Chehalis River Water Adjacent to the Live Box at Montesano, May 1989.

3. Analyses of the Receiving Environment of the Inner Harbor

   a. Chemical Characterization of Water from the Main Chehalis River Channel and the North and South Channels, June 1987.

   b. General Water Quality Monitoring during the Barging Study, May-June 1988.

   c. Chemical Characterizations of the Water Column in the Inner Harbor in 1988 and 1989.

   d. Analysis of Dioxin in the Sediments Below the Pulp Mill Outfalls by EPA, June 1987.

   e. Characterization of Sediments in Grays Harbor by Ecology, May 1988.

Table 1. Listing of water quality studies performed during the Grays Harbor study, 1987-1989 (continued).

f. Chemical Analyses of Fish and Shellfish Tissues from Grays Harbor as Part of the EPA National Bioaccumulation Study, 1987.

CHARACTERIZATION OF EFFLUENTS DISCHARGED INTO GRAYS HARBOR

To characterize pulp mill and STP effluents discharged into Grays Harbor, a variety of tasks were performed. Much of the work addressing this objective supported the continuous-flow coho salmon smolt bioassay (see PART II, Hypothesis 3). For example, effluent characterizations accomplished during these tests allowed an assessment of whether materials tested during the bioassay were typical and whether any observed toxic effects could be explained by chemicals found in the effluent.

Chemical Characterization of ITT, Weyerhaeuser, and Aberdeen STP Effluents, Spring 1987

Samples of effluent from the Weyerhaeuser and ITT pulp mills and the Aberdeen STP were collected by Ecology during spring 1987 and analyzed for EPA priority pollutants and hazardous substances list compounds (less pesticides, PCBs, cyanide, and dioxin), guaiacols, resin acids, and conventional water quality variables (Joy 1988). A major goal was to help refine water quality analyses which would be employed during the continuous-flow coho smolt bioassays in 1988 and 1989. Recommendations resulting from this work included using a flow-through system to prevent ammonia build-up, monitoring hardness to evaluate metals toxicity, improving analytical methods for quaiacols and resin acids, and adding additional compounds to the list of target chemicals.

Analyses of Suspended Particulate Matter from Pulp Mill Effluents by EPA, 1987

Between 5/28/87 and 5/30/87, EPA collected samples of

suspended particulate matter (SPM) from the ITT and Weyerhaeuser pulp mills. Analyses of these samples were restricted to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD or dioxin). Dioxin was detected in Weyerhaeuser effluent (4.42 ppt) but not in effluent from ITT (detection limit of 0.658 ppt).

Class II Inspections of Weyerhaeuser and ITT Rayonier Pulp Mills, May 1988

Class II Inspections of the Weyerhaeuser and ITT Rayonier pulp mills were conducted by Ecology from 5/23/88 to 5/25/88, just prior to beginning the continuous-flow coho smolt bioassay. Class II Inspections are conducted to ascertain if a discharger is meeting effluent permit limits, evaluate wastewater treatment efficiency, and examine effluent toxicity.

The Weyerhaeuser pulp mill was meeting all effluent permit limits during the inspection (Hallinan 1989). Results of effluent analyses for biochemical oxygen demand (BOD), total suspended solids (TSS), and the rainbow trout bioassay conducted independently by Ecology and Weyerhaeuser agreed closely. In the rainbow trout bioassay, which is an important component of the NPDES (National Pollution Discharge Elimination System) permits for the Grays Harbor mills, none of the rainbow trout exposed to a 65% concentration of effluent for 96 hrs died (Table 2). The permits require at least 80% survival of rainbow trout in 65% effluent during a 96 hr test.

Other effluent bioassays showed high toxicity to Pacific oyster larvae (EC50 of 0.3% effluent) and moderate toxicity to

Table 2. Summary of Ecology/EPA toxicity tests conducted on Grays Harbor pulp mill effluents in 1988. Ecology's tests were a part of Class II Inspections conducted May 23–25, 1988. Most of EPA'S tests were conducted during the continuous–flow coho salmon smolt bioassay.

Test	Effluent Concentration	Measurement	Result	
			Weyco	ITT
1. Ecology Results (samples collected May 23–25, 1988)				
Rainbow Trout Survival (96–hr.)	65%	% mortality	0	0
Daphnia Survival (48–hr.)	100%	% mortality	25%	0
	Control		5%	5%
Mysid Shrimp Survival (96–hr.)	100%	% mortality	90%	25%
	30%		5%	10%
	10%		0	5%
	3%		0	0
	1%		0	0
	Control		0	10%
Oyster Larvae Abnormality (48–hr.)	--	% effluent (EC–50)	0.3%	0.2%
Ames Test	100%	mutagenicity	negative	negative
Amphipod Test * (10–day)	*	survival, avoidance, re–burial	not different from control	not different from control
2. EPA Results				
Sea Urchin Fertilization	--	% effluent (NOEL)	4.8%	4.8%
Water Flea Reproduction	--	% effluent (NOEL)	12.5%	25%
Fathead Minnow Growth (168–hr.)	--	% effluent (NOEL)	12.5%	25%
Algal Growth	--	% effluent (NOEL)	50%	25%
Coho Smolt Survival (96–hr.)	100%	mortality rate	5/12	0/12
Fathead Minnow Survival (168–hr.)	100%	mortality rate	--	0/30
		% effluent (LC–50)	60% (LC–50)	--
Water Flea Survival (24– & 48–hr.)	100%	mortality rate	--	0/10
		% effluent (LC–50)	35% (LC–50)	--

* this test on outfall sediments

mysid shrimp (LC50 of 58% effluent) (Table 2). An Ames test of whole effluent showed no evidence of mutagenicity.

Except for detection of trace amounts (0.3-16 µg/L) of polyaromatic hydrocarbons (PAH), chemical analysis of Weyerhaeuser effluent indicated that levels of potentially toxic metals and organic compounds were low, and consistent with those found by Ecology during the 1988 coho smolt bioassay (see subsequent section).

The inspection at the ITT Rayonier pulp mill revealed that the mill exceeded the daily average limit for total suspended solids and that one of two fecal coliform samples was above the monthly average limit (Reif 1989a). These, however, were not technical violations of the mill's permit. Effluent samples split between Ecology and ITT for analysis of discharge permit parameters showed poor agreement for suspended solids and fecal coliform bacteria.

Results of bioassays of ITT effluent were similar to those conducted on Weyerhaeuser effluent (Table 2). The effluent was highly toxic to oyster larvae (EC50 of 0.2% effluent), was not acutely toxic to rainbow trout, and no evidence of mutagenicity was detected with the Ames test. ITT effluent differed from Weyerhaeuser effluent in that it was not toxic to mysid shrimp. The chemistry of ITT effluent was consistent with results of samples analyzed by Ecology during the 1988 coho smolt bioassay (see following section).

Analyses of Pulp Mill and Sewage Treatment Plant Effluents used during the 1988 Continuous-Flow Coho Smolt Bioassay

Ecology analyzed subsamples of effluents from the pulp mills and STPs that were used during the 1988 continuous-flow coho smolt bioassay.[3] In addition, EPA conducted a variety of bioassays of these effluents. Objectives of this work were to screen for potential toxicants, monitor general water quality, and assess toxicity of effluents (see Appendix A of Johnson et al. 1990 for the complete results of Ecology's analyses).

Chemistry and General Characteristics of Effluents

Ecology's samples were grabs of whole effluents from storage tanks at the bioassay tank farm complex. Weyerhaeuser effluents had been neutralized with NaOH to a pH of approximately 6.5 prior to being sampled. General water quality variables were analyzed once per day over the five day bioassay test period. On the second and fifth days of the bioassay, samples were collected for analysis of EPA priority pollutants/hazardous substances list compounds (except dioxin), herbicides, organophosphorus pesticides, quaiacols, catechols, resin acids, and fatty acids.

Results of the chemical analyses are summarized in Tables 3-6. Metals concentrations were low in effluents from STPs except for slight elevations in copper (5.6-8.7 µg/L) and zinc (22-50 µg/L).

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[3] Samples of dilution water and the reference toxicant (Cu^{++}) were also analyzed. No significant chemical contamination was found in the dilution water and it was effectively dechlorinated prior to use. The level of Cu^{++} in the reference toxicant was 13 µg/L, close to the intended spiking level of 12 µg/L.

Table 3. Metals concentrations in water samples collected by Ecology during the continuous flow coho smolt bioassay, Aberdeen, May 26-30, 1988 (μg/L; ppb-total recoverable metal) (from Johnson *et al.*, 1990).

Sample Type	Chehalis River Water		Weyerhaeuser Effluent		
Collection Date	5/27	5/30	5/28	-- 5/30 --	
Collection Time at Facility	0830	0855	1305	1225	
Time Subsample Collected	1610	1325	1510*	1630	1645
Sample Number (22-)	8234	8242	8257	8236	8237
Antimony	0.1 U	0.1 U	0.1 U	1 U	1 U
Arsenic	1 U	0.30	2 UJ	2 UJ	2 UJ
Cadmium	0.08	0.06	0.22	0.18	0.15
Chromium	0.6	0.9	1 U	5.7	5.2
Copper	1.3 B	1.4 B	6.8	8.4	8.4
Lead	2.2 J	1.2 J	2.0	1.7	1.8
Mercury	0.1 U	0.1 U	0.1 U	0.26	0.14
Nickel	1 U	1.0	6.6	6.0	5.8
Selenium	0.5 U	0.5 U	1 U	1 U	1 U
Silver	0.2	0.19	0.1 UJ	0.1 UJ	0.1 UJ
Thallium	0.1 U	0.1 U	1 U	1 U	1 U
Zinc	13 BJ	10 BJ	133 J	93 J	91 J

* Subsample was collected 5/29

Sample Type	ITT Effluent		Aberdeen STP Effluent		
Collection Date	5/27	5/30	5/27	----5/30----	
Collection Time at Facility	1500	1410	0852	1200	
Time Subsample Collected	1625	1510	1545	1610	1620
Sample Number (22-)	8231	8238	8232	8239	8240
Antimony	1 U	1 U	0.29	0.27	0.58
Arsenic	2 UJ	2 UJ	0.58	0.87	0.84
Cadmium	0.64	0.9	0.20	0.24	0.38
Chromium	486	227	1.4	1.2	1.7
Copper	5.8	8.6	8.7	8.7	5.6
Lead	4.3	5.9	2.4 J	3.3 J	1.4 J
Mercury	0.1 U	0.1 U	0.1 U	0.1	0.1 U
Nickel	7.2	6.2	2.2	2.2	2.1
Selenium	1 U	1 U	0.5 U	0.5 U	0.5 U
Silver	0.1 UJ	0.1 UJ	0.7	0.8	0.3
Thallium	1 U	1 U	0.1 U	0.1 U	0.1 U
Zinc	49 J	52 J	38	32	22

U = not detected at detection limit shown
J = an estimated value due to low matrix spike recoveries (75%)
B = detected value is less than 5 times reagent blank

Table 3. (continued)

Sample Type	Hoquiam STP Effluent		Dilution Water
Collection Date	5/27	5/30	5/27
Collection Time at Facility	0802	1308	-
Time Subsample Collected	1530	1450	1445
Sample Number (22-)	8233	8241	8235
Antimony	1.3	1.0	0.1 U
Arsenic	1.2	1.6	0.23
Cadmium	0.21	0.18	0.17
Chromium	2.2	1.6	0.8
Copper	6.1	6.9	1.2 B
Lead	5.8 J	2.0 J	0.9 J
Mercury	0.1 U	0.1	0.1 U
Nickel	2.3	2.0	1 U
Selenium	0.5 U	0.5 U	0.5 U
Silver	0.1	0.15	0.1
Thallium	0.1 U	0.1 U	0.1 U
Zinc	50 J	42 J	8 BJ

Sample Type	Transfer Blank	Transport Blank	Reference Toxicant	
Collection Date	5/30	-	5/27	5/30
Time Subsample Collected	-	-	1045	1335
Sample Number (22-)	8245	8246	8247	8263
Antimony	0.1 U	0.1 U	NA	NA
Arsenic	0.15	.18	NA	NA
Cadmium	0.05	0.05	NA	NA
Chromium	0.5 U	0.5 U	NA	NA
Copper	0.7 B	0.6 B	13	13
Lead	0.47 J	0.54 J	NA	NA
Mercury	0.1 U	0.1	NA	NA
Nickel	1 U	1 U	NA	NA
Selenium	0.5 U	0.5 U	NA	NA
Silver	0.18	0.20	NA	NA
Thallium	0.1 U	0.1 U	NA	NA
Zinc	4.2 BJ	5.3 BJ	NA	NA

U = not detected at detection limit shown
J = an estimated value due to low matrix spike recoveries (75%)
B = detected value is less than 5 times reagent blank
NA = not analyzed

Table 4. Organic priority pollutants/hazardous substances list compounds detected in water samples collected by Ecology during the continuous-flow coho smolt bioassay, Aberdeen, May 26-30, 1988 (µg/L;ppb) (from Johnson *et al.*, 1990).

Sample Type	Chehalis River Water		Weyerhaeuser Effluent			ITT Effluent	
Collection Date	5/27	5/30	5/28	--5/30--		5/27	5/30
Collection Time at Facility	0830	0855	1305	--1225--		1500	1410
Time Subsample Collected	1610	1325	1510*	1630	1645	1625	1510
Sample No. (22-)	8234	8242	8257	8236	8237	8231	8238
Volatile Organics							
Chloroform	5 U	5 U	140	180 J	170 J	220	190 J
Bromodichloromethane	5 U	5 U	5 U	5 U	5 U	5 U	5 U
Styrene	5 U	5 U	5 U	0.9 J	5 U	5 U	5 U
Carbon disulfide	5 U	5 U	0.5 J	0.3 J	0.3 J	5 U	5 U
Toluene	5 U	5 U	0.5 J	0.5 J	0.6 J	5 U	5 U
Acetone	3 U	10 U	30 B	29 U	35 U	3 U	2 U
2-Butanone	2 U	10 U	25 B	17 BJ	11 U	10 BU	10 U
Low Molecular Weight PAH							
Naphthalene	1 U	0.9 U	0.3 J	0.9 U	1 U	0.4 J	0.5 J
Acenaphthylene	1 U	0.9 U	1 U	0.9 U	1 U	0.1 J	0.9 U
Acenaphthene	1 U	0.9 U	1 U	0.9 U	1 U	0.7 J	0.9 U
Fluorene	1 U	0.9 U	1 U	0.9 U	1 U	0.06J	0.9U
Phenanthrene	1 U	0.9 U	1 U	0.9 U	1 U	1	0.9 U
High Molecular Weight PAH							
Fluoranthene	1 U	0.9 U	1 U	0.9 U	1 U	0.8	0.9 U
Pyrene	1 U	0.9 U	1 U	0.9 U	1 U	1.1	0.9 U
Benzo(a)anthracene	1 U	0.9 U	1 U	0.9 U	1 U	0.2 J	0.9 U
Chrysene	1 U	0.9 U	1 U	0.9 U	1 U	0.3 J	0.9 U
Benzo(b)fluoranthene	1 U	0.9 U	1 U	0.9 U	1 U	0.2 J	0.9 U
Benzo(k)fluoranthene	1 U	0.9 U	1 U	0.9 U	1 U	0.3 J	0.9 U
Chlorinated Organics							
bis(2-Chloroethyl)ether	1 U	0.9 U	1 U	0.9 U	1 U	5 J	0.9 U
1,4-Dichlorobenzene	1 U	0.9 U	1 U	0.9 U	1 U	0.9 U	0.9 U
Phthalates							
Dimethylphthalate	1 U	0.9 U	1	1	1 U	2	0.9 U
Di-n-butylphthalate	0.07BJ	0.9 BU	1 BU	0.9 BU	1 BU	0.9 BU	0.9BU
Butylbenzylphthalate	1 BU	0.9 BU	1 BU	0.9 BU	1 BU	0.5 BJ	1 B
bis(2-Ethylhexyl)phthalate	4 B	1 B	1 BU	0.7 BJ	1 BU	2 B	1 B
Di-n-octylphthalate	0.3 J	0.9 U	1 U	0.9 U	1 U	0.6 J	0.9 U
Phenols							
Phenol	0.5 U	0.9 U	1 U	0.9 U	1 U	0.9 U	0.9 U
4-Methylphenol	0.5 U	0.9 U	37	0.9 U	19	0.9 U	0.9 U
2,4-Dichlorophenol	0.5 U	0.9 U	1 U	2	1 U	0.9 U	0.9 U
2,4,6-Trichlorophenol	0.5 U	0.9 U	8	5	7	4 J	6
Miscellaneous Extractables							
Benzoic acid	2.5 U	5 U	5 U	5 U	11	5 U	4 U
Dibenzofuran	1 U	0.9 U	1 U	0.9 U	1 U	0.07J	0.9 U
N-Nitrosodiphenylamine	1 U	0.9 U	1 U	0.9 U	1 U	0.9 U	0.9 U
Organochlorine Pesticides	ND	ND	ND	ND	ND	ND	ND
Polychlorinated Biphenyls	ND	ND	ND	ND	ND	ND	ND

* = subsample was collected 5/29
U = not detected at detection limit shown
J = an estimated concentration
B = also detected in method blank
ND = not detected

Table 4. (continued)

Sample Type	Aberdeen STP Effluent			Hoquiam STP Effluent		Dilution Water	Transfer Blank	Transport Blank
Collection Date	5/27	--5/30--		5/27	5/30	5/27	5/30	--
Collection Time at Facility	0852	--1200--		0802	1308	--	--	--
Time Subsample Collected	1545	1610	1620	1530	1450	1445	--	--
Sample No. (22-)	8232	8239	8240	8233	8241	8235	8245	8246
Volatile Organics								
Chloroform	3 J	7 J	8 J	3 U	3 J	3 J	5 U	5 U
Bromodichloromethane	5 U	1 J	1 J	5 U	0.8 J	5 U	5 U	5 U
Styrene	2 J	1 J	1 J	5 U	5 U	5 U	5 U	5 U
Carbon disulfide	5 U	5 U	5 U	5 U	5 U	5 U	5 U	5 U
Toluene	5 U	5 U	5 U	5 U	5 U	5 U	5 U	5 U
Acetone	6 U	5 U	5 U	2 U	1 U	2 U	3 U	3 U
2-Butanone	46 B	10 BJ	13 BJ	1 U	10 U	10 U	10 U	1 U
Low Molecular Weight PAH								
Naphthalene	1 U	1 U	1 U	0.9 U	0.9 U	0.9 U	1 U	2 U
Acenaphthylene	1 U	1 U	1 U	0.9 U	0.9 U	0.9 U	1 U	2 U
Acenaphthene	1 U	1 U	1 U	0.9 U	0.9 U	0.9 U	1 U	2 U
Fluorene	1 U	1 U	1 U	0.9 U	0.9 U	0.9 U	1 U	2 U
Phenanthrene	1 U	1 U	1 U	0.9 U	0.9 U	0.9 U	1 U	2 U
High Molecular Weight PAH								
Fluoranthene	1 U	1 U	1 U	0.9 U	0.9 U	0.9 U	1 U	2 U
Pyrene	1 U	1 U	1 U	0.9 U	0.9 U	0.9 U	1 U	2 U
Benzo(a)anthracene	1 U	1 U	1 U	0.9 U	0.04J	0.9 U	1 U	2 U
Chrysene	1 U	1 U	1 U	0.9 U	0.9 U	0.08J	1 U	2 U
Benzo(b)fluoranthene	1 U	1 U	1 U	0.9 U	0.9 U	0.9 U	1 U	2 U
Benzo(k)fluoranthene	1 U	1 U	1 U	0.9 U	0.9 U	0.9 U	1 U	2 U
Chlorinated Organics								
bis(2-Chloroethyl)ether	1 U	1 U	1 U	0.9 U	0.9 U	0.9 U	1 U	2 U
1,4-Dichlorobenzene	0.3 J	0.3 J	0.3 J	0.9 U	0.9 U	0.9 U	1 U	2 U
Phthalates								
Dimethylphthalate	1	1 J	1 U	2	0.2 J	0.9 U	1 U	2 U
Di-n-butylphthalate	0.7 BJ	0.5 BJ	0.3 BJ	0.4 BJ	0.3 BJ	0.1 BJ	0.2 BJ	2 BU
Butylbenzylphthalate	0.3 BJ	0.3 BJ	1 BJ	0.3 BJ	0.1 BJ	0.2 BJ	1 BU	2 BU
bis(2-Ethylhexyl)phthalate	4 B	4 B	4 B	7 B	3 B	1 B	1 B	0.9 BJ
Di-n-octylphthalate	0.6 J	0.6 J	0.5 J	0.3 J	0.1 J	0.2 J	0.2 J	2 U
Phenols								
Phenol	0.5 J	1 J	1 U	0.9 U	0.9 U	0.9 U	1 U	2 U
4-Methylphenol	1 U	1 U	1 U	0.9 U	0.9 U	0.9 U	1 U	2 U
2,4-Dichlorophenol	1 U	1 U	1 U	0.9 U	0.9 U	0.9 U	1 U	2 U
2,4,6-Trichlorophenol	1 U	1 U	1 U	0.9 U	0.9 U	0.9 U	1 U	2 U
Miscellaneous Extractables								
Benzoic acid	2 J	0.5 J	6 U	0.5 J	5 U	0.9 U	5 U	10 U
Dibenzofuran	1 U	1 U	1 U	0.9 U	0.9 U	0.9 U	1 U	2 U
N-Nitrosodiphenylamine	5 U	1 U	1 U	0.9 U	0.1 J	0.9 U	1 U	2 U
Organochlorine Pesticides	ND	ND	ND	ND	ND	ND	ND	ND
Polychlorinated Biphenyls	ND	ND	ND	ND	ND	ND	ND	ND

* = subsample was collected 5/29
U = not detected at detection limit shown
J = an estimated concentration
B = also detected in method blank
ND = not detected

Table 5. Pesticides detected in water samples collected by Ecology during the continuous-flow coho smolt bioassay, Aberdeen, May 26-30, 1988 (μg/L; ppb) (from Johnson *et al.*, 1990).

Sample Type	Chehalis River Water		Weyerhaeuser Effluent			ITT Effluent	
Collection Date	5/27	5/30	5/28	--5/30--		5/27	5/30
Collection Time at Facility	0830	0855	1305	1225		1500	1410
Time Subsample Collected	1610	1325	1510*	1630	1645	1625	1510
Sample No. (22-)	8234	8242	8257	8236	8237	8231	8238
Pentachlorophenol	0.002	0.004	NA	NA	NA	NA	NA
2,3,4,5-Tetrachlorophenol	0.002 U	0.002 U	NA	NA	NA	NA	NA
Diuron	0.4 U	0.4 U	0.4 U	0.4 U	0.4 U	0.4 U	0.4 U

Sample Type	Aberdeen STP Effluent			Hoquiam STP Effluent		Dilution Water	Transfer Blank	Transport Blank
Collection Date	5/27	--5/30--		5/27	5/30	5/27	5/30	--
Collection Time at Facility	0852	1200		0802	1308	--	--	--
Time Subsample Collected	1545	1610	1620	1530	1450	1445	--	--
Sample No. (22-)	8232	8239	8240	8233	8241	8235	8245	8246
Pentachlorophenol	0.40	0.068	0.12	0.002 M	0.28	0.002 M	0.002 M	0.005
2,3,4,5-Tetrachlorophenol	0.066	0.032	0.08	0.002 U	0.01 M	0.002 U	0.002 U	0.005 U
Diuron	0.4 M	0.4 U	0.4 U	0.4 U	0.4 U	0.4 U	0.4 U	0.4 U

* = subsample was collected 5/29
M = presence of material verified but not quantified
NA = not analyzed

Table 6. Chlorophenols, resin acids, and fatty acids detected in water samples collected by Ecology during the continuous-flow coho smolt bioassay, Aberdeen, May 26-30, 1988 (μg/L;ppb) (from Johnson *et al.*, 1990).

Sample Type	Weyerhaeuser Effluent			ITT Effluent		Transfer Blank	Transport Blank
Collection Date	5/28	--5/30--		5/27	5/30	--	--
Collection Time at Mill	1305	--1225--		1500	1410	--	--
Time Subsample Collected	1510*	1630	1645	1625	1510	--	--
Sample No. (22-)	8257	8236	8237	8231	8238	8245	8246
Guaiacols							
Guaiacol	5	0.5 U	0.5	U 0.5	U 0.7	J 0.4	U 1U
4,5,6-Trichloroquaiacol	4	12	12	6	7	0.4	U 1U
Tetrachloroquaiacol	8	9	0.5	U 5	7	0.4	U 1U
Other Chlorophenols							
Trichlorosyringol	5	18	20	0.5 U	0.5	J 0.4	U 1U
Resin Acids							
Abietic	0.4 U	0.5	U 0.5	U 0.5	U 0.3	J 0.4	U 1U
Dehydroabietic	6	0.5	U 2	J 2	J 2	J 0.4	U 1U
Dichlorodehydroabietic	4	3 J	0.5	U 5	10	0.4	U 0.1J
Isopimaric	1 J	0.5	U 0.5	U 0.5	U 0.6	U 0.4	U 1U
Fatty Acids							
Oleic Acid	2 J	0.5	U 0.5	U 32	35	0.4	U 1U
Linoleic	2 J	0.5	U 0.5	U 0.5	U 0.6	U 0.4	U 1U

* = collected 5/29
U = not detected at detection limit shown
J = an estimated concentration

Note: Sample preservation and analysis methods used to obtain the above data where modified in 1989; the two data sets are not strictly comparable.

Several organic compounds were detected in both STP effluents such as chloroform (3-8 μg/L) bromodichloromethane (0.8-1 μg/L), benzoic acid (0.5-2 μg/L), pentachlorophenol (PCP) (0.002-0.4 μg/L), and 2,3,4,5-tetrachlorophenol (0.01-0.08 μg/L). Styrene (1-2 μg/L), 1,4-dichlorobenzene (0.3 μg/L), phenol (0.5-1 μg/L), and diuron (0.4 μg/L) were detected only in effluent from the Aberdeen plant while benzo(a)anthracene (0.04 μg/L), chrysene (0.08 μg/L), and N-nitrosodiphenylamine (0.1 μg/L) were only detected in effluent from the Hoquiam plant. Except for nitrosodiphenylamine and diuron, these compounds are commonly detected in sewage treatment plant effluents (Burns and Roe 1982).

Analyses of pulp mill effluents revealed that a number of toxicants were present at low concentrations, all of which have been previously reported in pulp mill effluents (e.g., Wallin and Condren 1981; NCASI 1981). Metals analysis showed a high concentration of chromium (227-486 ug/L) in ITT effluent. Concentrations of other metals were generally similar between the two mills and were also not substantially different than other materials used in the bioassay (e.g., STP effluents). The following concentration ranges were measured: cadmium (0.15-0.9 μg/L), chromium (<1-5.7 μg/L; Weyerhaeuser), copper (5.8-8.6 μg/L), lead (1.7-5.9 μg/L), mercury (0.14-0.26 μg/L; detected in Weyerhaeuser effluent only), nickel (6.0-7.2 μg/L), and zinc (49-133 μg/L).

Although a number of organic compounds on the EPA priority pollutants/hazardous substances list were detected, they were not

important effluent constituents. This is consistent with other studies of pulp mill effluents (NCASI 1981) and has been Ecology's general experience when analyzing effluent from other Washington pulp mills. The predominant priority pollutants/hazardous substances compounds detected were volatiles and phenols, especially chloroform (140-220 µg/L), 4-methylphenol (19-37 µg/L; detected in Weyerhaeuser effluent only), and 2,4,6-trichlorophenol (4-8 µg/L).

Concentrations of quaiacols, resin acids, and fatty acids in pulp mill effluent ranged from 0.5-20 µg/L, 0.3-10 µg/L, and 2-35 µg/L, respectively. Because of problems encountered in extraction and derivitization of catechols, concentrations of these compounds could not be measured in 1988. Sample preservation and analytical methods were modified in 1989. As a result, the 1988 and 1989 data for quaiacols, resin acids, and fatty acids are not strictly comparable.

Results of the 1988 coho bioassay showed that the most significant adverse effects were for smolts exposed to 30% Weyerhaeuser effluent (see PART II, Hypothesis 3). At a 30% dilution, concentrations of most potentially toxic chemicals detected by Ecology would be at levels near or below detection limits (generally 1 µg/L or less). Somewhat higher concentrations of zinc (30-40 µg/L), chloroform (40-50 µg/L), and 4-methylphenol (5-10 µg/L) may have occurred. The above concentrations are below known thresholds of toxicity to salmonids (e.g., Lorz et al. 1978b; EPA 1986).

EPA Bioassays

EPA conducted a number of bioassays in 1988 to evaluate toxicity of pulp mill and STP effluents including the following:

1. A freshwater algae (*Selenastrum capricornutum*)- growth following a 96 hr exposure to effluent.

2. Sea urchin (*Strongylocentrotus purpuratus*)- percent fertilization success.

3. *Ceriodaphnia dubia* (a freshwater zooplankter sometimes referred to as the water flea)- effects on reproduction and mortality after 7 day exposures.

4. Coho salmon (*Oncorhynchus kisutch*)- survival of coho in a 96 hr exposure.

5. Fathead minnow (*Pimephales promelas*)- growth and survival in a 7 day exposure.

The *Selenastrum*, sea urchin, and *Ceriodaphnia* tests were conducted by an EPA contractor, ERC Environmental of San Diego, California. Effluent samples for these tests were shipped to San Diego where the bioassays were performed. The sea urchin test was performed only with pulp mill effluents and the *Selenastrum* and *Ceriodaphnia* tests were performed with effluents from pulp mills and STPs.

The coho salmon and fathead minnow tests were performed in EPA's Region 10 mobile bioassay laboratory (EPA unpublished). Coho salmon bioassays were 96 hr flow-through tests using the same pulp mill effluents as those used in the continuous-flow bioassays described previously in PART II, Hypothesis 3. Bioassays performed

in EPA's mobile laboratory used fish from the same source (i.e., Humptulips Hatchery) and tested the same effluents as those used in the continuous-flow tests. Flow-through tests utilized procedures presented in EPA (1985) and APHA (1985).

Fathead minnow experiments were static renewal tests that included some of the same effluents used in the continuous-flow coho experiments. Tests ran for 7 days (168 hrs) and followed EPA methods described in Norberg and Mount (1985) and EPA (1988). Survival and mean weight (gr) of fish remaining after the exposure period were determined for each dilution tested.

STP effluents either elicited no effect or a variable response, indicating no consistent effect while pulp mill effluents demonstrated more toxicity (Table 2). In the sea urchin fertilization tests, the NOEC for both mills was 4.8% effluent. An LC-50 of 35% was calculated for Weyerhaeuser effluent in the *Ceriodaphnia* bioassay while there was no significant mortality in tests with ITT effluent. In the flow-through tests with coho, the only mortalities were in 100% Weyerhaeuser effluent where 5/12 coho died after 96 hrs (Table 2).

In the fathead minnow bioassays, the test protocol requiring dissolved oxygen to be greater than 40% saturation could not be met for effluent concentrations greater than 50%; thus, data for these dilutions may be biased. The estimated LC-50 for Weyerhaeuser effluent after 168 hours was 60% (95% C.I. = 44 to 81%). The ITT and STP effluents did not cause acute toxicity in the fathead minnow test. Fathead minnow growth data suggested that both pulp

mill effluents were chronically toxic (Fig. 1) with an NOEC for Weyerhaeuser and ITT effluents of 12.5% and 25%, respectively.

Chemical Characterization of Grays Harbor Pulp Mill Effluents, March-June 1989

The most extensive analysis of pulp mill effluent was conducted in 1989 by Ecology and EPA (Johnson et al. 1990; EPA unpublished). Other effluents (e.g., STP's) were not evaluated in 1989 because 1988 results indicated they did not affect survival of coho salmon smolts. The major objectives were to: 1) further define the chemical character and toxicity (i.e., via bioassays) of effluents, 2) test for relationships between toxicity and concentrations of effluents, and 3) assess effluent variability, in particular determine if effluents discharged during the salmon bioassay and live box experiments in 1989 were representative of typical discharges. Ecology monitored effluent quality from 3/7/89 to 6/20/89 to encompass the time period when the coho smolt bioassay and live box experiments were conducted (4/21-5/26) and to cover the time period most coho smolts move through the inner harbor. EPA collected samples of effluent in late April and early May at the bioassay tank farm using a continuous-flow centrifuge and XAD resin columns.

General Methods

Several different procedures were followed to obtain samples. Most samples for chemical analyses and bioassays were collected by Ecology with an ISCO automatic compositor at intervals of 5 min at Weyerhaeuser and 15 min at ITT (approximately 15 liters total were

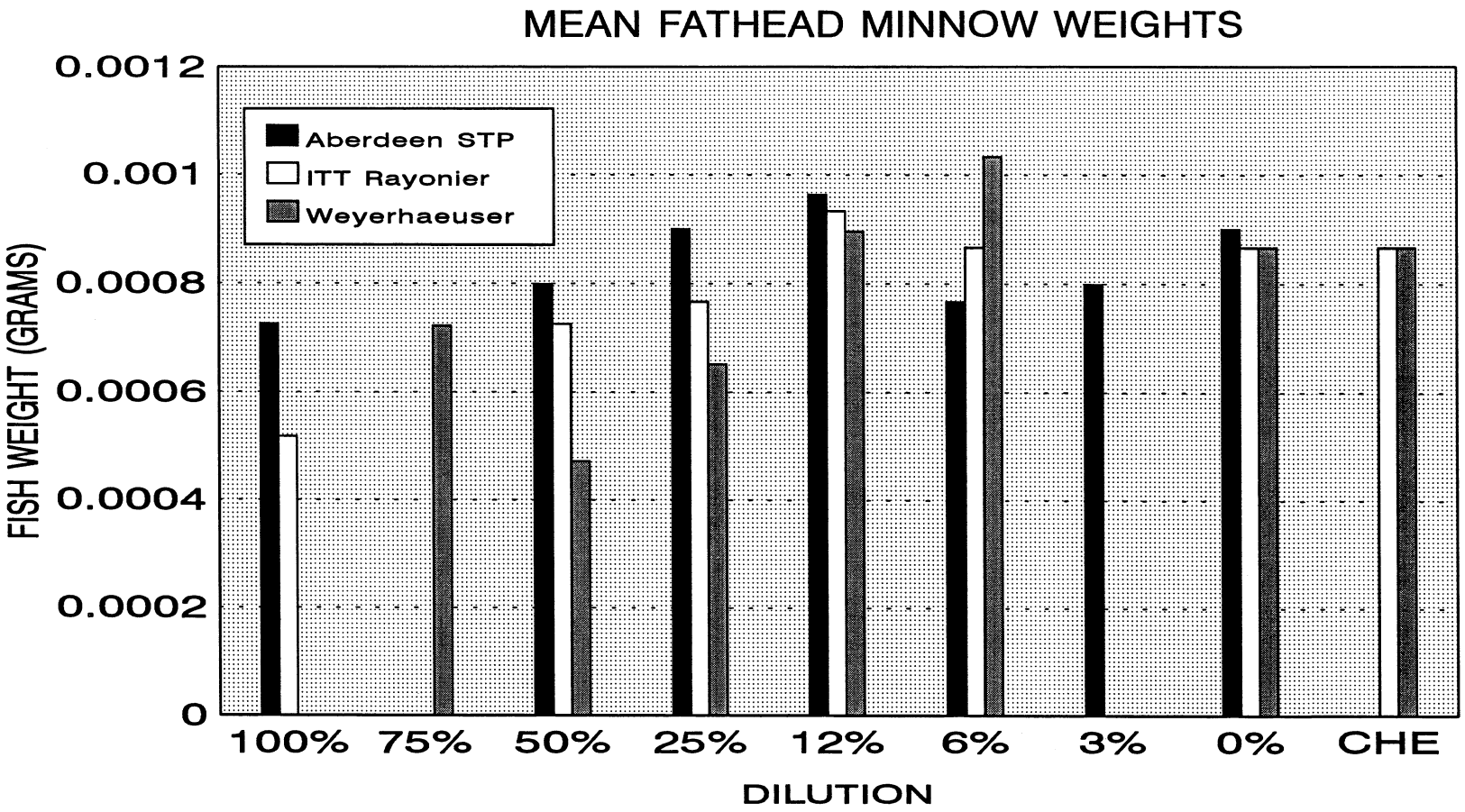

Fig. 1. Mean weights of fathead minnows exposed to serial dilutions of Weyerhaeuser and ITT Rayonier pulp mill effluent, Aberdeen sewage treatment water and Chehalis River water for 168 hours in 1988.

obtained at each mill). However, TOX and volatile samples were single grabs since compositing was not appropriate for these analyses. Because compositors could not hold the large volumes required for bioassays (approximately 90 liters), bioassay samples were from composites of four grabs spaced over the sampling period.

Weyerhaeuser effluent samples were collected over a period of two hours during one of its twice-daily discharge cycles. During this study, Weyerhaeuser was discharging only during ebb tide for a period of 2 to 2 1/2 hours. ITT samples were 4-hour composites. Sampling time was longer at ITT because of its continuous discharge.

Effluent samples were collected on eight separate occasions at Weyerhaeuser and four at ITT. Greater sampling effort was devoted to Weyerhaeuser because of its history of failing the rainbow trout bioassays, and because the mill produces multiple grades of pulp. Two effluent collections were made at Weyerhaeuser and one at ITT during the coho salmon survival experiments in April and May. The mills were not told in advance when samples would be collected.

EPA obtained samples of pulp mill effluents at the bioassay tank farm during the coho smolt bioassay using a continuous-flow centrifuge to concentrate suspended solids and accompanying hydrophobics. Water from the centrifuged sample portion was then run through XAD resin columns to concentrate hydrophilics. Centrifuges and resin columns were used because many toxicants often found in pulp mill effluents occur in such minute quantities that they can be too dilute for detection in whole water samples.

Although the XAD resin columns were employed both in 1988 and 1989, the 1988 data are suspect because of QA/QC problems and thus were not included in this report. These problems were not encountered in 1989 so those results are included.

Chemical analyses conducted in 1987 and 1988 were used to select the chemicals analyzed in 1989. Target chemicals in Ecology's studies included metals (cadmium, chromium, copper, lead, mercury, nickel, silver, and zinc), a subset of EPA priority pollutant compounds (volatiles and selected phenols), guaiacols, catechols, resin acids, and fatty acids (Table 7). Chemical analyses by EPA focused on chlorinated compounds, quaiacols, catechols, and phenols. Ecology also measured conventional variables including temperature, pH, specific conductance, color, TSS, total recoverable phenolics, and total organic halogens (TOX). Flow data were obtained from the mills.

Results of Ecology's Analyses

Table 8 summarizes general characteristics of the effluents. Weyerhaeuser and ITT discharges were comparable in terms of average flow (22.2 vs. 20.4 MGD), temperature (20.5 vs. 21.6 °C), and TOX (27.2 vs. 22.2 mg/L). Specific conductance (3,300 vs. 1,600 μmhos/cm), TSS (152 vs. 68 mg/L), and total recoverable phenolics (13.9 vs. 6.2 mg/L) in Weyerhaeuser effluent averaged approximately twice that found in ITT effluent. The average pH of the effluents was 2.6 at Weyerhaeuser and 7.0 at ITT.

Concentrations of metals and organic compounds are summarized in Table 9. Metals concentrations compared closely between the two

Table 7. Effluent analyses conducted by Ecology during their evaluation of pulp mill effluents from March to June 1989. The analyses included conventional measurements, metals, volatile organic chemicals, phenols/guaiacols/catechols, resin acids/fatty acids, and bioassays (from Johnson *et al.*, 1990).

Conventionals

pH
specific conductance
color
total suspended solids
total recoverable phenolics
total organic halides

Metals

cadmium
chromium
copper
lead
mercury
nickel
silver
zinc

Volatiles

chloromethane
bromomethane
vinyl chloride
chloroethane
methylene chloride
acetone
carbon disulfide
1,1-dichloroethene
1,1-dichloroethane
trans-1,2-dichloroethene
cis-1,2-dichloroethene
chloroform
1,2-dichloroethane
2-butanone
1,1,1-trichloroethane
carbon tetrachloride
vinyl acetate
bromodichloromethane
1,2-dichloropropane
trans-1,3-dichloropropene
trichloroethene
dibromochloromethane
1,1,2-trichloroethane
benzene
cis-1,3-dichloropropene
2-chloroethylvinylether

Volatiles (Continued)

bromoform
4-methly-2-pentanone
2-hexanone
tetrachloroethene
1,1,2,2-tetrachloroethane
toluene
chlorobenzene
ethylbenzene
styrene
total xylenes

Phenols/Guaiacols/Catechols

4-chloro-3-methylphenol
pentachlorophenol
2,4,6-trichlorophenol
2-nitrophenol
guaiacol
2-methylphenol
2-chlorophenol
2,4,5-trichlorophenol
4-allylguaiacol
4-propenylguaiacol
acetophenone
4-nitrophenol
2,4-dimethylphenol
4-methylphenol
phenol
2,4-dichlorophenol
tetrachloroguaiacol
4,5,6-trichloroguaiacol
6-chlorovanillin
5,6-dichlorovanillin
tetrachlorocatechol
4-chlorocatechol
4,5-dichloroguaiacol
trichlorosyringol
4,5-dichlorocatechol
a-terpineol
4-chloroguaiacol
3,4,5-trichlorocatechol

Resin Acids/Fatty Acids

hexadecanoic acid
octadecanoic acid

Table 7. (Continued)

Resin Acids/Fatty Acids (Continued)

linoleic acid
levopimaric acid
oleic acid
pimaric acid
palmitoleic acid
sandaracopimaric acid
neoabietic acid
retene
abietic acid
9,10-dichlorostearic acid
dichlorodehydroabietic acid
14-chlorodehydroabietic acid
12-chlorodehydroabietic acid
dehydroabietic acid
eicosatrienoic acid
palustric acid
isopimaric acid

Bioassays

rainbow trout
microtox^{R}
oyster larvae
echinoderm sperm cell

Table 8. General characteristics of pulp mill effluents measured by Ecology, March-June 1989 (from Johnson *et. al.*, 1990).

Variable	Weyerhaeuser (n=8) Average (Range)	ITT (n=4) Average (Range)
Flow (MGD)	22.2 (18.4 - 27.0)	20.4 (20.0 - 20.9)
Temperature (°C)	20.5 (17.4 - 23.4)	21.6 (18.4 - 24.9)
pH (S.U.)	2.6 (2.2 - 3.0)	7.0 (6.8 - 7.5)
Spec. Conductance (μmhos/cm)	3300 (2200 - 4300)	1600 (1350 - 1850)
Color (units)	1840 (1070 - 2460)	1840 (1130 - 2420)
TSS (mg/L)	152 (74 - 380)	68 (37 - 150)
t. rec. phenolics (μg/L)	13.9 (10.0 - 18.8)	6.2 (4.1 - 8.0)
TOX (mg/L)	27.2 (22.5 - 39.0)	22.2 (16.0 - 26.0)

Table 9. A listing of potentially toxic chemicals detected by Ecology in pulp mill effluents (μg/L), March-June 1989. For each chemical detected, the detection frequency, average concentration and range are presented (from Johnson *et al.*, 1990).

Chemical	Weyerhaeuser Detection Frequency (n=8)	Weyerhaeuser Average Conc. (Range)	ITT Detection Frequency (n=4)	ITT Average Conc. (Range)
Metals				
cadmium	4	**0.3** (0.2U - **1.0**)	4	**0.7** (**0.5** - **0.9**)
chromium	6	**8.7** (**5.1** - **23**)	4	**67** (**45** - **87**)
copper	8	**10** (**4** - **16**)	4	**8.8** (**3.9** - **13**)
lead	8	**2.7** (**1.0** - **5.4**)	4	**2.2** (**1.3** - **3.3**)
nickel	8	**19** (**15** - **27**)	3	**7.6** (**1.9** - 20U)
zinc	8	**44** (**21** - **110**)	4	**30** (**22** - **39**)
Volatiles				
chloroform	8	**13** (**0.7** - **18**)	4	**135** (**110** - **170**)
2-butanone	6	**20** (1.0U - **39**)	0	1.0U (0.1U-6.2U)
toluene	8	**4.6** (**1.4** - **13**)	1	0.6U (0.6U - 0.8U)
bromodichloromethane	1	0.2U (0.2U - **0.4**)	3	**0.6** (0.2U - **1.1**)
1,2-dichloropropane	0	0.6U (0.6U all)	1	0.6U (**0.5** - 0.7U)
Phenols				
4-methylphenol	8	**28** (**10** - **54**)	3	**0.4** (**0.2** - 1U)
2,4-dimethylphenol	5	**0.2** (**0.1** - 1U)	0	0.5U (0.5U - 1U)
2-chlorophenol	5	**0.3** (**0.1** - 1U)	0	0.5U (0.5U - 1U)
2,4-dichlorophenol	8	**0.2** (**0.6** - **5**)	3	**0.4** (**0.2** - 1U)
2,4,6-trichlorophenol	8	**7** (**2** - **15**)	3	**0.7** (**0.5** - **1**)
Guaiacols				
guaiacol	8	**2** (**0.5** - **5**)	3	**0.4** (**0.1** - 1U)
4-allylguaiacol	4	**0.4** (**0.2** - 1U)	0	0.5U (0.5U - 1U)
4-chloroguaiacol	5	**0.3** (**0.1** - 1U)	0	0.5U (0.5U - 1U)
4,5-dichloroguaiacol	8	**2** (**0.2** - **3**)	4	**0.8** (**0.3** - **2**)
4,5,6-trichloroguaiacol	7	**1** (**0.3** - **3**)	4	**0.3** (**0.1** - **0.6**)
tetrachloroguaiacol	8	**4** (**1** - **7**)	4	**1** (**0.6** - **2**)
Catechols				
4-chlorocatechol	7	**0.9** (**0.4** - **2**)	2	**0.3** (**0.1** - 1U)
4,5-dichlorocatechol	8	**6** (**1** - **14**)	4	**0.6** (**0.3** - **1**)
3,4,5-trichlorocatechol	8	**27** (**5** - **56**)	4	**4** (**1** - **5**)
Other Chlorophenols				
trichlorosyringol	7	**9** (**0.4** - **35**)	0	0.5U (0.5U - 1U)
6-chlorovanillin	4	**0.9** (0.5U - **2**)	4	**0.9** (**0.4** - **2**)
5,6-dichlorovanillin	5	**1** (0.5U - **2**)	4	**0.9** (**0.4** - **2**)
Resin Acids				
abietic	1	0.6U (0.4U - **3**)	0	0.4U (0.4U - 0.5U)
dehydroabietic	8	**15** (**1** - **36**)	2	0.4U (**0.3** - 0.5U)
12-chlorodehydroabietic	8	**25** (**1** - **50**)	3	**0.6** (0.5U - **1.6**)
14-chlorodehydroabietic	8	**10** (**0.5** - **22**)	1	0.2U (**0.2** - 0.5U)
dichlorodehydroabietic	8	**11** (**1** - **27**)	0	0.5U (0.4U - 0.5U)
palustric	2	0.6U (0.4U - **3**)	0	0.5U (0.4U - 0.5U)
isopimaric	3	**0.9** (0.4U - **2**)	0	0.5U (0.4U - 0.5U)

U = not detected at detection limit

Table 9. (Continued)

Chemical	Weyerhaeuser Detection Frequency (n=8)	Weyerhaeuser Average Conc. (Range)	ITT Detection Frequency (n=4)	ITT Average Conc. (Range)
Fatty Acids				
Oleic	7	**30** (0.4U - **97**)	2	**2** (0.4U - **3**)
linoleic	3	**0.6** (0.4U - **4**)	0	0.5U (0.4U - 0.5U)
hexadecanoic	6	**32** (**5** - **64**)	3	**4** (**2** -**5**)
octadecanoic	6	**32** (**3** - **70**)	3	**2** (**2** -**2**)
palmitoleic	4	**6** (0.4U - **16**)	2	**1** (0.5U - **3**)
9,10-dichlorostearic	6	**6** (**2** - **10**)	3	**2** (**1** - **3**)
Miscellaneous				
a-terpineol	1	0.5U (0.4U - 1U)	0	0.5U (0.5U - 1U)

U = not detected at the detection limit shown

mills, with the exception of chromium which was consistently elevated in ITT's wastewater (average concentration of 67 vs. 8.7 μg/L). ITT traced the source of chromium to contaminated sodium chloride utilized in generating chlorine dioxide for bleaching pulp and now uses a more highly refined salt. Chromium concentrations in ITT effluent are currently about 10 μg/L (D. Kjosness, Washington Department of Ecology, pers. comm.).

More organic chemicals were detected in Weyerhaeuser effluent than in ITT effluent (Table 9). Concentrations of organics in Weyerhaeuser effluent were also higher than in ITT effluent, probably reflecting the fact that only 15-20% of the total plant flow was subjected to activated sludge treatment. The predominant compounds detected in Weyerhaeuser effluent were chloroform, butanone, methylphenol, trichlorocatechol, dehydroabietic acid, chlorodehydroabietic acid (2 isomers), dichlorodehydroabietic acid, oleic acid, hexadecanoic acid, and octadecanoic acid, each of which had an average concentration of 10 μg/L or more. In ITT effluent, the compounds present in the highest concentrations were chloroform, tetrachloroguaiacol, trichlorocatechol, tetrachlorocatechol, and several fatty acids. Other than chloroform, individual organic compounds in ITT effluent never exceeded 5 μg/L and were typically about 1 μg/L or less.

There were several noteworthy findings revealed by Ecology's analyses of organic chemicals. First, the concentration of chloroform in ITT's discharge was considerably greater than in Weyerhaeuser's (110-170 μg/L vs. 0.7-18 μg/L). Lower chloroform

levels in Weyerhaeuser effluent may be due to the greater opportunity for volatilization afforded by the longer retention time of material during treatment (approximately 40 hours vs. 8 hours at ITT) and greater surface area provided by Weyerhaeuser's four treatment ponds.

Second, concentrations of chlorinated compounds (phenols, guaiacols, and catechols) were greater in Weyerhaeuser's effluent than in ITT's (Table 9). Because Weyerhaeuser is now utilizing oxygen delignification in the bleaching process to reduce the amount of chlorine used in pulp production, concentrations of chlorinated compounds in their effluent should decrease.

Daily load estimates of selected potentially toxic constituents discharged into Grays Harbor were made using Ecology's data. The estimated, combined daily load for the mills ranged from 0.09 kg/day of cadmium to 13.5 kg/day of resin and fatty acids. The combined loading estimate of TOX was approximately 3,960 kg/day, indicating that individual chlorinated compounds analyzed during the study represented only a small fraction of the total halogenated material released into the harbor.

Results of EPA's Analyses

EPA analyzed the SPM and XAD from three different effluent samples obtained at the bioassay tank farm: 1) Weyerhaeuser effluent collected on 4/22/89, 2) ITT effluent collected on 4/23/89, and 3) a 50% Weyerhaeuser and 50% ITT mixture collected on 5/5/89. Results of the analyses of these samples are presented in Tables 10 and 11.

Table 10. Organic compounds detected in suspended particulate matter obtained from Weyerhaeuser and ITT Rayonier pulp mill effluent. Mill effluent was processed through a continuous-flow centrifuge during the 1989 continuous-flow coho salmon bioassays. Concentrations are in μg/kg.

Organic Compound	TYPE OF EFFLUENT Weyerhaeuser (4/22/89)	 ITT Rayonier (4/23/89)	 50/50 Mix (5/05/89)
RESIN AND FATTY ACIDS			
Hexadecanoic Acid	380,000 B[1]	260,000 BJ[2]	660,000 BJ
Octadecanoic Acid	210,000 B	130,000 BJ	240,000 BJ
Linoleic Acid	**230,000**	3,000 J	37,000 J
Oleic Acid	280,000 BJ	88,000 BJ	56,000 BJ
Palmitoleic Acid	370,000 J	290,000 J	610,000 J
Abietic Acid	4,000 J	**9,000**	4,900 J
9,10-Dichlorostearic Acid	11,000 B		
Dichlorodehydroabietic Acid	82,000 J	6,000 J	55,000 J
14-Chlorodehydroabietic Acid	40,000 J	8,000 J	
12-Chlorodehydroabietic Acid	110,000 J	**23,000**	63,000 J
Dehydroabietic Acid	11,000 B	12,000 B	7,300 J
Eicosatrienoic Acid	4,000 J	**24,000**	
Isopimaric Acid	6,000 J		
PHENOLS, GUAIACOLS AND CATECHOLS			
Phenol	3,100 BJ	3,400 BJ	4,400 BJ
2,4-Dichlorophenol	**3,900**	**2,900**	2,000 J
Trichlorosyringol	24,000 J		**5,100**
Pentachlorophenol			1,300 B
2,4,6-Trichlorophenol	**11,000**	**4,600**	**4,600**
2-Methylphenol	270 BJ	430 BJ	210 BJ
Acetophenone	330 BJ	670 BJ	260 BJ
4-Methylphenol	**14,000**	250,000 J	120,000 J
4-Allylguaiacol	460 J	**2,200**	460 J
Guaiacol	**1,800**	28,000 J	**4,400**
Tetrachloroguaiacol	**8,500**	2,600 J	**2,500**
4,5,6-Trichloroguaiacol	**2,800**		
4,5-Dichloroguaiacol	1,700 J	1,200 J	670 J
4,5-Dichlorocatechol	**9,500**	1,500 J	**4,300**
3,4,5-Trichlorocatechol	110,000 J	6,700 J	44,000 J
Tetrachlorocatechol	20,000 J	2,000 J	10,000 J
4-Chlorocatechol	700 J	410 J	**250**

B[1] = Contaminated blank
J[2] = Estimated value

Table 11. Organic compounds detected in Weyerhaeuser and ITT Rayonier pulp mill effluents using a XAD resin column. Samples were collected during the 1989 continuous-flow coho salmon bioassays. Concentrations are in ng/L.

Organic Compound	TYPE OF EFFLUENT Weyerhaeuser (4/22/89)	ITT Rayonier (4/23/89)	50/50 Mix (5/05/89)
RESIN AND FATTY ACIDS			
Hexadecanoic Acid	5,000 B[1]	4,000 B	15,000 BJ
Octadecanoic Acid	5,900 B	3,200 B	9,500 BJ
Oleic Acid			2,500 B
Palmitoleic Acid			2,000 J
9,10-Dichlorostearic Acid	**4,400**	**1,700**	**1,000**
Dichlorodehydroabietic Acid	**2,400**		1,300 J
14-Chlorodehydroabietic Acid	1,100 J		
12-Chlorodehydroabietic Acid	2,300 J		9,600 J
PHENOLS, GUAIACOLS AND CATECHOLS			
Phenol	560 BJ	98 BJ	660 BJ
2,4-Dichlorophenol	**2,100**	290 J	**960**
Trichlorosyringol	12,000 J		**3,900**
o-Chlorophenol			55 J
Pentachlorophenol			**280**
2,4,6-Trichlorophenol	**6,000**	**1,000**	**2,800**
2-Methylphenol	150 BJ	23 BJ	
4-Methylphenol	9,300 J	76 J	**13,000**
2,4-Dimethylphenol	38 J		25 J
5,6-Dichlorovanillan		210 J	
6-Chlorovanillan	640 J	270 J	**180**
Acetophenone	1,100 BJ	370 BJ	380 B
4-Allylguaiacol	440 J		
Guaiacol	**1,900**	130 J	530 J
Tetrachloroguaiacol	**2,600**	**1,100**	**1,200**
4-Chloroguaiacol	240 J		
4,5,6-Trichloroguaiacol	**1,300**	120 J	
4,5-Dichloroguaiacol	**1,300**	190 J	**140**
4,5-Dichlorocatechol	**4,400**	84 J	**1,100**
3,4,5-Trichlorocatechol	18,000 J	660 J	4,900 J
2-Cyclopenten-1-one,2+	260 J		**130**
Tetrachlorocatechol	3,700 J	520 J	**1,900** J
4-Chlorocatechol	250 J		**60**

B[1] = Contaminated blank
J[2] = Estimated value

A total of 38 chemicals were detected[4] in the SPM and XAD. In general, there were more resin and fatty acids in the SPM than the XAD while there were more guaiacols, phenols, and catechols in the XAD than the SPM.

The list of chemicals found in the SPM from the three effluent samples was generally comparable. There were 25 chemicals detected in the Weyerhaeuser effluent, 24 in the ITT effluent, and 24 in the 50:50 mixture (Table 10). The chemicals detected at the highest concentration in each SPM sample were 3,4,5-trichlorocatechol (110,000 µg/kg, estimate) in the Weyerhaeuser effluent, 4-methylphenol (250,000 µg/kg, estimate) in the ITT effluent, and 4-methylphenol (120,000 µg/kg, estimate) in the 50:50 mixture (Table 10).

In the XAD samples, 24 compounds were detected in Weyerhaeuser effluent, 16 in the ITT sample, and 23 in the 50:50 mixture (Table 11). One notable difference between the three XAD samples was the occurrence of PCP in the mixture but not in either of the single effluent samples. Dominant compounds detected in the XAD from Weyerhaeuser effluent were 3,4,5-trichlorocatechol, trichloro-syringol, and 4-methylphenol. In the XAD sample from ITT effluent, the major compounds present were 2,4,6-trichlorophenol, 9,10-dichlorostearic acid, and tetrachloroguaiacol. Significant compounds present in the mixed effluent sample were hexadecanoic

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[4] In the analyses of SPM and XAD samples, compounds were considered detected if: 1) their concentration could be estimated (even if there was some blank contamination), or 2) their concentration could be accurately quantified.

acid, 4-methylphenol, 2,4,6-trichlorophenol, and 12-chloro-dehydroabietic acid (Table 11).

Effluent Bioassays

Recognizing that it would not be possible to analyze all of the several hundred potentially toxic chemicals reported to occur in pulp mill effluent, the monitoring program relied heavily on bioassays to assess effluent quality. Coho or other migratory salmonids were not used because standard bioassay protocols have not been developed for these species. The following tests were conducted:

1. Rainbow trout (*Oncorhynchus mykiss*, formerly *Salmo gairdneri*) - percent survival during 96 hr exposure to 65% effluent.

2. Microtox (*Photobacterium phosphoreum*, a luminescent marine bacterium) - percent decrease in light output after 15 minute exposure to 5.7-45.5% effluent.

3. Pacific oyster larvae (*Crassostrea gigas*) - percent developmental abnormality after 48 hr exposure to 0.1-18% effluent.

4. Echinoderm sperm cell (sea urchin, *Strongylocentrotus* spp. and sand dollar, *Dendraster excentricus*) - percent fertilization success following a 60 minute exposure to 0.1 to 50% effluent.

5. Fathead minnows (*Pimephales promelas*) - survival and growth in a 168 hr exposure.

The first four bioassays were performed by Ecology each time

effluent was collected at the mills while the fathead minnow bioassays were performed by EPA when they collected effluent at the bioassay tank farm. Results of the bioassays are presented in Table 12. Weyerhaeuser effluent elicited a toxic response in all five tests. ITT effluent was non-toxic in the rainbow trout and Microtox bioassays (one Microtox assay showed marginal evidence of toxicity), but was toxic in the oyster and echinoderm bioassays. Results of each test are discussed in greater detail below.

Depending on when samples were collected, Weyerhaeuser effluent was either highly toxic or non-toxic to rainbow trout. There was essentially 100% mortality (0-13% survival) of trout in the samples collected 3/21, 5/31, and 6/20 while there was 100% survival in effluent samples collected on the five other occasions. Low survival was never observed on two consecutive sampling dates.

The results of Microtox assays of Weyerhaeusers' effluent were variable, showing EC50's ranging through, approximately, 24%, 30%, 56%, 87%, and >100% (no effect). Four of the eight Weyerhaeuser effluent samples showed little or no toxicity using this test.

Weyerhaeuser and ITT discharges were consistently extremely toxic to oyster larvae and echinoderm sperm (Table 12). For both effluents, the oyster bioassay was the more sensitive of the two tests. ITT effluent was about four times more toxic to oyster larvae than Weyerhaeuser effluent, with average EC50s of 0.2% (range 0.1-0.3%) and 0.8% (range 0.4-1.3%), respectively. Both effluents exhibited similar toxicity in the echinoderm bioassay; EC50s averaged 6.8% and 5.9% for ITT and Weyerhaeuser effluents,

Table 12. Results of bioassays of pulp mill effluents conducted by EPA and Ecology in 1989. Values shown are percent of effluent which resulted in a defined adverse effect (from Johnson et al. (1990) and EPA (unpublished data)).

Date	Rainbow Trout Survival[a]	Microtox EC50[b]	Pacific Oyster Larvae Abnormality EC50[c]	LOEC[d]	NOEC[e]	Echinoderm Fertilization Success EC50	LOEC	NOEC	Species	Fathead Minnow NOEC[f]
Weyerhaeuser										
3/07	100	29.7	0.5	0.5	0.1	4.3	3.0	1.0	Purple urchin	-
3/21	13	24.0	0.6	0.1	<0.1	14.6	3.0	1.0	Green urchin	-
4/05	100	86.7	1.0	0.5	0.1	5.0	3.0	1.0	Green urchin	-
4/24	100	>100.0	1.1	3.2	1.0	9.2	3.0	1.0	Green urchin	50
5/17	100	>100.0	0.6	0.5	0.1	1.1	1.0	0.1	Sand Dollar	-
5/31	3	>100.0	0.9	1.0	0.5	6.4	1.0	0.1	Sand Dollar	-
6/12	100	56.1	1.3	1.0	0.5	2.6	1.0	0.1	Sand Dollar	-
6/20	0	56.9	0.4	0.5	0.1	4.0	3.0	1.0	Sand Dollar	-
ITT Rayonier										
3/07	100	>100	0.1	0.1	<0.1	5.1	6.1	3.0	Purple urchin	-
4/15	100	>100	0.2	0.5	0.1	9.4	3.0	1.0	Green urchin	-
4/24	100	>100	0.3	0.5	0.1	8.7	0.1	<0.1	Green urchin	100
5/31	100	85.5	0.3	0.5	0.1	3.9	1.0	0.1	Sand dollar	-

a Percent survival in 65% effluent.
b Percent effluent concentration causing 50% loss of light emmission.
c Percent effluent concentration at which 50% of test population shows an effect
d Lowest observed effect concentration.
e No observed effect concentration.
f The EPA fathead minnow test was begun 4/25.

respectively.

In the fathead minnow test conducted by EPA, all specimens in the 100% Weyerhaeuser effluent dilution were dead by day 2 (Table 12); the LC-50 for Weyerhaeuser effluent, based upon this test, was 50%. Few mortalities were observed at any dilution of ITT effluent.

Comparison of the above results from Grays Harbor mills with the limited number of Microtox, oyster larvae, and echinoderm bioassays conducted by Ecology at other Washington pulp mills suggested that Grays Harbor effluents were not uniquely toxic. The following EC50s have been observed at other mills: Microtox - 58.9%, 67.3%, >100%; oyster larvae abnormality - 0.5%, 1.7%, 3.0%; echinoderm sperm cell 0.6%, 2.4% (Reif 1989b, 1990).

Receiving water dilutions needed to reduce effluent concentrations to the average NOECs observed in the oyster and echinoderm bioassays were calculated. Review of dye studies done by Weyerhaeuser during June, 1988 (Weyerhaeuser Technical Center 1988) indicated effluent dilution in much of inner Grays Harbor may not be sufficient to reduce concentrations to average NOEC levels. Low salinity in receiving waters and uncertainty regarding correspondence between laboratory bioassays and receiving water toxicity need to be considered in assessing dilution requirements using these types of data.

Chemistry/Bioassay Correlations

The chemical and bioassay data obtained by Ecology for Weyerhaeuser effluent were examined for links among effluent

constituents and responses of bioassay organisms using Spearman's non-parametric test of correlation. Similar calculations were not done for ITT because of the small sample size.

Although a number of chemical constituents were significantly correlated, only one significant correlation, total phenols with the Microtox bioassay results (r_s = .73 (α=.05)), was found between chemical constituents and bioassay responses. Moreover, none of the bioassay results were significantly correlated. A review of relevant toxicity data showed that levels of metals and organic compounds measured in Weyerhaeuser and ITT effluents were one or more orders of magnitude below concentrations shown to produce adverse effects in these tests (e.g., Woelke 1972; Bitton 1982; Cherr et al. 1987; Ribio and Kaiser 1987). Thus, test organisms were apparently responding to different chemicals, concentration levels, additive or synergistic effects of pollutants, and/or other toxicants not analyzed.

Variability in Effluent Quality

Concentrations of metals and organic compounds measured by Ecology in Weyerhaeuser and ITT effluent samples collected during the continuous-flow coho smolt bioassay and live box experiments were compared with similar data obtained from other time periods (Figures 2 and 3). One of the primary reasons for making this comparison was to assess if effluents used during experiments were "representative". For both Weyerhaeuser and ITT, concentrations of most constituents measured in effluent samples during the coho salmon experiments were comparable to those measured during other

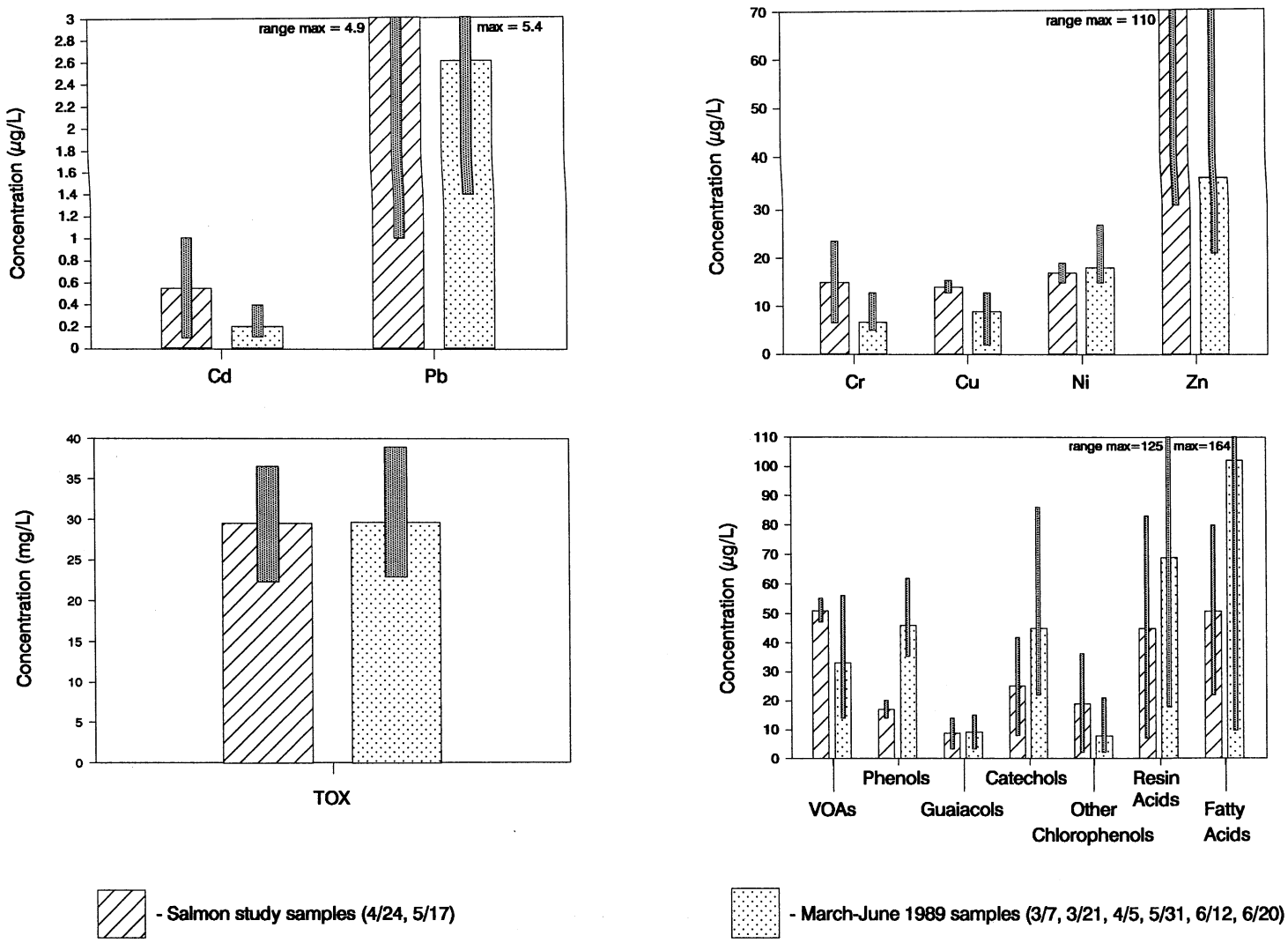

Fig. 2. Toxic constituents in Weyerhaeuser effluent samples collected during salmon survival studies compared to other samples collected March-June 1989. Small bars within graphs represent data ranges (Johnson et al., 1990)

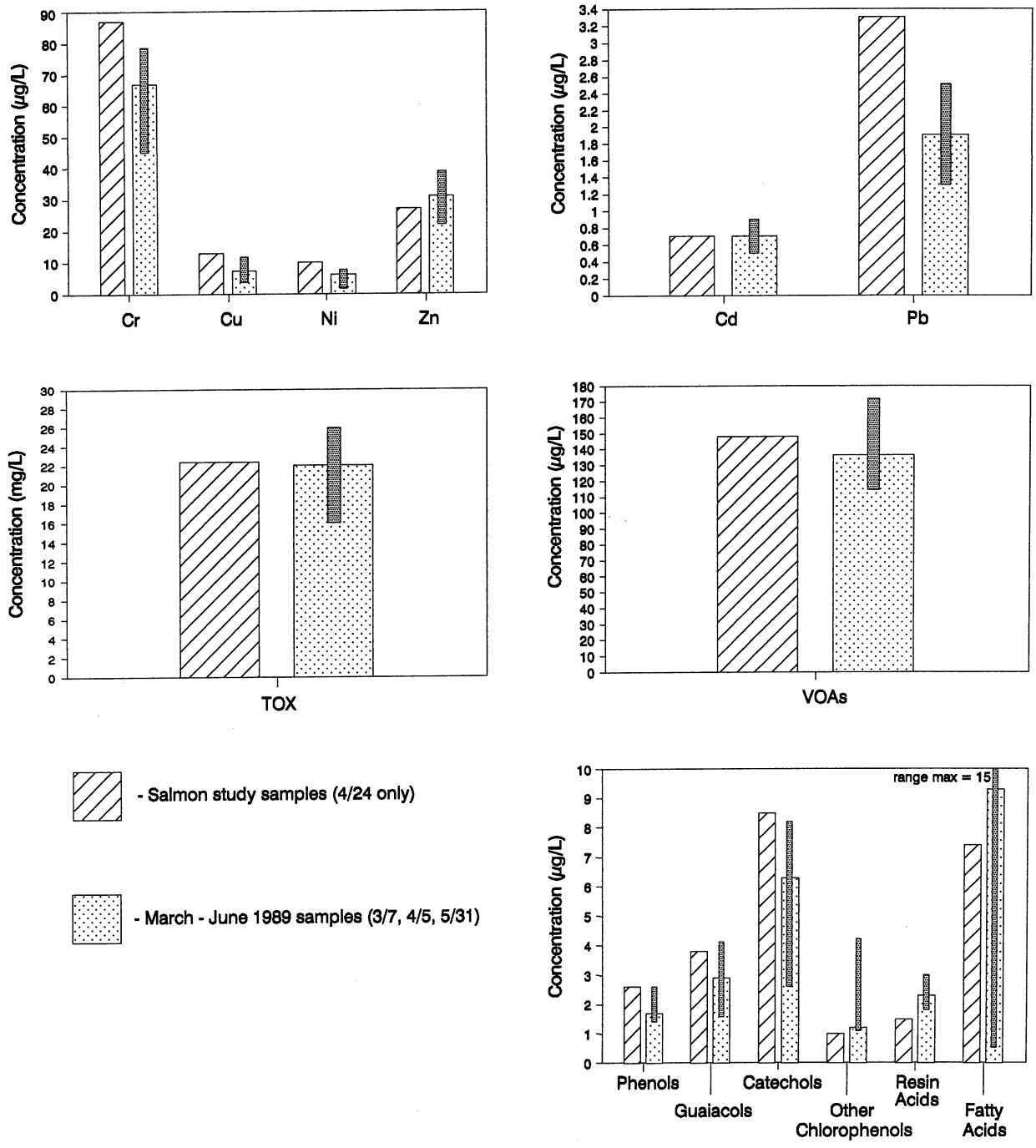

Fig. 3. Toxic constituents in ITT effluent samples collected during salmon survival studies compared to other samples collected March-June 1989. Small bars within graphs represent data ranges (from Johnson et al., 1990).

time periods.

Results of oyster larvae and echinoderm bioassays (and in the case of ITT, the rainbow trout and Microtox bioassays) also indicated no appreciable differences in effluent toxicity with respect to time period (Table 12). Rainbow trout and Microtox bioassays of Weyerhaeuser effluent, however, showed toxicity only prior to and after the study period.

Results of the rainbow trout bioassays of Weyerhaeuser's effluents conducted independently by Ecology and Weyerhaeuser were compared from January to September, 1989 (Table 13). The toxicity of Weyerhaeuser effluent to trout appeared to vary from lethal to non-toxic over periods as short as one day and, at most, three days. Although Ecology's tests showed more frequent toxicity, the sample collected by Ecology the day after Weyerhaeuser reported a bioassay failure (3/6/89) had 100% survival.

Weyerhaeuser's rainbow trout bioassay data were reviewed from 1986 to 1989 to determine if any patterns in toxicity existed (Figure 4). The general pattern during this time period was increased effluent toxicity during spring and summer and relatively infrequent toxicity during fall and winter. As a result of the bioassay failures in 1986, Ecology instructed Weyerhaeuser to conduct a study aimed at achieving compliance with the bioassay requirement in its discharge permit. Control measures implemented as a result of this study have reduced the frequency of bioassay failures. Bioassays conducted by Weyerhaeuser in 1989 were consistent with the historical pattern and improving trend in

Table 13. A comparison of rainbow trout effluent bioassays conducted by Weyerhaeuser and Ecology during 1989 (% survival) (from Johnson et al. 1990).

Date	Weyerhaeuser Data	Ecology Data
1/08	100%	--
1/22	100%	--
2/15	100%	--
2/26	100%	--
3/06	40%	--
3/07	--	100%
3/20	100%	--
3/21	--	13%
4/03	100%	--
4/05	--	100%
4/16	90%	--
4/21	--	100%
5/07	90%	--
5/17	--	100%
5/29	100%	--
5/31	--	3%
6/04	100%	--
6/12	--	100%
6/17	100%	--
6/20	--	0%
7/09	100%	--
7/23	100%	--
8/06	100%	--
8/20	100%	--
9/04	100%	--
9/17	100%	--

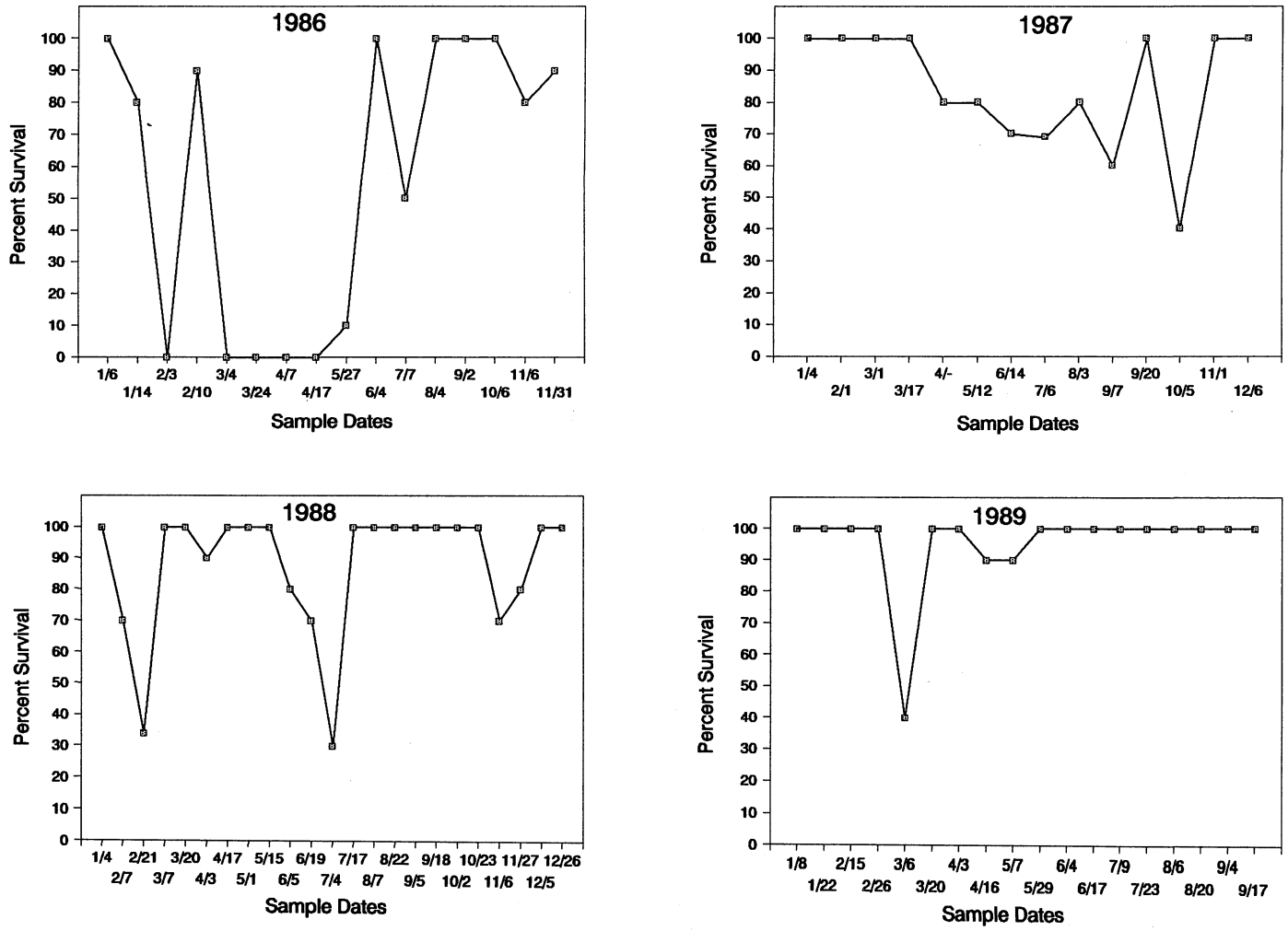

Fig. 4. Historical record of results of Weyerhaeuser's rainbow trout bioassays from 1986-1989 (from Johnson et al., 1990).

effluent toxicity since 1986. Results obtained by Ecology, however, indicated that the effluent toxicity problem was not solved as of 1989.

Factors other than effluent quality may at least partially account for differences in rainbow trout bioassay results. These include differences between methods employed by Ecology and Weyerhaeuser to collect and handle samples and to conduct the bioassay. Bioassay procedures are being standardized in all new discharge permits.

The schedule of pulp grades produced by Weyerhaeuser was examined to determine if there was a relationship with bioassay failures or results of the salmon study (Table 14). It appeared that five of the six grades in greatest production from March through June, 1989 were made at one time or another during the salmon study. The remaining grades were each manufactured for periods of one to three days only. Four separate pulp grades were made during bioassay failures; three of these were also produced during the salmon study.

A final concern with regard to the salmon study was whether effluents used in the 1989 continuous-flow coho smolt bioassay were representative. This concern was addressed in several ways. Monthly discharge monitoring reports (DMRs) submitted by Weyerhaeuser and ITT to Ecology were examined for the time period March to June 1989. DMRs include data on flow, BOD, and TSS (Fig. 5 and 6). Based on these general indicators of plant performance and effluent quality, discharges during the salmon study were

Table 14. Weyerhaeuser pulp grades in production during 1989 salmon survival experiments (i.e., live box and continuous-flow bioassays) (from Johnson et al. 1990).

Weyerhaeuser Pulp Grade Code	Number of Days in Production During 1989 Salmon Survival Experiments		Total Number of Days in Production During March - June 1989
	April 21-May 7	May 12-28	
006[a]	11		35
096	2	8	23
094[b]			14
093[c]		2	12
029[d]	3		10
008	1	2	4
010			3
016			3
023			3
009			2
099			2
025			1

a - in production during rainbow trout bioassay failure (Weyerhaeuser March 6 sample)

b - in production during rainbow trout bioassay failure (Ecology March 27 sample)

c - in production during rainbow trout bioassay failure (Ecology May 31 sample)

d - in production during rainbow trout bioassay failure (Ecology June 20 sample)

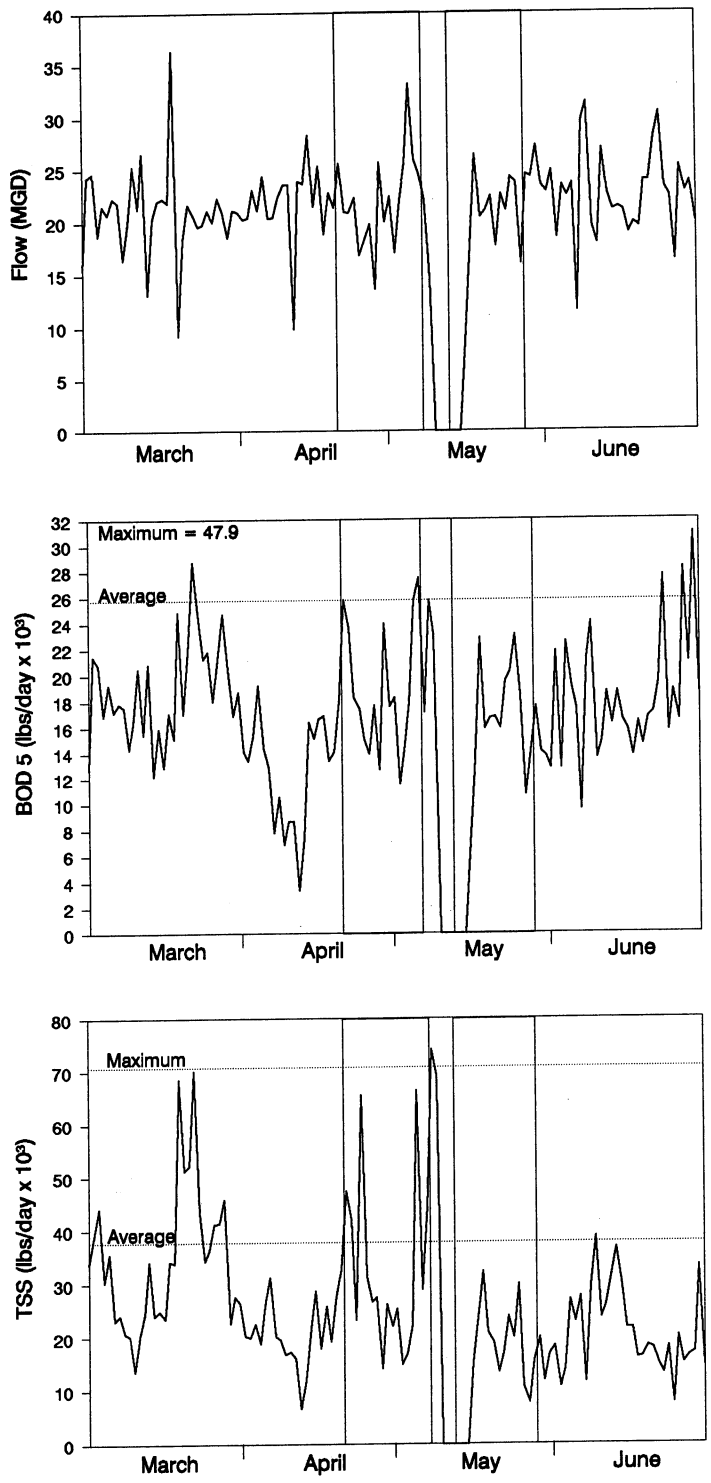

Fig. 5. Weyerhaeuser discharge monitoring report data for March-June 1989. Periods of salmon survival studies (April 21- May 7 and May 12-May 28) indicated by vertical lines. Horizontal lines represent daily maximum and monthly average permit limits (from Johnson et al., 1990).

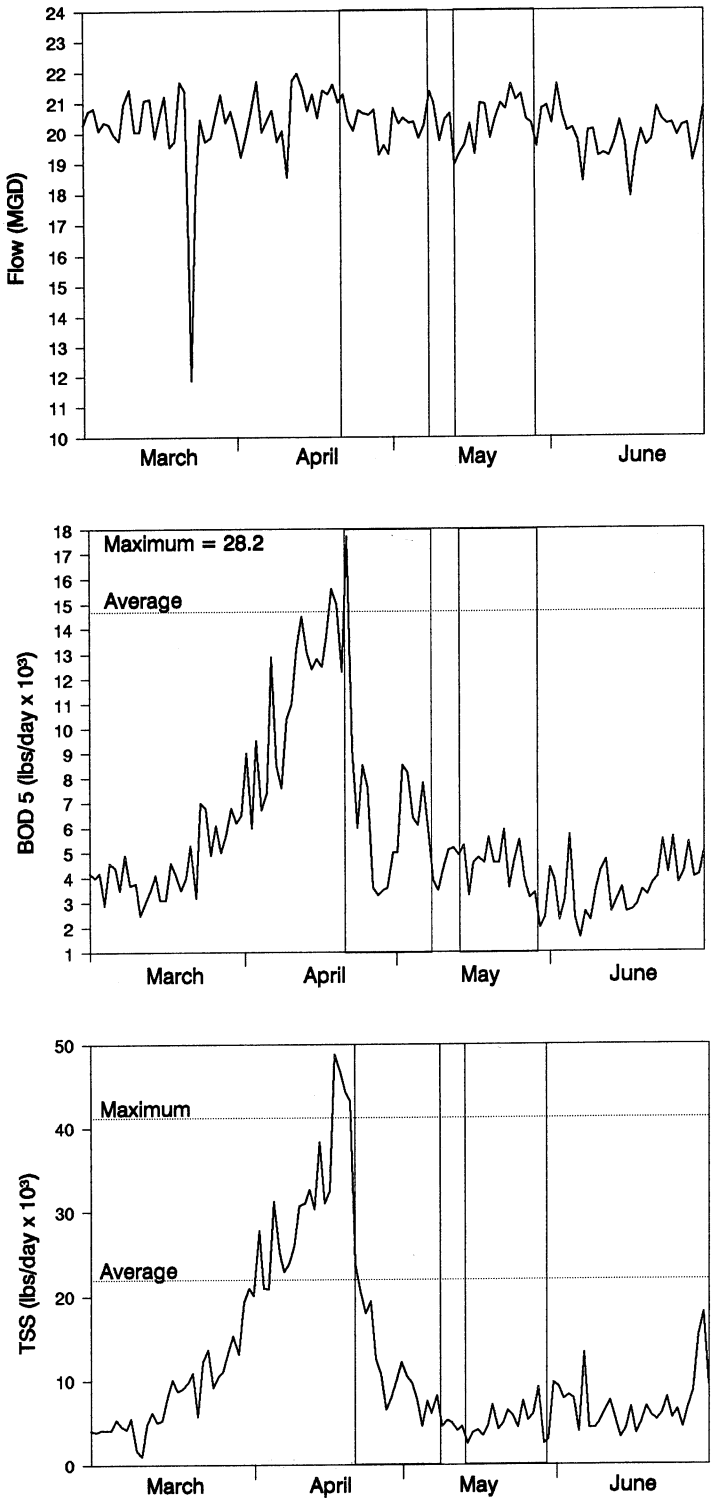

Fig. 6. ITT discharge monitoring report data for March-June 1989. Periods of salmon survival studies (April 21-May 7 and May 12-May 28) indicated by vertical lines. Horizontal lines represent daily maximum and monthly average permit limits (from Johnson et al., 1990).

"normal", except for one instance at each mill[5].

Another method of examining if effluents tested in the salmon experiments were representative involved taking grab samples every other day from tanker truck loads of effluents used in the bioassay. Samples were analyzed by Ecology for specific conductance, color, TSS, total recoverable phenolics, and TOX, and results compared to values measured in effluent samples collected by Ecology at the mill outfalls between March and June (Figures 7 and 8).

Specific conductance, color, TSS, and total recoverable phenolics in samples of effluents obtained at the tank farm during the continuous-flow bioassay were generally within ranges Ecology found in their unannounced collections at the mills. High TSS concentrations occurred in ITT samples obtained during the smolt bioassay, probably reflecting a treatment plant upset that occurred in mid-April (see Figure 6). TOX appeared elevated in effluent samples obtained from both mills during the bioassay. Although the reason for this discrepancy could not be determined, it may have been an artifact of sample handling methods since the change occurred simultaneously in samples of effluent from both mills.

CHEMICAL CHARACTERIZATION OF CHEHALIS RIVER WATER

The potential chemical toxicity of Chehalis River water was examined by conducting bioassays of river water in 1987 and 1988

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[5] The Weyerhaeuser pulp mill closed for routine maintenance from 5/9/89 to 5/16/89. Study participants were told in advance that this would occur.

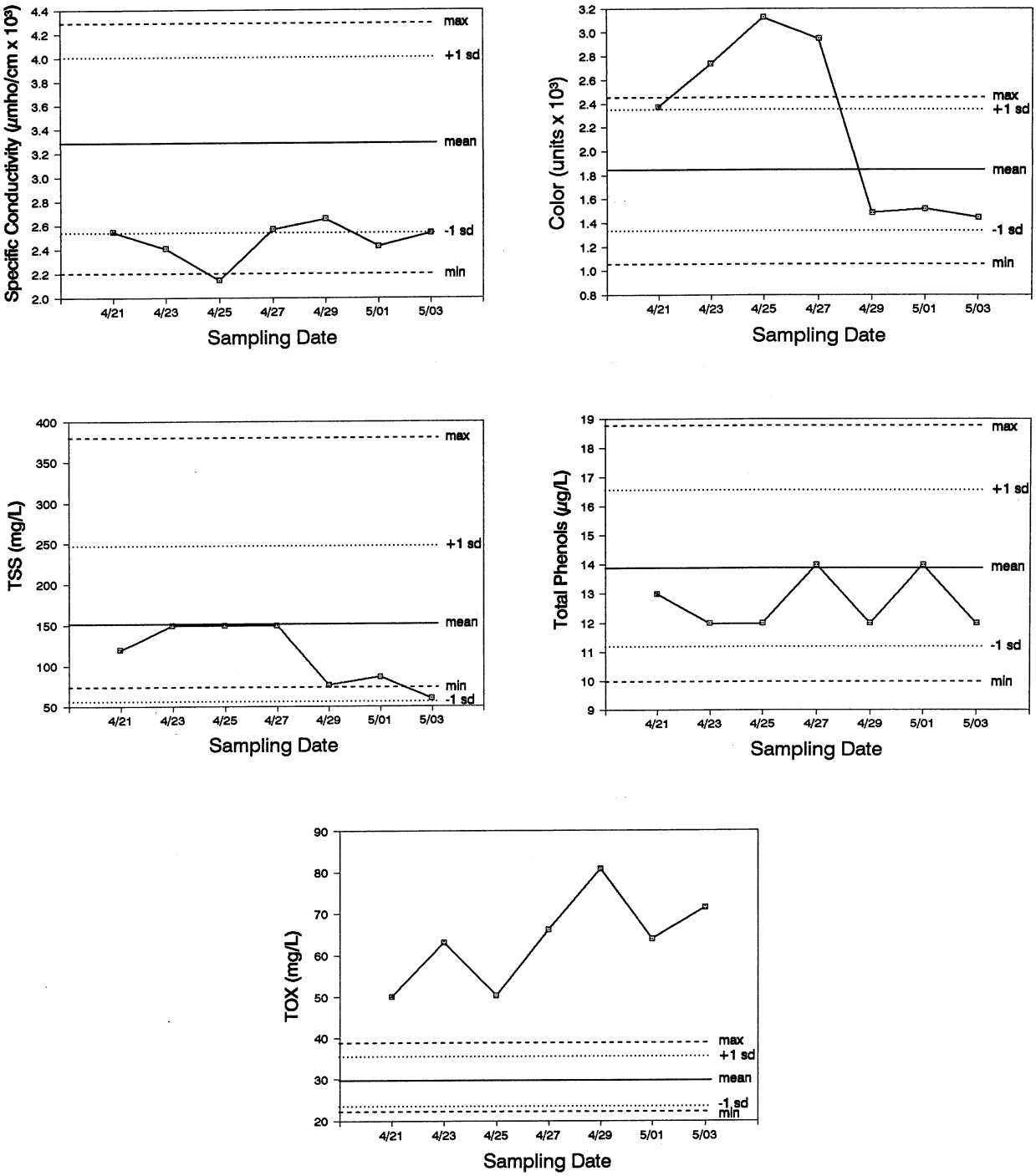

Fig. 7. Quality of Fisheries' Weyerhaeuser effluent samples collected for coho smolt bioassay (line graphs) compared to results of Ecology's March-June 1989 monitoring program (represented by data means ± sd and ranges) (from Johnson et al., 1990).

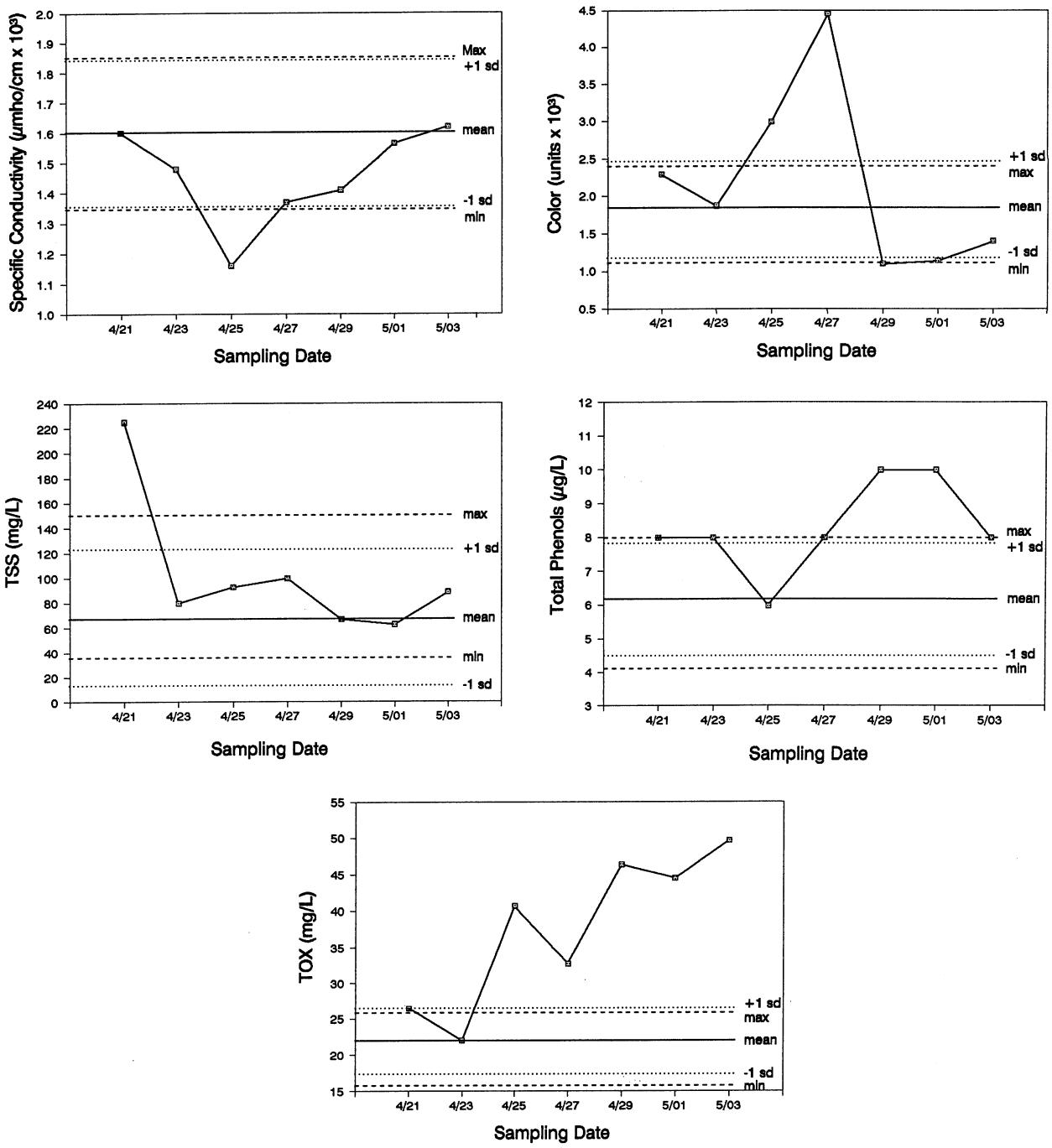

Fig. 8. Quality of Fisheries' ITT effluent samples collected for coho smolt bioassay (line graphs) compared to results of Ecology's March-June 1989 monitoring program (represented by data means ± sd and ranges) (from Johnson et al., 1990).

using *Ceriodaphnia* and fathead minnows. In addition, several analyses of chemical constituents present in Chehalis River water were conducted during spring 1988 and 1989.

Bioassays of Chehalis River Water, 1987-88

Ceriodaphnia Bioassay

In 1987-88, a study was conducted by Michaud (1988) to test whether toxic conditions occurred upstream of discharges to inner Grays Harbor and to compare conditions in the Chehalis and Humptulips basins. To accomplish this, water samples were collected in February, June, and September, 1987, and in February, 1988 at the following sites:

Location	River Km
Chehalis R. @ Dryad	157.3
" " " Centralia	107.0
" " " Porter	53.3
" " " Montesano	18.6
Satsop R. @ Satsop	3.5[6]
Humptulips R., West Fork	58.2
" " @ Humptulips	37.8
" " " Mouth	2.4

Seven-day bioassays measuring survival and reproductive rates of the freshwater crustacean *Ceriodaphnia dupia* were conducted at

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[6] This represents the distance in the Satsop River upstream of its confluence with the Chehalis River.

the EPA Environmental Research Laboratory in Duluth, Minnesota. Consistent toxicity was not observed at any one site. In most cases, survival was >90%, with reproductive rates comparable between the two drainages.

In February 1987, a downstream trend of increasing toxicity was observed in the Humptulips River. Survival and the number of young-per-female were 70% and 18.9 in the West Fork, 50% and 23.6 at Humptulips, and 0% and 10.3 at the river mouth. Toxicity did not recur during subsequent sampling. Another toxicity episode was observed at two sites in the Chehalis River drainage in September 1987. On this occasion survival was 0% at both Dryad and Satsop. Toxic conditions were not encountered in the Chehalis basin during other collection periods. Non-point agricultural sources were hypothesized as the most likely cause of the transient toxicity observed during this investigation.

In summary, Michaud (1988) found no evidence of chronic toxicity in the Chehalis River drainage. Moreover, the toxicity potential of the Chehalis and Humptulips basins were comparable.

Fathead Minnow Bioassay

A bioassay using fathead minnows was performed in May and June 1988 to evaluate acute and chronic effects of Chehalis River water. Tests were conducted with EPA's Region 10 mobile bioassay laboratory and partly overlapped with the continuous-flow coho smolt bioassay (EPA unpublished). Chehalis River water was collected from the same location used during the continuous-flow coho smolt experiments. Two tests using three replicates each were

conducted; there was no mortality or significant impacts on growth in any group.

Characterization of Chehalis River Water during the Continuous-Flow Coho Smolt Bioassay, May 1988

During the continuous-flow smolt bioassay conducted in late May 1988, smolts were exposed to Chehalis River water. Ecology monitored general water quality and the occurrence of potential toxicants in samples of river water used in the bioassay (see Appendix A of Johnson et al. 1990 for complete results). A fresh load of river water was obtained each day of the bioassay near Montesano at about RK 22.4. General water quality was evaluated daily over the five day test period. On the second and fifth days, samples were collected for analysis of the same priority pollutants/hazardous substance list compounds included in the evaluation of pulp mill and STP samples.

Water quality of the Chehalis River appeared good (Tables 3-5). Only trace amounts of metals were present, including arsenic (0.3 µg/L), cadmium (0.06-0.08 µg/L), chromium (0.6-0.9 µg/L), lead (1.2-2.2 µg/L), nickel (1.0 µg/L), silver (0.2 µg/L), and zinc (approximately 5 µg/L, blank corrected). These concentrations are comparable to those typically reported in other Washington rivers (USGS 1989).

The only organic compounds detected in river water were very low concentrations of pentachlorophenol (0.002-0.004 µg/L) and several phthalate acid esters (0.07-4 µg/L). Similar levels of these compounds, however, were also detected in field and/or

laboratory blanks indicating samples were contaminated during sample collection and/or analysis.

Characterization of Chehalis River Water Adjacent to the Live Box at Montesano, May 1989

A sample of Chehalis River water was obtained by EPA in early May, 1989 immediately adjacent to the location of the Montesano live box underneath the State Route 107 bridge over the Chehalis River; a continuous-flow centrifuge with an XAD resin column was used to obtain samples. The analysis of this sample is included in the section dealing with the receiving environment of the inner harbor.

ANALYSES OF THE RECEIVING ENVIRONMENT OF THE INNER HARBOR

Water Column Analyses

Analysis of Dioxin in Water from the Main Chehalis River Channel and the North and South Channels, June 1987

EPA analyzed samples of SPM from the main river above the pulp mill outfalls near the confluence with the Wishkah River and from the North and South channels below the outfalls in early June, 1987. Analysis of these samples was restricted to measuring the concentration of 2,3,7,8-TCDD. 2,3,7,8-TCDD was not detected in the Chehalis River in the vicinity of the Wishkah River, although the detection limit was quite high (7.11 ppt). Dioxin was, however, detected in both the North Channel (1.53 ppt) and South Channel samples (2.00 ppt).

General Water Quality Monitoring during the Barging Study, May-June 1988

Dissolved oxygen (D.O.), temperature, pH, and salinity were monitored by Ecology during the 1988 coho barging experiments within the pens holding the smolts using a Hydrolab 8000 and Beckman salinometer (Coots 1988). D.O., pH, and temperature varied little during barging between Cosmopolis and the North and South Chehalis River channels while salinity increased slightly moving down the estuary. Water quality was also uniform during barging in North Bay. Salinity and pH were much higher in North Bay than inner Grays Harbor because of its proximity to the ocean. All water quality measurements were within a range suitable for coho salmon.

Chemical Characterizations of the Water Column in the Inner Harbor in 1988 and 1989

During the barging bioassay in 1988 and the live box experiments in 1989, EPA collected and analyzed water column samples to evaluate the chemical constituents present in the inner harbor, North Bay, and Chehalis River. This effort was made primarily to assess what chemicals the coho in the bioassays were being exposed to.

Three types of samples were obtained (Table 15). First, whole water samples were collected by taking a single "grab" or using an ISCO compositor. Second, SPM samples were secured with a continuous-flow centrifuge. The SPM removed from the water column by the centrifuge contained hydrophobic organic compounds bound to

Table 15. The number of whole water, suspended particulate matter (SPM), and XAD samples taken in 1988 and 1989 at various locations in Grays Harbor.

	SAMPLING YEAR: 1988 TYPE OF SAMPLE		
Location	Whole H_2O	SPM	XAD
Montesano	-	-	-
Wishkah	3[A]	1	0[B]
Elliott Slough	-	-	-
Main Channel	-	3	0[B]
S. Channel	2[A]	1	0[B]
N. Channel	3[A]	1	0[B]
North Bay	4[A]	1	0[B]

	SAMPLING YEAR: 1989 TYPE OF SAMPLE		
Location	Whole H_2O	SPM	XAD
Montesano	2	1	1
Elliott Slough	3	1	1
Wishkah	-	-	-
Main Channel	-	-	-
S. Channel	6	2	2
N. Channel	2	1	1
North Bay	2	1	1

A= Includes both grab and composite samples.
B= Samples were collected and analyzed but data are not included here because of QA/QC problems.
(-)= Samples were not collected.

particulate matter. Third, water from the centrifuge was run through an XAD resin column to remove hydrophilic compounds. The 1988 XAD results were not included because of the previously mentioned QA/QC problems.

The number of samples taken at a site depended on the type (i.e., whole water, XAD, or SPM) and year (Table 15). Up to six whole water samples were obtained per site with composite samples obtained only in 1988. Only one SPM sample and one XAD sample were collected at a site each year with two exceptions: 1) the South Channel live box site in 1989 when samples were collected on both 5/2 and 5/17, and 2) in the Chehalis River in 1988 when SPM samples were collected on three separate days. Sampling locations are shown in Figure 9.

In 1989, all samples were obtained immediately adjacent to a live box site. The 1988 samples from the North Channel and Wishkah River were also from fixed points; the North Channel sample was collected from the Corps of Engineers dock at Terminal One while a dock at the confluence of the Wishkah and Chehalis rivers was used for the Wishkah River sample (Figure 9). The 1988 samples from North Bay, South Channel, and the Chehalis River were collected from the bioassay barge while it was being moved and thus came from an area rather than a specific site. For example, the 1988 Chehalis River samples were obtained from a section of the river near the Weyerhaeuser pulp mill (this was approximately 10 km above the point where the mill's effluent is discharged) that was several kilometers long.

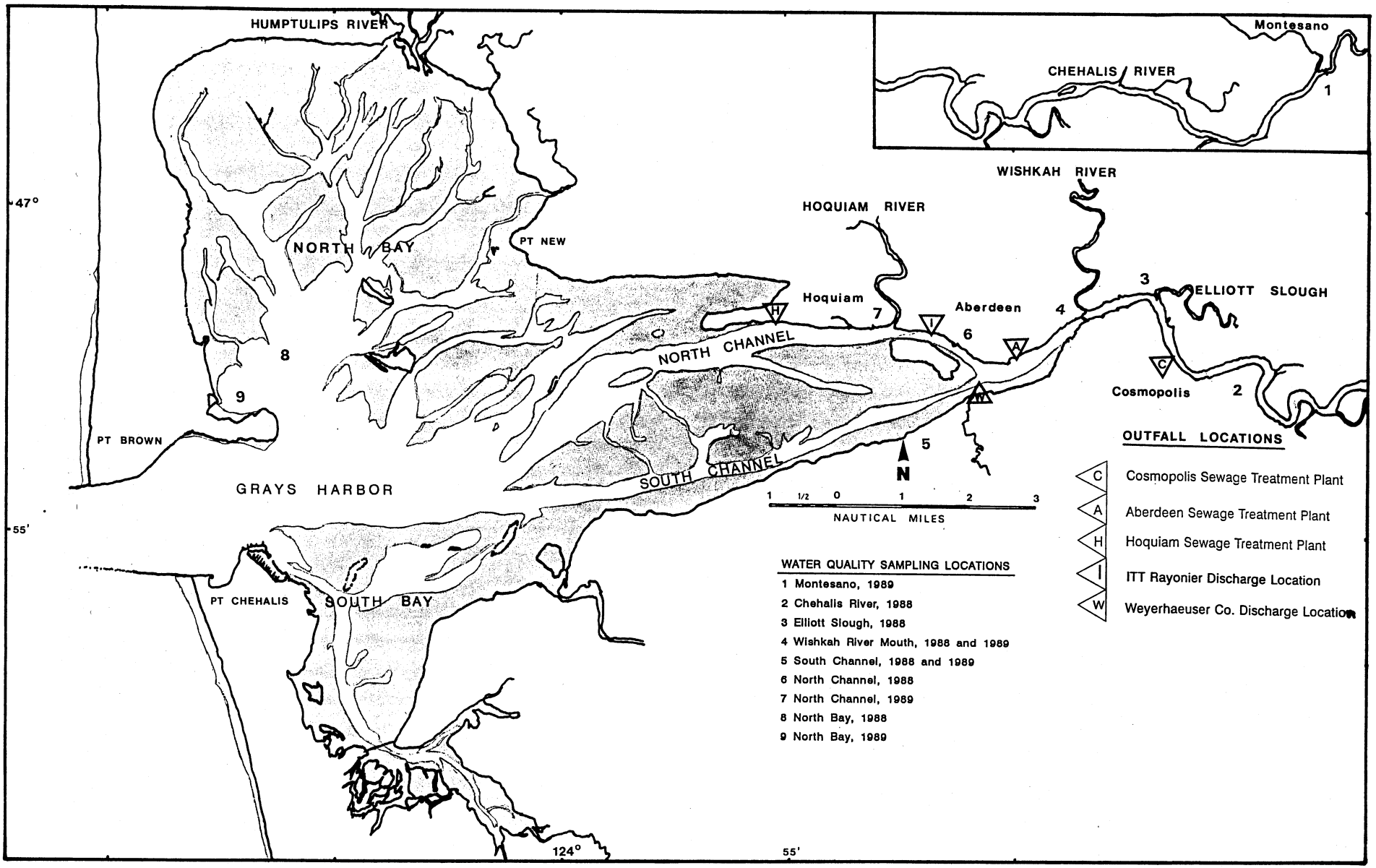

Fig. 9. Map of Grays Harbor showing where water quality samples were taken in 1988 and 1989. Shaded areas are intertidal mud flats.

The chemical analyses performed depended on the type of sample (i.e., whole water, SPM, and XAD) and when and where it was collected. Most whole water samples were analyzed for EPA's organic priority pollutants/hazardous substances list compounds (except dioxin). Whole water composite samples taken in 1988 were analyzed for resin acids, guaiacols, catechols, herbicides, organophosphorus pesticides, and fatty acids. The focus of the SPM and XAD analyses was on chlorinated phenols, guaiacols, catechols, and resin acids, although target compounds differed somewhat each year. Concentrations of metals are reported as mg/kg dry weight for SPM, while concentrations of organic compounds are given as μg/L for whole water, μg/kg- dry weight for SPM samples, and ng/L for XAD samples.

Metals data were available from SPM samples taken from: 1) the Chehalis River near the Weyerhaeuser pulp mill in 1988, 2) the Wishkah River site in 1988, and 3) the Elliott Slough and North Channel live box sites in 1989 (Table 16). Concentrations of commonly targeted metals, such as chromium, copper, lead and zinc, obtained from the two sites in 1988 were comparable. In 1989, concentrations of metals were also comparable at the two sites, with the exception of zinc which was an order of magnitude higher at Elliott Slough than the North Channel (948 mg/kg vs. 92 mg/kg). Because the zinc concentration in the Elliott Slough sample was almost exactly an order of magnitude higher than in the other three samples, it suggests that the Elliott Slough value may have been reported incorrectly (i.e., the decimal point was placed in the

Table 16. Metals concentrations obtained from suspended particulate matter samples collected in 1988 (main channel and mouth of the Wishkah River) and 1989 (North Channel and Elliott Slough). Concentrations are in mg/kg - dry weight[1].

	YEAR: 1988		YEAR: 1989	
Element	Main Channel (5/31-6/01)	Wishkah (5/25-28)	North Channel (5/04)	Elliott Slough (5/03)
Antimony	114.0	106.0	5.0 U	5.0 U
Arsenic	4.9	5.7	30.0 J	27.0 J
Beryllium	0.7	0.7	1.1	1.1
Cadmium	0.5 U	0.5 U	0.5 U	0.5 U
Chromium	40.1	42.4	74.0	58.2
Copper	57.1	53.9	109.0	88.6
Lead	5.0 U	5.0 U	17.0	16.0
Mercury	0.04	0.04	-	-
Nickel	24.9	22.9	34.0	28.5
Selenium	0.2	0.4	10.0 U	10.0 U
Silver	2.7	2.3	9.2	9.4
Thallium	0.1	0.1 U	5.0 U	5.0 U
Zinc	93.8	94.3	92.0	948.0[2]

[1] U= Compound was analyzed for, but not detected. Number represents the minimum detection limit.
J= Estimated value only.
(-)= Analysis not conducted.

[2] This value is an order of magnitude greater than the other three zinc measurements, suggesting it may have been reported incorrectly; however, this possibility could not be confirmed.

wrong position). However, this could not be confirmed. With the exception of zinc at Elliott Slough (assuming this value is correct), concentrations of metals were generally low and did not appear to represent a threat to aquatic life.

Results of whole water analyses are presented in Tables 17 and 18. Twenty-three compounds were present in whole water samples. The greatest number of compounds detected in grab samples were 11 in 1989 at the North Channel live box site while the fewest (one) were found at the Wishkah River live box site in 1989, North Bay 1988, and South Channel in 1988. Concentrations of all compounds detected in whole water samples were very low. The highest concentration of any compound detected from whole water samples was dichloroethane (15 μg/L) which was present at the Wishkah River site in 1988. Very low levels of PCP (0.009-0.021 μg/L) and 2,3,4,5-tetrachlorophenol (0.002-0.011 μg/L) were detected in all composite samples from 1988 (Tables 17 and 18).

Organic compounds detected in SPM samples from the continuous-flow centrifuge and XAD resin columns are listed in Tables 19-22. Many more compounds were detected in SPM and XAD column samples than in whole water samples. However, because of differences in target analytes these two sets of samples cannot be directly compared.

A total of 74 organic chemicals were found in SPM and XAD samples from the water column of Grays Harbor, North Bay, and the Chehalis River in 1988 and 1989. In general, the same resin and fatty acids were detected at the same site in SPM and XAD samples

Table 17. Organic compounds detected in grab and composite whole water samples obtained from the inner harbor (North and South Channels) and North Bay in 1988 and 1989. Concentrations are in μg/L; samples collected on 5/17/89 in the South Channel were pooled[1].

Organic Compound	North Channel (6/02-03/88) Grab	North Channel (6/03-04/88) Composite	North Channel (5/04/89) Grab	South Channel (6/04/88) Grab	South Channel (6/04/88) Composite	South Channel (5/02/89) Grab	South Channel (5/17/89) Grab	North Bay (6/05-07/88) Grab	North Bay (6/05-07/88) Composite	North Bay (5/17/89) Grab
PAHs										
Naphthalene	-	-	1.0 J	-	-	-	-	-	-	-
VOLATILES										
1,2,3-Trichlorobenzene	-		0.5 J	-		-	-	-		-
1,2-Dichlorobenzene	-	-	0.3 J	-	-	-	-	-	-	-
1,4-Dichlorobenzene	-		0.4 J	-		-	-	-		-
1,2,4-Trichlorobenzene	-	-	-	-	-	-	-	-	-	-
1,3-Dichlorobenzene	-	-	0.3 J	-	-	-	-	-	-	-
Acetone	-		0.6 BJ	-		-	-	-		-
Chloroform	0.5 J		0.5 J	-		0.4 J	-	-		-
Bromomethane	-		0.5 J	-		0.3 J	-	-		-
Chloromethane	-		0.3 BJ	-		0.3 BJ	0.4 J	-		0.3 J
Methylene Chloride	-		0.4 BJ	-		-	-	-		18.0 B
Benzene Ethyl-	-		0.2 J	-		-	0.2 J	-		-
Toluene	-		-	-		-	0.2 J	-		-
1,2-Dichloroethane	3.0 J		-	0.9 J				0.9 J		-
PHTHALATES										
Diethylphthalate		0.3 J			0.1 J				0.1 J	
Di-n-butylphthalate		-			0.9 J				0.1 J	
Bis (2-ethylhexyl)phthalate		-			0.4 J				0.3 J	
Di-n-octyl Phthalate		-			-				-	
Dimethylphthalate		0.1 J			0.1 J				0.2 J	
RESIN ACIDS										
Dehydroabietic Acid		-			-				-	
PHENOLS										
Pentachlorophenol		0.009			0.016				0.004	
2,3,4,5-Tetrachlorophenol	-	0.006			0.009				0.002	

[1] B= Contaminated blank
J= Estimated concentration
(-)= Chemical analyzed for but not detected
Blank= Chemical not analyzed for

Table 18. Organic compounds detected in grab and composite whole water samples obtained from Montesano, Elliott Slough, and the mouth of the Wishkah River in 1988 and 1989. Concentrations are in μg/L.

Organic Compound	Montesano (5/02/89) Grab	Elliott Slough (5/03/89) Grab	Wishkah River Mouth (5/25-27/88) Composite	Wishkah River Mouth (5/27/89) Grab
PAHs				
Naphthalene	0.9 J	-	-	-
VOLATILES				
1,2,3-Trichlorobenzene	0.6 J	-		-
1,2-Dichlorobenzene	0.3 J	-	-	-
1,4-Dichlorobenzene	0.4 J	-		-
1,2,4-Trichlorobenzene	0.5 J	-	-	-
1,3-Dichlorobenzene	0.3 J	-	-	-
Acetone	-	1.0 J		-
Chloroform	-	0.4 J		-
Bromomethane	-	-		-
Chloromethane	-	-		-
Methylene Chloride	-	-		-
Benzene Ethyl-	-	-		-
Toluene	-	-		-
1,2-Dichloroethane	0.2 J	-		-
PHTHLATES				
Diethylphthalate			2.0 J	
Di-n-butylphthalate			0.1 BJ	
Bis (2-ethylhexyl)phthalate			10.0 BJ	
Di-n-octyl Phthalate			0.4 J	
Dimethylphthalate			-	
RESIN ACIDS				
Dehydroabietic Acid			0.8 J	
PHENOLS				
Pentachlorophenol			**0.021**	
2,3,4,5-Tetrachlorophenol			**0.011**	

[1] B= Contaminated blank
J= Estimated concentration
(-)= Chemical analyzed for but not detected
Blank= Chemical not analyzed for

Table 19. Concentrations of organic chemicals in suspended particulate matter (SPM reported in µg/kg- dry weight (=ppb)) samples obtained from the water column in Grays Harbor, 1988[1].

Organic Compound	Wishkah R. 5/25-28	N. Channel 6/03	Chehalis River 5/31-6/01	Chehalis River 6/01-6/02	Chehalis River 6/02-6/03	S.Channel 6/04	N. Bay 6/05
PAH'S							
Acenaphthylene	-	-	13J	17J	17J	18J	-
Chrysene	130J	-	36J	41J	52J	-	-
Fluoranthene	240J	170J	140J	170J	210J	170J	31J
Isophorone	-	46J	21J	16J	20J	36J	71J
Naphthalene	43J	93J	45J	47J	70J	77J	25J
Phenanthrene	140J	150J	93J	120J	180J	160J	47J
Pyrene	180J	160J	93J	120J	160J	140J	30J
Benzo(a)anthracene	-	-	51J	-	74J	-	-
Fluorene	-	-	12J	14J	19J	-	-
Dibenzofuran	-	-	19J	23J	26J	-	-
Benzo(b)fluoranthene	-	-	85J	-	-	-	-
Benzo(a)pyrene	-	-	-	**860**	-	-	-
Naphthalene, 1-Methyl	-	-	-	17J	27J	-	-
Naphthalene, 2-Methyl	-	-	7J	-	-	-	-
Benzo(ghi)perylene	-	-	-	54J	-	-	-
Ideno(1,2,3-cd)pyrene	-	-	-	54J	-	-	-
Benzo(k)fluoranthene	-	-	-	130J	36J	-	-
PHTHALATES							
Di-n-octyl phthalate	-	-	260BJ	3600B	-	140BJ	140BJ
Di-n-butyl phthalate	-	-	-	21BJ	-	-	31BJ
Bis(2-Ethylhexyl)phthalate	470BJ	440BJ	1600B	8200B	690B	360BJ	220BJ
Butylbenzylphthalate	-	-	-	190J	-	-	-
RESIN AND FATTY ACIDS							
Oleic Acid	4000J	-	**3200**	**1500**	**6900**	3500J	-
Linoleic Acid	**2700**	-	**2100**	**690**	**1900**	-	-
Dehydroabietic Acid	440J	1100J	690J	340J	**1200**	510J	-
Retene	560J	-	**530**	**400**	**350**	-	-
PHENOLS							
4-Methylphenol	-	-	-	190J	-	-	-
Phenol	-	-	24BJ	39BJ	52BJ	-	-

Table 19. Concentrations of organic chemicals in suspended particulate matter (SPM reported in µg/kg- dry weight (=ppb)) samples obtained from the water column in Grays Harbor, 1988[1] (continued)

Organic Compound	Wishkah R. 5/25-28	N. Channel 6/03	Chehalis River 5/31-6/01	Chehalis River 6/01-6/02	Chehalis River 6/02-6/03	S.Channel 6/04	N. Bay 6/05
MISCELLANEOUS							
N-Nitrosodiphenylamine	-	190BJ	28BJ	17BJ	43BJ	130BJ	-
Hexazinone	-	-	-	380	-	-	-

[1] J= Value is an estimate
B= Blank was contaminated with the analyte
M= Value is an estimate that is above the detection limit but below the quantification limit.
(-)= Chemical analyzed for but not detected

Table 20. Concentrations of PAHs, resin acids, and fatty acids in suspended particulate matter (SPM reported in μg/kg-dry weight (= ppb)) and XAD resin column (XAD reported in ng/L (= ppt)) samples obtained from the water column of Grays Harbor, 1989[1].

Organic Compound	N. Bay		N. Channel		S. Channel		Elliott Slough		Montesano	
	SPM	XAD	SPM	XAD	SPM	XAD	SPM	XAD	SPM	XAD
PAH										
Retene	-	-	120J	-	210J	-	240J	-	-	-
RESIN AND FATTY ACIDS										
Oleic Acid	-	810B	16000BJ	430J	170000BJ	1600J	8000BJ	870J	220000BJ	560J
Linoleic Acid	-	120J	-	-	-	220J	**2700**	-	**44000**	-
Abietic Acid	-	-	350J	-	430J	-	450J	-	-	-
Dehydroabietic Acid	-	55J	740J	-	1700J	**210**	1000J	-	-	-
Decanoic Acid,Hexa	300000BJ	2800B	130000BJ	1700B	230000BJ	9300BJ	51000B	2700B	320000B	2400B
Octadecanoic Acid	120000BJ	890B	22000BJ	550BJ	50000BJ	1400BJ	1800B	770B	82000B	820B
Palmitoleic Acid	-	1500J	47000J	**620**	110000J	**2100**	34000J	1200J	7000J	**640**
Eicosanoic Acid	-	-	-	-	-	-	-	-	2000J	-
Dichlorodehydro-abietic Acid	-	-	-	-	4000J	85J	-	-	-	-
14-Chlorodehydro-abietic Acid	-	-	-	-	-	100J	-	-	-	-
12-Chlorodehydro-abietic Acid	-	-	-	-	37000J	230J	-	-	-	-
Isopimaric Acid	-	-	-	-	-	-	300J	-	-	-
9,10-Dichlorostearic Acid	-	29J	650J	-	810BJ	26J	460BJ	-	-	-

[1] J= Value is an estimate
B= Blank was contaminated with the analyte
M= Value is an estimate that is above the detection limit but below the quantification limit.
(-)= Chemical analyzed for but not detected

Table 21. Concentrations of phenols, catechols, and guaiacols in suspended particulate matter (SPM reported in μg/kg- dry weight (= ppb)), and XAD resin column (XAD reported in ng/L = ppt)) samples obtained from the water column of Grays Harbor, 1989[1].

Organic Compound	N. Bay		N. Channel		S. Channel		Elliott Slough		Montesano	
	SPM	XAD	SPM	XAD	SPM	XAD	SPM	XAD	SPM	XAD
PHENOLS										
Phenol	6200B	94BJ	1200BJ	97BJ	18000B	320BJ	150BJ	110BJ	1500BJ	160BJ
2-Methylphenol	-	6BJ	27BJ	12BJ	41BJ	17BJ	-	13BJ	250BJ	16BJ
4-Methylphenol	**47000**	13J	**2000**	10J	**12000**	**59**	160J	8J	260J	-
Acetophenone	810BJ	350B	-	380B	540J	600B	-	480B	390BJ	450B
2,4,6-Trichlorophenol	-	-	-	-	-	**34**	-	-	-	-
Trichlorosyringol	-	-	-	-	-	**80**	-	-	-	-
2,4-Dimethylphenol	-	-	-	-	-	5J	-	6J	-	-
2,4-Dichlorophenol	-	-	-	-	-	11J	-	-	-	31J
GUAIACOLS										
Guaiacol	-	-	39J	-	60J	22J	-	-	-	-
Tetrachloroguaiacol	-	-	-	-	-	27J	-	-	-	-
CATECHOLS										
4,5-Dichlorocatechol	-	-	-	-	-	11J	-	-	-	-
3,4,5-Trichlorocatechol	-	-	-	-	-	41J	-	-	-	-
Tetrachlorocatechol	-	-	-	-	-	30J	-	-	-	-
MISC.										
a-Terpineol	-	-	-	-	-	**49**	-	-	-	55J

[1] J= value is an estimate
B= Blank was contaminated with the analyte
M= Value is an estimate that is above the detection limit but below the quantification limit.
(-)= Chemical analyzed for but not detected

Table 22. Concentrations of selected herbicides and pesticides in suspended particulate matter (SPM reported in µg/kg- dry weight (= ppb)), and XAD resin column (XAD reported in ng/L (= ppt)) samples obtained from the water column of Grays Harbor, 1988 and 1989[1].

Location and Type of Sample	2,3,4,5-TCP	Triphenyl Phosphate		PCP[2]	
	1988	1988	1989	1988	1989
North Bay					
SPM	NA	-	NA	NA	1100BJ
XAD	NA	NA	NA	NA	**84**
North Channel					
SPM	10M	-	NA	**7**	140BJ
XAD	NA	NA	NA	NA	77J
South Channel					
SPM	**17**	20M	NA	**160**	800BJ
XAD	NA	NA	NA	NA	**76**
Elliott Slough					
SPM	NA	NA	NA	NA	100BJ
XAD	NA	NA	NA	NA	82J
Montesano					
SPM	NA	NA	NA	NA	-
XAD	NA	NA	NA	NA	93J
Wishkah River					
SPM	-	-	NA	**99**	NA
Chehalis River					
SPM	**14**	**160**	NA	**36**	-

[1] J= Value is an estimate
B= Blank was contaminated with the analyte
M= the value is an estimate above the detection limit but below the quantification limit
(-)= chemical was analyzed for but not detected
NA= the chemical was not analyzed for

[2] PCP is pentachlorophenol and TCP is tetrachlorophenol.

while there tended to be more guaiacols, catechols, and phenols detected in the XAD than in the SPM samples.

The most significant finding of the analyses of SPM and XAD water column samples was that the concentrations of most organic chemicals were very low. For the majority of chemicals detected, concentrations could only be estimated. Some of the more important compounds detected in the SPM were a number of resin and fatty acids in 1989 (e.g., oleic and hexadecanoic acid), 4-methylphenol in 1989, and oleic acid in 1988. Significant compounds detected in the XAD (1989 only) included resin and fatty acids (e.g., palmitoleic acid) (Tables 19-22).

One objective of the water column analyses was to compare chemicals present at inner harbor stations to those present at "control" or reference stations in North Bay (1988 and 1989) and the Montesano live box site (R.K. 10 on the Chehalis River) (1989 only). Data from 1988 and 1989 are considered separately because of different target analytes and sampling locations. In 1988 (SPM samples only), more chemicals were detected in samples from the Chehalis River near the Weyerhaeuser mill at Cosmopolis than from any other sample (Tables 19 and 22). Thirty-one chemicals were present in the three samples collected from 5/31/88 to 6/03/88. At the other sites in the Chehalis River estuary, between 10 and 14 compounds were detected while at the North Bay reference station, 8 chemicals were detected.

All chemicals detected in SPM samples from North Bay in 1988 occurred at concentrations that were low relative to stations in

the Chehalis River estuary. Compounds that were present at elevated levels in the Chehalis River estuary, included retene (350-530 µg/kg, Chehalis River only), oleic acid (1,500-6,900 µg/kg, Chehalis River only), linoleic acid (690-2,700 µg/kg, Chehalis River and Wishkah River site), dehydroabietic acid (440-1,200 µg/kg, detected at all sites other than North Bay), PCP (7-160 µg/kg, detected at all sites other than North Bay), and benzo(a)pyrene (860 µg/kg, Chehalis River only).

In 1989 (both XAD and SPM samples), more compounds (27) were detected at the South Channel live box site (data for the two samples collected on 5/2/89 and 5/17/89 were combined) than at any other location (Tables 20-22). A total of 14 and 13 chemicals were detected at the Elliott Slough and North Channel live box sites, respectively. At the two control sites, 12 chemicals were detected at Montesano and 11 at North Bay.

In general, concentrations of resin and fatty acids were greater at the South Channel net pen site than at any other site in 1989. Only two resin and fatty acids (hexadecanoic and dehydroabietic acid) were detected in the SPM sample from the control site in North Bay. Comparable concentrations of these two chemicals were also found at several of the other sites, including the other control site at Montesano. The control site at Montesano had high concentrations of several fatty and resin acids (linoleic acid, oleic acid, and eicosanoic acid) relative to other sites.

In the SPM samples collected in 1989, between two and four phenols, guaiacols, and catechols were detected per site (Table

21). One major difference in the concentration of phenols, guaiacols, and catechols was that 4-methylphenol was present at much higher levels in North Bay than at other sites (47,000 µg/kg).

In the XAD samples obtained in 1989, concentrations of chemicals were generally low and comparable at sites (Table 21). Most exceptions to this pattern were compounds that were found at the South Channel net pen site but no where else. Examples of this include 2,4,6-trichlorophenol (34 ng/L) and trichlorosyringol (80 ng/L).

Besides comparing water column chemistry from different sites in Grays Harbor and the Chehalis River, short and long term variability in water column chemistry was also addressed by examining three data sets. First, annual variability was examined by comparing SPM data from 1988 and 1989 at three sites sampled in both years (South Channel, North Channel, and North Bay). Second, variation within a year was examined by comparing SPM and XAD data collected in 1989 at the South Channel site on 5/2/89 and 5/17/89 and from the mainstem Chehalis River in 1988 on several dates (SPM only). And finally, whole water data were used to examine variability over a time span as short as several hours.

In comparing data from 1988 and 1989, only those compounds that were analyzed in common were considered because the list of target analytes was different each year. Twelve compounds were analyzed (and detected) in both the 1988 and 1989 SPM samples (Table 23). In the South Channel, ten compounds were detected in 1989 and five in 1988; several of these, such as PCP, were detected

Table 23. A comparison of the concentrations of organic compounds found in suspended particulate matter samples collected in 1988 and 1989. Concentrations are in μg/kg- dry weight (ppb)[1].

Organic Compound	South Channel 1988	South Channel 1989[2]	North Channel 1988	North Channel 1989	North Bay 1988	North Bay 1989
RESIN AND FATTY ACIDS						
Oleic Acid	3500 J	170000 BJ		16000 BJ		
Abietic Acid		430 J		350 J		
Dehydroabietic Acid	510 J	1700 J	1100 J	1100 J		
Dichlorodehydroabietic Acid		4000 J				
Retene		210 J		120 J		
PHENOLS AND GUIACOLS						
2-Methylphenol		41 BJ		27 BJ		47000
4-Methylphenol		12000		2000		
Phenol		18000 B		1200 BJ		6200 B
Pentachlorophenol	160	810 BJ	7	140 BJ		1100 BJ
2,3,4,5-Tetrachlorophenol	17		10 M			
Guaiacol		60 J		39 J		
MISCELLANEOUS						
Triphenylphosphate	20 M				30.0	

[1] Data from the two 1989 samples were combined

[2] B= Contaminated blank
J= Value is an estimate
M= Estimate. Above detection limit but below quantification limit
Blank= Chemical not detected

in common with higher concentrations generally occurring in 1989. In the North Channel, nine compounds were detected in 1989 and three in 1988. In North Bay, the control area, three chemicals were detected in 1989 and one in 1988, only one of which, PCP, was detected in both years.

To assess short term variability, multiple samples taken in one year at the same site were compared. At the South Channel live box site on 5/2/89, the total concentration of chlorinated phenols, guaiacols, catechols and resin acids was 0.13 μg/L (this was almost entirely phenols) while on 5/17/89 the total concentration was 1.10 μg/L (the largest component was chlorinated resin acids, 0.9 μg/L). The difference between these two dates appeared to be a function of the behavior of the mill's effluent stream. On 5/2/89, the stream missed the live box site by about 30 m while on 5/17/89, the effluent stream passed directly through the site where the fish were being held.

In the Chehalis River in the vicinity of Cosmopolis, SPM samples were taken on three consecutive days in 1988. A number of compounds were detected in common on the three dates. Concentrations of oleic acid and linoleic acid, which occurred above detection limits on all three dates, varied by about four-fold in the case of oleic acid and three-fold in the case of linoleic acid over the three days. Some noteworthy differences in concentrations of chemicals were: 1) triphenyl phosphate was detected on days two and three (99-160 μg/kg) but not on day one; 2) dehydroabietic acid occurred above detection limits on day three

(1,200 μg/kg) but not on day one or two; and 3) benzo(a)pyrene was detected on day two (860 μg/kg) but not on the other two sampling dates.

An examination of the whole water samples indicated that changes in water column chemistry could occur over a time span as short as several hours. For example, at the North Channel live box site in 1989, 11 compounds were detected in a sample collected at 1250 while three compounds were detected seven hours later.

Although the origin of compounds detected in the water column cannot be determined with certainty, a number have been associated with forest product activities. Acetophenone, for example, was only detected in Elliott Slough and at Montesano, perhaps due to decaying wood material on the bottom. We compared the compounds detected in the water column with those detected in mill effluent and found that several constituents of mill effluents also occurred at quantifiable levels in the water column. For instance, trichlorosyringol, which results from chlorination activity during pulp production, was found in the XAD samples taken in the South Channel in 1989. This compound was also detected in the XAD from the mixed effluent sample analyzed on 5/5/89 and SPM from the Weyerhaeuser mill in 1989. Moreover, 4-methylphenol, which was detected in SPM samples from several sites in 1988 and 1989 and from the XAD in the South Channel in 1989, occurred in SPM from Weyerhaeuser effluent collected on 4/22/89 and in mixed effluent collected on 5/5/89.

PCP was detected at a number of sites in both years, occurring

in whole water, SPM and XAD samples. Although it is a well known herbicide and a wood preservative, it is also apparently on occasion disharged from the mills. It occurred at a concentration of 280 ng/L in the XAD sample of the mixed effluents taken on 5/5/89 at the bioassay tank farm.

Chemical Characterization of Bottom Sediments of the Inner Harbor

Analysis of Dioxin in the Sediments Below the Pulp Mill Outfalls by EPA, June 1987

On 6/25/87, one sediment sample from below (i.e., downstream) the Weyerhaeuser mill and one sample from below the ITT mill were collected. Dioxin (i.e., 2,3,7,8-TCDD) was the only chemical analyzed for and was detected in the sediment samples from below both the Weyerhaeuser (1.3 ng/kg) and ITT outfalls (3.5 ng/kg).

Characterization of Sediments in Grays Harbor by Ecology, May 1988

To evaluate the occurrence of chemical contaminants in Grays Harbor, Ecology (Johnson and Coots 1989) sampled bottom sediments at 10 sites in the Grays Harbor estuary during May 1988 (Fig. 10). A site in the Humptulips River Channel in North Bay was selected as a reference (i.e., control) station. North Bay was also used as the control for portions of the biological assessments during the salmon survival experiments.

Each sediment sample consisted of a composite of the top 2-cm of the surface layer from three separate grabs collected with a stainless steel Van Veen sampler. EPA priority pollutants/ hazardous substances list compounds, herbicides, quaiacols, resin

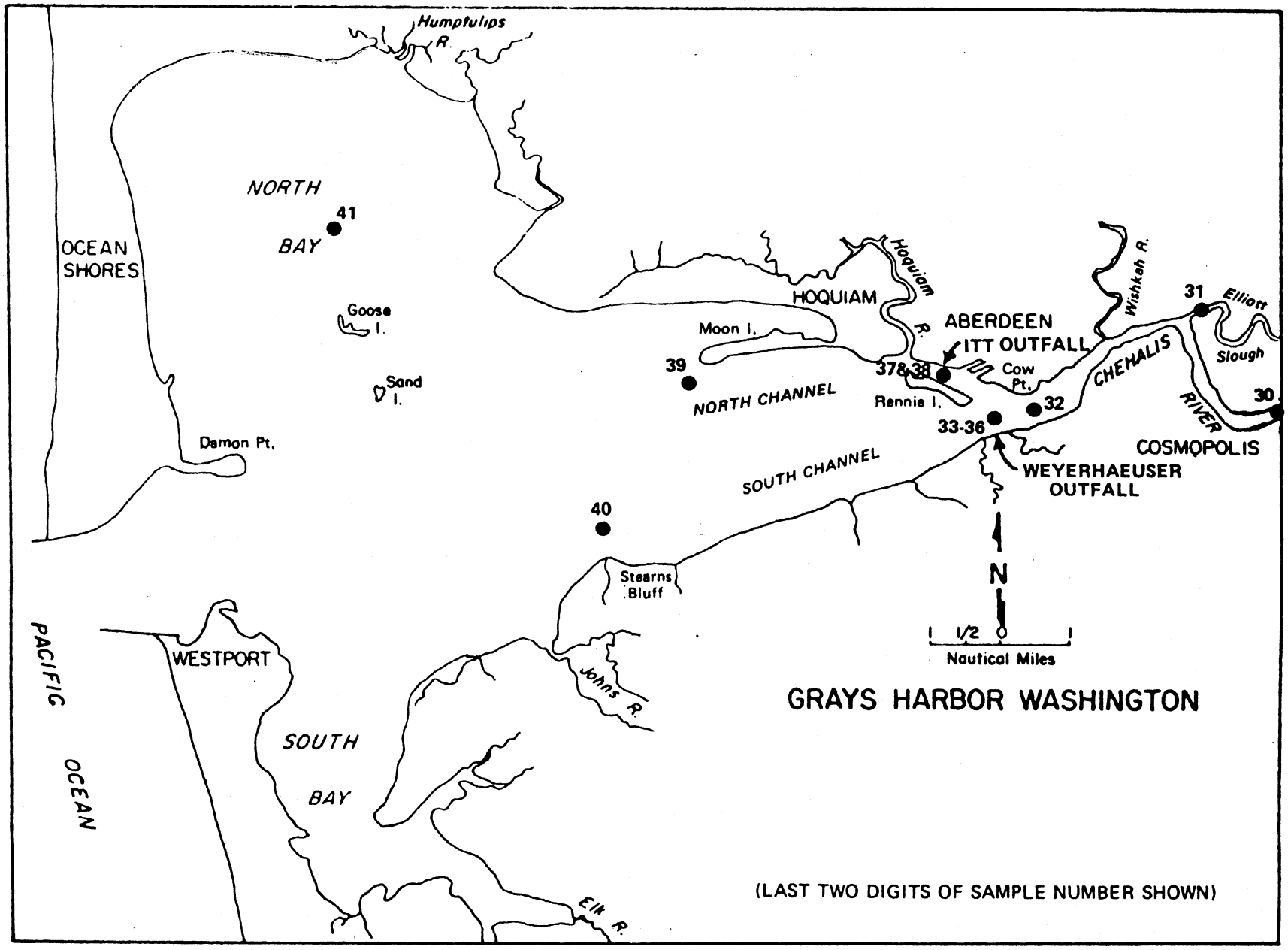

Fig. 10. Ecology sediment sampling locations in the Grays Harbor estuary, May 23-24, 1988.

acids, fatty acids, grain size, and total organic carbon were analyzed in all samples (Table 24). Samples collected near the Weyerhaeuser and ITT outfalls were also analyzed for dioxins and furans, and subjected to ten day bioassays with the amphipod *Rhepoxynius abronius*.

Metals concentrations in Grays Harbor sediments were, in most cases, comparable to concentrations measured in the Humptulips River Channel (Table 25). Organic priority pollutants/hazardous substances list compounds were primarily detected in Grays Harbor sediments and included several volatile compounds, phenol, nitrosodiphenylamine, PAHs, 4-methylphenol, retene, and phthalate acid esters (PAE). Only trace amounts of trichlorofluoromethane and PAE (also detected in method blanks) were detected in North Bay sediments. Chlorinated pesticides, PCBs, cyanide, and herbicides were not found in any samples at detection limits of 1 μg/kg, 10 μg/kg, 30-60 μg/kg, and 40-140 μg/kg, respectively.

Compared to Puget Sound sediments, concentrations of metals, organic priority pollutants, and hazardous substances list compounds detected in Grays Harbor sediments were relatively low and generally comparable to non-urban reference areas (Table 26). Concentrations of several organic compounds in sediments collected at the Weyerhaeuser and/or ITT outfalls appeared elevated relative to North Bay and Puget Sound reference areas. These were total low molecular weight PAH (864 μg/kg), total high molecular weight PAH (1,700 μg/kg), 4-methylphenol (520 μg/kg), dibenzofuran (31 μg/kg), retene 540 μg/kg), and PAE (510-610 μg/kg). However, none of the

Table 24. Target chemicals and ancillary variables analyzed for the Ecology survey of sediments in the Grays Harbor estuary, May 23-24, 1988.

1. EPA Priority Pollutants/HSL Compounds:

Metals	Volatiles	Volatiles (Continued)
Antimony	Carbon Tetrachloride	Butylbenzene
Arsenic	Acetone	4-Chlorotoluene
Beryllium	Chloroform	1,4-Dichlorobenzene
Cadmium	Benzene	1,2-Dibromoethane
Chromium	1,1,1-Trichloroethane	1,2-Dichloroethane
Copper	Bromomethane	Vinyl Acetate
Lead	Chloromethane	4-Methyl-2-Pentanone
Mercury	Vinyl Chloride	1,3,5-Trimethylbenzene
Nickel	Methylene Chloride	Bromobenzene
Selenium	Carbon Disulfide	Toluene
Silver	Bromoform	Chlorobenzene
Thallium	Bromodichloromethane	1,2,4-Trichlorobenzene
Zinc	1,1-Dichloroethane	Dibromochloromethane
	1,1-Dichloroethene	Tetrachloroethene
	Trichlorofluoromethane	sec-Butylbenzene
	Dichlorodifluoromethane	1,3-Dichloropropane
	1,2-Dichloropropane	cis-1,2-Dichloroethene
	2-Butanone	trans-1, 2-Dichloroethene
	1,1,2-Trichloroethane	1,3-Dichlorobenzene
	Trichloroethene	1,1-Dichloropropene
	1,1,2,2-Tetrachloroethylene	2,2-Dichloropropane
	1,2,3-Trichlorobenzene	2-Hexanone
	Hexachlorobutadiene	1,1,1,2-Tetrachloroethane
	Naphthalene	cis-1,3-Dichloropropene
	Total Xylenes	trans-1,3-Dichloropropene
	2-Chlorotoluene	Dibromomethane
	1,2-Dichlorobenzene	Chloroethane
	1,2,4-Trimethylbenzene	
	DBCP	
	1,2,3-Trichloropropane	
	tert-Butylbenzene	
	Isopropylbenzene	
	p-Isopropyltoluene	
	Ethylbenzene	
	Styrene	
	Propylbenzene	

Table 24. Target chemicals and ancillary variables analyzed for the Ecology survey of sediments in the Grays Harbor estuary, May 23-24, 1988 (continued)

1. **EPA Priority Pollutants/HSL Compounds (Continued):**

Semivolatiles	Semivolatiles (Continued)	Pesticides/PCBs
Benzo(a)pyrene	4-Bromophenyl-phenylether	4,4'-DDT
2,4-Dinitrophenol	2,4-Dimethylphenol	Chlordane
Dibenzo(a,h)anthracene	4-Methylphenol	Gamma-BHC (Lindane)
Benzo(a)anthracene	1,4-Dichlorobenzene	Dieldrin
4-Chloro-3-Methylphenol	4-Chloroaniline	Endrin
Benzoic Acid	Phenol	4,4'-DDD
Hexachloroethane	bis(2-Chloroethyl)ether	4,4'-DDE
Hexachlorocyclopentadiene	bis(2-Chloroethoxy)methane	Heptachlor
Isophorone	bis(2-Ethylhexyl)phthalate	Aldrin
Acenaphthene	Di-n-Octyl phthalate	alpha-BHC
Diethylphthalate	Hexachlorobenzene	beta-BHC
Di-n-Butylphthalate	Anthracene	delta-BHC
Phenanthrene	1,2,4-Trichlorobenzene	alpha-Endosulfan
Butylbenzylphthalate	2,4-Dichlorophenol	beta-Endosulfan
N-Nitrosodiphenylamine	2,4-Dinitrotoluene	Heptachlor Epoxide
Fluorene	Pyrene	Endosulfan Sulfate
Carbazole	Dimethylphthalate	Endrin Aldehyde
Hexachlorobutadiene	Dibenzofuran	Toxaphene
Pentachlorophenol	Benzo(ghi)perylene	PCB - 1260
2,4,6-Trichlorophenol	Indeno(1,2,3-cd)pyrene	PCB - 1254
2-Nitroaniline	Benzo(b)fluoranthene	PCB - 1221
2-Nitrophenol	Fluoranthene	PCB - 1232
1-Methylnaphthalene	Benzo(k)fluoranthene	PCB - 1248
Naphthalene	Acenaphthylene	PCB - 1016
2-Methylnaphthalene	Chrysene	PCB - 1242
2-Chloronaphthalene	Retene	
3,3-Dichlorobenzidine	4,6-Dinitro-2-methylphenol	Dioxin
2-Methylphenol	1,3-Dichlorobenzene	
1,2-Dichlorobenzene	2,6-Dinitrotoluene	2,3,7,8-TCDD
o-Chlorophenol	N-Nitroso-di-n-propylamine	
Nitrobenzene	4-Chlorophenyl-phenylether	Miscellaneous
3-Nitroaniline	bis (2-Chloroisopropyl)ether	
4-Nitroaniline		Cyanide
4-Nitrophenol		
Benzyl Alcohol		

Table 24. Target chemicals and ancillary variables analyzed for the Ecology survey of sediments in the Grays Harbor estuary, May 23-24, 1988 (continued)

2. Other Chemicals:

Resin Acids, Guaiacols, Fatty Acids

Linoleic Acid
Levopimaric Acid
Guaiacol
4-Allylguaiacol
Oleic Acid
Linolenic Acid
Sandaracopimaric Acid
Neoabietic Acid
Abietic Acid
Tetrachloroguaiacol
4,5,6-Trichloroguaiacol
Dichlorodehydroabietic Acid
9,10-Dichlorosteric
Dehydroabietic Acid
Palustric Acid
4,5-Dichloroguaiacol
Trichlorosyringol
Isopimaric Acid
4-Chloroguaiacol

Polychlorinated Dioxins

TCDD (total)
2,3,7,8-TCDD
PeCDD (total)
1,2,3,7,8-PeCDD
HxCDD (total)
1,2,3,4,7,8-HxCDD
1,2,3,6,7,8-HxCDD
1,2,3,7,8,9-HxCDD
HpCDD (total)
1,2,3,4,6,7,8-HpCDD
OCDD (total)

Polychlorinated Furans

TCDF (total)
2,3,7,8-TCDF
PeCDF (total)
1,2,3,7,8-PeCDF
2,3,4,7,8-PeCDF
HxCDF (total)
1,2,3,4,7,8-HxCDF
1,2,3,6,7,8-HxCDF
2,3,4,6,7,8-HxCDF
1,2,3,7,8,9-HxCDF
HpCDF (total)
1,2,3,4,6,7,8-HpCDF
1,2,3,4,7,8,9-HpCDF
OCDF (total)

Miscellaneous Herbicides, Pesticides

Aldicarb
Simazine
Diuron
Atrazine
Butylate
Metribuzin
Fenamiphos
Pronamide
Hexazinone

3. Ancillary Variables

Grain Size
Total Organic Carbon
Dry Weight

Table 25. Summary of the occurrence of priority pollutants and hazardous substance list compounds in Grays Harbor estuary sediment samples collected by Ecology May 23-25, 1988 (metals in mg/kg, dry wt. (ppm); organics in µg/kg dry wt. (ppb)) (from Johnson and Coots 1989).

Chemical	Detection Frequency (a)	Concentration Range in Grays Harbor	Concentration in North Bay	Location of Maximum
Metals:				
antimony	0/10	0.1 U	0.1 U	---
arsenic	10/10	3.4 - 4.8	3.8	lower S. Channel
beryllium	10/10	0.7 - 1.1	0.8	various
cadmium	1/10	0.5 U - 0.9	0.5 U	lower N. Channel
chromium	10/10	16.3 - 35.0	20.1	ITT Outfall
copper	10/10	19.3 - 59.0	30.6	Cosmopolis
lead	8/10	0.5 U - 5.1	0.5 U	ITT Outfall
mercury	10/10	0.014 - 0.081	0.017	Cosmopolis
nickel	10/10	45.7 - 64.0	56.3	Weyco Outfall
selenium	10/10	0.1 - 1.9	0.8	Elliott Slough
silver	3/10	0.02 U - 0.33	0.02 U	Cosmopolis
thallium	0/10	0.1 U	0.01 U	---
zinc	10/10	51.6 -80.8	60.3	ITT Outfall
Volatile Organics:				
carbon disulfide	1/10	2 U - 8 J	6 U	Cosmopolis
acetone	1/10	4 U - 130	8 U	Cosmopolis
2-butanone	1/10	1 BU - 21 B	1 BU	Cosmopolis
methylene chloride	1/10	4 BU - 77 B	23 BU	Cow Point
trichlorofluoromethane	1/10	6 U - 12 U	7	North Bay
Total PAH (b):				
low molecular weight	9/10	20 BJ - 860 J	75 U	Weyco Outfall
high molecular weight	9/10	71 U - 1700 J	75 U	Weyco Outfall
Phenols:				
phenol	2/10	16 BJ - 24 BJ	75 BU	Weyco Outfall
4-methylphenol	6/10	46 J - 529	75 U	Weyco Outfall
Miscellaneous Extractables:				
retene	9/10	64 J - 540	75 U	Cosmop./ITT
dibenzofuran	5/10	19 J - 31 J	75 U	Weyco Outfall
N-nitrosodiphenylamine	1/10	71 U - 12 J	75 U	Weyco Outfall
Phthalates:				
di-n-butylphthalate	8/10	13 BJ - 35 BJ	20 BJ	Weyco Outfall
di-n-octylphthalate	4/10	15 BJ - 510 B	75 BJ	ITT Outfall
bis(2-ethylhexyl)phthalate	10/10	46 BJ - 610 B	57 BJ	ITT Outfall

(a) number of samples where chemical detected/ total number of samples analyzed

(b) polyaromatic hydrocarbons; sum of detected compounds

U - not detected at detection limit shown

J - estimated concentration

B - also detected in method blank

Table 26. Priority pollutants/hazardous substances list compounds detected in Grays Harbor sediment samples compared to Puget Sound reference areas and Apparent Effects Threshold (AET) values (metals in mg/kg, dry wt. (ppm); organics in µg/kg dry wt. (ppb)) (from Johnson and Coots 1989).

	Grays Harbor:		Puget Sound Reference Areas (a):		Puget Sound AET Values (b):	
Chemical	Detection Frequency (c):	Concentration Range	Detection Frequency	Concentration Range	Lowest AET	Highest AET
Metals:						
arsenic	10/10	3.4 - 4.8	38/38	1.9 - 17	57	700
beryllium	10/10	0.7 - 1.1	-	-	-	-
cadmium	1/10	0.5 U - 0.9	28/28	0.1 - 1.9	5.1	9.6
chromium	10/10	16.3 - 35.0	42/42	9.6 - 255	260	270
copper	10/10	19.3 - 59.0	32/32	5 - 74	390	1300
lead	8/10	0.5 U - 5.1	25/32	0.1 U - 24	450	660
mercury	10/10	0.014 - 0.081	42/42	0.01 - 0.28	0.41	2.1
nickel	10/10	45.7 - 64.0	30/30	4 - 140	>140	>140
selenium	10/10	0.1 - 1.9	18/28	0.1 U - 1.0	-	-
silver	3/10	0.02 U - 0.33	28/30	0.02 U - 3.3	0.56	>6.1
zinc	10/10	51.6 - 80.8	30/30	15 - 102	410	1600
Volatile Organics:						
Carbon disulfide	1/10	2 U - 8 J	0/10	-	-	-
acetone	1/10	4 U - 130	0/10	-	-	-
2-butanone	1/10	1 BU - 21 B	0/10	-	-	-
methylene chloride	1/10	4 BU - 77 B	0/10	-	-	-
Total PAH (d):						
low molecular weight PAH	9/10	20 BJ - 860 J	10/25	2.5 - 170	5200	24000
high molecular weight PAH	9/10	71 U - 1700 J	12/15	22 - 217	12000	69000
Phenols:						
phenol	2/10	16 BJ - 24 BJ	8/20	3.3 U - 62	420	1200
4-methylphenol	6/10	46 J - 520	7/11	2 U - 290	670	3600
Miscellaneous Extractables:						
retene	9/10	64 J - 540	8/13	U - 130 J	-	-
dibenzofuran	5/10	19 J - 31 J	4/11	5 U - 14	540	1700
n-nitrosodiphenylamine	1/10	71 U - 12 J	0/8	0.5 U - 10 U	28	130
Phthalates:						
di-n-butylphthalate	8/10	13 BJ - 35 BJ	6/8	20 U - 760	1400	>5100
di-n-octylphthalate	4/10	15 BJ - 510 B	5/12	0.5 U - 56 U	>420	6200
bis(2-ethylhexyl)phthalate	10/10	46 BJ - 610 B	3/8	0.5 U - 58	1300	>3100

(a) Tetra Tech (1986a, 1988a)

(b) Tetra Tech (1988a)

(c) number of samples where chemical detected/total number of samples analyzed

(d) polyaromatic hydrocarbons

U = not detected at detection limit shown

J = estimated concentration

chemical concentrations in Grays Harbor sediments were above thresholds for adverse biological effects that have been determined through sediment bioassays and assessment of benthic invertebrate communities in Puget Sound sediments. Amphipod bioassays of pulp mill outfall sediments in Grays Harbor showed no acute toxicity or evidence of sublethal effects.

Resin acids were not consistently elevated at any one location (Table 27). Guaiacols were detected only at the ITT outfall while fatty acids (oleic and linoleic acids) were detected at all sites in Grays Harbor. Except for a low concentration of oleic acid (76 µg/kg), resin acids, guaiacols, and fatty acids were not detected in North Bay. Concentrations of resin acids (16-210 µg/kg) and quaiacols (9-19 µg/kg) in Grays Harbor sediments appeared low relative to Puget Sound pulp mills and comparable to those reported below Columbia River mills (Tetra Tech 1988b; Johnson and Norton 1988). Similar, comparative data were not available for fatty acids.

2,3,7,8-TCDD, the most toxic of the dioxins and furans, was not detected in Ecology's sediment samples from either pulp mill outfall or in nearby Cow Point sediments at detection limits of 0.7-0.8 ng/kg. Higher chlorinated dioxins (hepta- and octa-chlorodibenzodioxin) were detected at similar levels at all three sites in concentrations ranging from 11 to 140 ng/kg. Trace amounts of 2,3,7,8-tetrachlorodibenzofuran (TCDF) were also detected at the ITT outfall (2.4 ng/kg) and Cow Point (2.8 ng/kg), but not at the Weyerhaeuser outfall.

Table 27. Concentrations of resin acids and guaiacols detected in Grays Harbor sediments compared to Puget Sound reference area sediments and sediments near other northwest pulp mills (concentration range: µg/kg, dry wt. (ppb)) (from Johnson and Coots 1989).

			Sediments Near Other Northwest Pulp Mills :			
Chemical	Grays Harbor	Puget Sound Reference Areas (a) (n=3)	Everett Harbor, East Waterway (a) (n=14)	Everett Harbor Port Gardner (a) (n=5)	Port Townsend Bay (b) (n=3)	Columbia River @ Camas Vancouver & Longview (c) (n=3)
Resin Acids:						
abietic acid	29 J - 130 J	130 U - 180 U	590 - 98000	280 J - 1700	160 J - 4400	110 - 500
dehydroabietic acid	16 BJ - 980 B	20 J - 130 U	1200 - 83000	560 - 1500	230 J - 33000	240 - 920
dichlorodehydroabietic acid	70 J - 80 J	130 U - 180 U	170 J - 900 U	140 U - 240 U	270 U - 450 U	42 J - 300 U
isopimaric acid	61 J - 210 J	130 U - 180 U	330 J - 6200	85 J - 350 J	200 U - 1700	130 - 500
sandaracopimaric acid	71 U - 92 J	130 U - 180 U	200 J - 8800	43 J - 110 J	21 J - 970	32 J - 130 J
12-chlorodehydroabietic acid	NA	130 U - 180 U	200 J - 11000	78 J - 270 J	NA	NA
14-chlorodehydroabietic acid	NA	130 U - 180 U	220 U - 1400	46 J - 190 U	NA	NA
neoabietic acid	NA	130 U - 180 U	79 J - 14000 J	220 U - 3400	NA	NA
levopimaric acid	NA	NA	NA	NA	270 U - 450 U	100 U - 300 U
palustric acid	NA	NA	NA	NA	270 U - 450 U	100 U - 300 U
Guaiacols:						
guaiacol	19 J - 130 U	NA	NA	NA	200 U - 450 U	100 U - 300 U
4,5,6-trichloroguaiacol	9 J - 130 U	3 U (all)	4 U - 48	4 U (all)	200 U - 450 U	100 U - 300 U
3,4,5-trichloroguaiacol	NA	3 U (all)	4 U - 110	4 U (all)	NA	NA
tetrachloroguaiacol	71 U - 130 U	3 U (all)	4 U - 50	4 U (all)	200 U - 450 U	100 U - 300 U

(a) Tetra Tech (1988b)

(b) Johnson (1988)

(c) Johnson and Norton (1988)

U = not detected at detection limit shown

J = estimated concentration

NA = not analyzed

An extensive evaluation of dioxin, furan and resin acid levels in Grays Harbor sediments was conducted by the Army Corps of Engineers in 1990 (COE 1990). They concluded that resin acids were "not present at levels sufficient to cause toxicity to benthic biota" and "would not be released from sediments to the water column at measurable levels during dredging". Consistent with Ecology's findings, TCDD and TCDF were detected in Grays Harbor sediments at several locations at, or slightly above, method detection limits (approximately 1 ng/kg). The COE concluded that the observed concentrations should not cause acute toxicity to, or significant accumulation in, biota. The EPA concurred with the COE's conclusions and also determined that dredged material was suitable for unconfined open water disposal in estuarine and ocean waters.

A number of other chemical and biological evaluations of Grays Harbor sediments have been conducted, particularly over the last decade (e.g., Am. Test, Inc. and Environmental Resources Section 1981; Pierson et al. 1983; Word 1987; Brown et al. 1984; Lattin 1986). The results of these other chemical evaluations of sediments are generally comparable to Ecology's in terms of both the types and levels of contaminants detected. Moreover, these studies have found that sediments were non-toxic to Dungeness crab (*Cancer magister*), amphipods (*Rhepoxynius abronius* and *Grandifoxus grandis*), and chum salmon (*Oncorhynchus keta*). Bioaccumulation potential for sediment-associated chemicals also appeared to be low and sediments did not appear to affect the osmoregulatory capacity

of coho smolts. Thus, these other studies support Ecology's findings that low levels of contaminants are present in sediments.

Chemical Analyses of Fish and Shellfish Tissues from Grays Harbor as Part of the EPA National Bioaccumulation Study, 1987

EPA's National Bioaccumulation Study, initiated in 1986, was a one time screening survey with two primary objectives: 1) determine the prevalence of selected bioaccumulative pollutants in fish and shellfish in the United States, and 2) identify correlations with sources of these pollutants (Tetra Tech 1990). As part of this effort and in support of the salmon survival study, EPA collected samples of starry flounder (*Platichthys stellatus*) and soft shell clams (*Mya arenaria*) from inner Grays Harbor below the Weyerhaeuser and ITT mills during 1987. Samples were analyzed for 15 dioxins/furans and 44 additional potentially toxic organic compounds determined by EPA to have high potential to accumulate in fish. The list of target analytes are provided in Table 28.

Contaminants detected in the Grays Harbor samples are summarized in Table 29. Five dioxins/furans and nine other compounds were detected in one or more samples. The dioxin/furan present at the highest concentration was 2,3,7,8-TCDF in flounder (9.1 ppt) and clams (13 ppt) below the Weyerhaeuser outfall. 2,3,7,8-TCDD was only detected in flounder caught below the Weyerhaeuser outfall (0.4 ppt).

Of the other target compounds, PCBs and DDE (a metabolite of the pesticide DDT) were detected most frequently and in the highest

Table 28. Target analytes for EPA's National Bioaccumulation Study, 1987.

Compound Name	Chemical Abstracts Registry No.
1,3,5- Trichlorobenzene	108-70-3
1,2,4- Trichlorobenzene	120-82-1
1,2,3- Trichlorobenzene	87-61-6
Hexachlorobutadiene	87-68-3
1,2,4,5- Tetrachlorobenzene	95-94-3
1,2,3,5- Tetrachlorobenzene	634-90-2
Biphenyl	92-52-4
1,2,3,4- Tetrachlorobenzene	634-66-2
Pentachlorobenzene	608-93-5
Trifluralin	1582-09-8
Alpha-BHC	319-84-6
Hexachlorobenzene	118-74-1
Pentachloroanisole	1825-21-4
Gamma-BHC (Lindane)	58-89-9
Pentachloronitrobenzene	82-68-8
Diphenyl Disulfide	882-33-7
Heptachlor	76-44-8
Chlorpyrifos	2921-88-2
Isopropalin	33820-53-0
Octachlorostyrene	29082-74-4
Heptachlor epoxide	1024-57-3
Oxychlordane	27304-13-8
Chlordane, trans	5103-74-2
Chlordane, cis	5103-73-1
Nonachlor, trans	39765-80-5
p,p'- DDE	72-55-9
Dieldrin	60-57-1
Nitrofen	1836-75-5
Endrin	72-20-8
Perthane	72-56-0
Nonachlor, cis	3734-49-4
Methoxychlor	72-43-5
Dicofol (Kelthane)	115-32-2
Mirex	2385-85-5
Total Monochlorobiphenyl	27323-18-8
Total Dichlorobiphenyl	25512-42-9
Total Trichlorobiphenyl	25323-68-6
Total Tetrachlorobiphenyl	26914-33-0
Total Pentachlorobiphenyl	25429-29-2
Total Hexachlorobiphenyl	26601-64-4
Total Heptachlorobiphenyl	28655-71-2
Total Octachlorobiphenyl	31472-83-0
Total Nonachlorobiphenyl	53742-07-7
Total Decachlorobiphenyl	2051-24-3
Total Polychlorinated Biphenyls	1336-36-3

Table 29. Results of tissue analyses (wet weight basis) of starry flounder and softshell clams obtained during EPA's National Bioaccumulation Study. The animals were captured below the Weyerhaeuser and ITT pulp mill outfalls in spring 1987.

Location:	Below Weyerhaeuser Outfall		Below ITT Outfall	
Species:	Starry Flounder	Softshell Clam	Starry Flounder	Softshell Clam
Tissue:	Whole Body	Soft Parts	Whole Body	Soft Parts
Dioxins (ppt):				
2,3,7,8-TCDD	0.4	ND	ND	ND
1,2,3,4,6,7,8-HpCDD	0.7	2.2	0.5	1.7
Furans (ppt):				
2,3,7,8-TCDF	9.1	13	ND	1.9
2,3,4,7,8-PeCDF	0.2	ND	ND	ND
1,2,3,4,6,7,8-HpCDF	0.6	0.4	ND	0.3
Other Compounds (ppb):				
total PCBs	7.3	5.8	8.0	ND
DDE	1.4 D	ND	0.9 D	1.0 D
gamma BHC	1.5 D	ND	ND	ND
alpha BHC	ND	ND	0.2 D	ND
methoxychlor	ND	ND	0.3 D	ND
biphenyl	0.2 D	0.1 D	0.2 D	ND
1,2,3-trichlorobenzene	0.1 D	ND	0.1 D	ND
1,2,4-trichlorobenzene	0.1 D	ND	0.1 D	ND
1,2,3,4-tetrachlorobenzene	0.1 D	ND	0.1 D	ND

ND = not detected

D = an estimated value, below limits of quantification

ppt = parts per trillion

ppb = parts per billion

concentrations. Similar levels were measured in flounder from both sites (7.3 - 8.0 ppb for PCBs and 0.9 - 1.4 ppb for DDE).

To put these results in perspective, Table 30 compares Grays Harbor data with median values reported by EPA from the national survey. Medians are grouped according to type of upstream source and background sites. Except for 2,3,7,8-TCDF, levels of compounds from tissues collectedfrom Grays Harbor are typical of, or lower than, background levels. 2,3,7,8-TCDF concentrations in flounder and clams below the Weyerhaeuser outfall compare closely to the EPA national median for areas below bleach pulp mills.

Since completion of the salmon survival study, some additional data on dioxins and furans in Grays Harbor organisms, including salmon smolts, have become available (Table 31). The U.S. Fish and Wildlife Service (Benkert personal communication) analyzed samples of juvenile chinook salmon and clams collected near the Weyerhaeuser outfall and one sample of amphipods (*Corophium* spp.) from Bowerman Basin. In addition, the Army Corps of Engineers and EPA jointly analyzed Dungeness crab samples from North Bay and South Bay for use in developing risk assessments. All samples, except for the amphipods, were consistent with EPA's data from their national survey. Concentrations of 2,3,7,8-TCDD (23.4 ppt) and 2,3,7,8-TCDF (27.5 ppt) in the amphipod samples from Bowerman Basin are sufficiently high to raise concerns about adverse effects on coho smolts or other organisms that feed on amphipods. Additional samples are needed to confirm this finding. In summary, the preponderance of data presented in this section (tissue

Table 30. The results of animal tissue analyses conducted during EPA's National Bioaccumulation Study in Grays Harbor compared to the national medians.

Compound	Grays Harbor (range)	Background Sites	Sewage Treatment Plant Sites	Bleach Pulp Mill Sites	Other Urban/ Industrial Sites	Agricultural Sites
Dioxins (ppt):						
2,3,7,8-TCDD	ND-0.4	0.5	0.6	4.7	1.3	0.6
1,2,3,4,6,7,8-HpCDD	0.5-2.2	- - - - - - - - - - - statistic not available - - - - - - - - -				
Furans (ppt):						
2,3,7,8-TCDF	ND-13	0.9	0.5	13	2.2	0.6
2,3,4,7,8-PeCDF	ND-0.2	0.4	0.5	1.3	0.9	0.4
1,2,3,4,6,7,8-HpCDF	ND-0.6	- - - - - - - - - - - statistic not available - - - - - - - - -				
Other Compounds (ppb):						
total PCBs	ND-8.0	9	22	--	326	7
DDE	ND-1.4	15	34	--	62	197
gamma BHC	ND-1.5	- - - - - - - - - - - statistic not available - - - - - - - - -				
alpha BHC	ND-0.2	ND	1	--	0.8	ND
methoxychlor	ND-0.3	- - - - - - - - - - - statistic not available - - - - - - - - -				
biphenyl	ND-0.2	0.2	0.6	--	0.7	0.5
1,2,3-trichlorobenzene	ND-0.1	- - - - - - - - - - - statistic not available - - - - - - - - -				
1,2,4-trichlorobenzene	ND-0.1	- - - - - - - - - - - statistic not available - - - - - - - - -				
1,2,3,4-tetrachlorobenzene	ND-0.1	- - - - - - - - - - - statistic not available - - - - - - - - -				

ND = not detected
ppt = parts per trillion
ppb = parts per billion

Table 31. Results of other chemical analyses of animal tissues (parts per trillion, wet weight basis) from Grays Harbor.

Investigator:	U.S. Fish and Wildlife Service (unpub. data)			Army Corps of Engineers (Word et al., 1990)			
Location:	Near Weyerhaeuser		Bowerman Basin	North Bay		South Bay	
Organism:	Chinook Smolts	Clams	Amphipods	Dungeness Crabs		Dungeness Crabs	
Tissue:	Whole Body	Soft Parts	Whole Body	Muscle	Hepatopancreas	Muscle	Hepatopancreas
Dioxins:							
2,3,7,8-TCDD	1.3	ND	23.4	ND	ND	ND	2.1
1,2,3,7,8-PeCDD	ND	ND	ND	ND	3.8	ND	3.7
1,2,3,4,7,8-HxCDD	ND	ND	14.9	ND	11	ND	ND
1,2,3,6,7,8-HxCDD	ND	ND	ND	ND	ND	ND	7.3
1,2,3,7,8,9-HxCDD	ND	ND	ND	ND	11	ND	ND
1,2,3,4,6,7,8-HpCDD	1.8	2.0	114	2.2	9.9	1.9	6.4
OCDD	9.9	12.2	2300	22	20	24	1.5
Furans:							
2,3,7,8-TCDF	1.6	1.1	27.5	1.9	28	1.5	38
1,2,3,7,8-PeCDF	ND	ND	ND	ND	ND	ND	0.6
2,3,4,7,8-PeCDF	ND	ND	ND	ND	1.8	ND	2.1
1,2,3,4,7,8-HxCDF	ND	ND	41.1	ND	1.7	ND	1.1
1,2,3,6,7,8-HxCDF	ND	ND	34.8	ND	ND	ND	ND
1,2,3,7,8,9-HxCDF	ND	ND	ND	ND	ND	ND	ND
2,3,4,6,7,8-HxCDF	ND	ND	ND	ND	ND	ND	ND
1,2,3,4,6,7,8-HpCDF	ND	ND	85.1	ND	ND	ND	ND
1,2,3,4,7,8,9-HpCDF	ND	ND	ND	ND	ND	ND	ND
OCDF	ND	ND	97.1	ND	ND	ND	ND

ND = not detected

analyses) and elsewhere in the report (sediments and water column evaluations) indicates that levels of dioxins and furans present in Grays Harbor are relatively low.

SUMMARY AND CONCLUSIONS

There were three major objectives of Grays Harbor water quality studies. The first was to evaluate the chemical composition of several major effluent streams that enter the estuary. Attention was focused on the Weyerhaeuser and ITT pulp mills and Aberdeen and Hoquiam sewage treatment plants because they comprise the greatest volume of material discharged into the inner harbor. Data obtained on the sewage treatment plant effluents indicated they were typical for these types of discharges, had low volumes, and did not elicit effects in bioassays; thus, it is unlikely that they have significant adverse effects on smolts moving through the inner harbor.

A large number of potentially toxic chemicals were detected in both ITT and Weyerhaeuser pulp mill effluents. The detection frequency and concentrations of chemicals tended to be higher in Weyerhaeuser effluent. All detected chemicals have been previously reported in pulp mill effluents. Furthermore, concentrations were below those expected to have adverse effects on aquatic life in the receiving environment. None of the chemicals found in the effluents of either mill could be linked to the salmon survival problem.

Because it was possible to analyze only a small subset of chemicals present in the pulp mill effluents, a number of bioassays

were employed to assess toxic effects of pulp mill effluents. Although both effluents elicited toxic responses in some bioassays, Weyerhaeuser effluent had greater potential for causing toxic conditions in the receiving waters. However, concentrations of chemicals and bioassays could not be correlated, suggesting the organisms were responding to effluent constituents (or combinations of them) other than those measured. Receiving water dilutions were calculated for the inner harbor from results of the echinoderm sperm and oyster larvae bioassays and studies of effluent movements. Although it is difficult to relate results of these calculations to the salmon survival problem, it appears that effluent dilution in much of the inner harbor may not be sufficient to reduce concentrations to average NOEC levels.

An additional goal of the analyses of mill effluents was to determine whether effluent quality during the salmon survival study was representative of "normal" discharges. A comprehensive inspection of each mill conducted before the 1988 salmon survival experiments revealed that both mills were in compliance with discharge permits at the time of the inspection. In late winter and spring 1989, repetitive and unannounced sample collections were conducted at each mill to obtain an unbiased evaluation of effluents. Results of these collections indicated that mill effluent quality was inherently variable but that normal discharges appeared to be occurring.

Historically, Weyerhaeuser has had a high rate of failure in the rainbow trout bioassay requirement of their permit. They have

instituted a number of process changes in an effort to solve the persistent toxicity problem. Rainbow trout bioassays conducted by Ecology and Weyerhaeuser in 1989 indicated that as of 1989, toxic conditions occurred less frequently. However, the toxicity problem had not been completely resolved at the time this study was conducted. Effluent quality varied between lethal and benign over a period as short as one day. Moreover, the pattern of bioassay results indicated that a toxic property of the effluent may have been absent or reduced during the salmon smolt bioassay. Clearly, other factors could also explain the bioassay results.

The second objective of the water quality analyses was to evaluate quality of Chehalis River water. Although *Ceriodaphnia* bioassays conducted by EPA showed some evidence of toxicity of river water, this was transient and was also observed in the Humptulips River. From the standpoint of chemical contamination, water quality of the Chehalis River as assessed near Montesano was good, consistent with results of the continuous-flow coho smolt bioassay. Levels of metals were normal (i.e., background) while only trace amounts of several organic compounds occurred.

The third objective was to evaluate the occurrence of selected potentially toxic chemicals in the receiving environment of Grays Harbor. Bottom sediments, the water column, and animal tissues were included in this evaluation.

The extensive evaluation of sediments in 1988 indicated that the estuary does not have a high level of contamination relative to other urban embayments, such as those in Puget Sound. Metals

concentrations in sediments were similar throughout the harbor and in North Bay. Organic compounds detected in sediments were below levels that produce adverse effects in Puget Sound. Constituents such as resin acids, dioxins, furans and chlorophenols were not found at high concentrations in inner harbor sediments. One factor that may have a significant influence on results is the environment from which samples were taken. Much of the sediment sampling was conducted in channels where there is a higher rate of removal of materials due to currents and, in some places, routine dredging. This fact, coupled with high rate of suspended sediment discharge from the Chehalis drainage, may limit conclusions one can draw between contaminant levels in bottom sediments and the water column.

The chemical evaluation of the water column included analyses of suspended particulate matter, soluble materials removed with an XAD resin column, and whole water. Although some chemicals were found in the inner harbor, Chehalis River, and North Bay, concentrations of most materials were generally very low. The concentrations of chemicals were, for the most part, greater in the inner harbor than in the North Bay and Montesano area of the Chehalis River. Chemistry of the water column varied as a function of location and when the samples were collected (e.g., 1988 versus 1989). A number of materials found in the water column can be associated with wood products activities; some of the same materials were also found in samples of pulp mill effluent.

As part of the National Bioaccumulation Study, EPA collected

starry flounder and soft shell clams from the inner harbor below the Weyerhaeuser and ITT mills in 1987. Samples were analyzed for 15 dioxins and furans and 44 additional toxic organic compunds determined by EPA to have high potential to accumulate in fish. Five dioxins/furans and nine other compounds were detected in one or more samples. Except for 2,3,7,8-TCDF, concentrations of these compounds were typical of, or lower than, background levels. TCDF concentrations were similar to levels that EPA found below other bleach pulp mills. Tissue samples analyzed by various agencies as part of other Grays Harbor studies also indicated that levels are generally consistent with EPA's bioaccumulation study. Thus, the results of the chemical analyses of the receiving environment indicate that although a variety of chemicals were detected throughout the inner harbor, concentrations were generally low to non-detectable and below levels known to adversely affect aquatic life. And, in addition, none of the chemicals detected in Grays Harbor could be conclusively linked to the salmon survival problem.

There are several limitations to the chemical analyses conducted during this study. First, because of the large number of potential chemicals involved, it was not possible to include in the analyses all those that might affect survival of Chehalis River coho. Not only would an analysis of all possible chemicals have been far too costly, but analytical methods are not yet available for many compounds. This limitation was illustrated by analyses of pulp mill effluent samples collected by Ecology during spring, 1989. Individual chlorinated compounds targeted in these analyses

represented less than one percent of the total load of chlorinated material discharged into the inner harbor each day by the Weyerhaeuser and ITT pulp mills.

A second major limitation of the chemical analyses was that the significance of detecting a particular chemical or concentration was unknown in many cases. Moreover, effects of combinations of chemicals were also unknown. This was exacerbated by the general lack of data on toxicity of specific organic chemicals to smolts.

A third limitation is that conditions in the inner harbor constantly change. As a result, chemicals present in the inner harbor can vary from year-to-year. Both mills, for instance, have recently gone to high substitution of chlorine in their bleaching processes to reduce the quantity of chlorinated organics discharged into the inner harbor. Additionally, both have instituted new measures to prevent or decrease the inadvertent introduction of spills or chemicals into their waste streams. ITT has improved its solids removal, stopped discharging activited sludge, and increased aeration of its large treatment pond. Weyerhaeuser has installed oxygen delignification, a process which requires less use of chemicals, surfactants, and bleaching agents. Furthermore, the mill has stopped using sulfuric acid for coliform control and now continuously discharges effluent into Grays Harbor as opposed to discharging twice a day.

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APPENDICES

Appendix Table 1A. Numbers of wild coho salmon assayed for ATPase activity and thyroid hormone levels in the Humptulips and Chehalis River basins, March-June 1987. (R=Residents, M=Migrants)

Site/Parameter	Date															
	Mar 12-13		Mar 25-26		Apr 8-9		Apr 22-23		May 3-5		May 13-15		May 27-28		June 8	
	R	M	R	M	R	M	R	M	R	M	R	M	R	M	R	M
Stevens Cr.																
ATPase	15	-	15	-	15	-	15	-	15	15	15	15	-	15	-	14
T3, T4	-	-	-	-	12	-	-	-	-	-	15	-	-	-	-	-
Bingham Creek																
ATPase	10	-	15	-	15	15	15	15	15	15	15	15	-	15	-	15
T3, T4	-	-	15	-	15	15	-	-	-	-	-	15	-	-	-	-
Stillman Creek																
ATPase	15	-	15	-	15	15	15	15	15	15	8	15	-	15	-	-
T3, T4	-	-	15	-	15	-	-	-	15	-	-	-	-	-	-	-
Scoop Trap																
ATPase	-	-	-	-	-	6	-	15	-	14	-	15	-	15	-	-
T3, T4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Appendix Table 1B. Numbers of wild coho salmon measured for morphometrics, color pattern changes, growth and condition, and hematocrits, March-June 1987. (R=Residents, M=Migrants)

Site/Parameter	Date															
	Mar 12-13		Mar 25-26		Apr 8-9		Apr 22-23		May 3-5		May 13-15		May 27-28		June 8	
	R	M	R	M	R	M	R	M	R	M	R	M	R	M	R	M
Stevens Cr.																
Morphometrics	15	-	15	-	15	-	15	-	15	-	-	-	-	15	-	-
Color Patterns	15	-	15	-	15	-	15	-	15	15	15	15	-	15	-	14
Growth, Condition	15	-	15	-	15	-	15	-	15	15	15	15	-	15	-	14
Hematocrits	14	-	15	-	15	-	15	-	14	15	14	15	-	15	-	14
Bingham Creek																
Morphometrics	15	-	15	-	15	-	15	-	15	-	15	-	-	-	-	-
Color Patterns	15	-	15	-	15	15	15	15	15	15	15	15	-	15	-	15
Growth, Condition	15	-	15	-	15	15	15	15	15	15	15	15	-	15	-	15
Hematocrits	13	-	15	-	15	15	14	15	15	15	14	15	-	15	-	15
Stillman Creek																
Morphometrics	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Color Patterns	10	-	15	-	15	15	15	15	15	15	8	15	-	15	-	-
Growth, Condition	10	-	15	-	15	15	15	15	15	15	8	15	-	15	-	-
Hematocrits	10	-	15	-	15	15	11	14	15	15	7	15	-	15	-	-
Scoop Trap																
Morphometrics	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Color Patterns	-	-	-	-	-	6	-	15	-	14	-	15	-	15	-	-
Growth, Condition	-	-	-	-	-	6	-	15	-	14	-	15	-	15	-	-
Hematocrits	-	-	-	-	-	6	-	15	-	14	-	15	-	-	-	-

Appendix Table 1C. Numbers of wild coho salmon used to determine the general health and *Nanophyetus* loadings in the Humptulips and Chehalis River basins, March-June 1987. (R=Residents, M=Migrants)

Site/Parameter	Date															
	Mar 12-13		Mar 25-26		Apr 8-9		Apr 22-23		May 3-5		May 13-15		May 27-28		June 8	
	R	M	R	M	R	M	R	M	R	M	R	M	R	M	R	M
Stevens Cr.																
General Health	15	-	15	-	-	-	16	-	13	14	15	15	-	15	-	14
Histology	-	-	-	-	-	-	-	-	-	14	-	-	-	-	-	-
Nanophyetus	15	-	15	-	-	-	16	-	13	14	15	15	-	15	-	14
Bingham Creek																
General Health	15	-	15	-	-	15	14	14	15	14	15	13	-	15	-	15
Histology	-	-	-	-	-	-	-	-	-	14	-	-	-	-	-	-
Nanophyetus	15	-	15	-	-	15	14	14	15	14	15	13	-	15	-	15
Stillman Creek																
General Health	10	-	15	-	15	15	-	14	15	10	-	15	-	15	-	-
Histology	-	-	-	-	-	-	-	-	-	10	-	-	-	-	-	-
Nanophyetus	10	-	15	-	15	15	-	14	15	10	-	15	-	15	-	-
Scoop Trap																
General Health	-	-	-	-	-	6	-	15	-	15	-	15	-	15	-	-
Histology	-	-	-	-	-	-	-	-	-	15	-	-	-	-	-	-
Nanophyetus	-	-	-	-	-	6	-	15	-	15	-	15	-	15	-	-

Appendix Table 1D. Numbers of wild coho salmon assayed for ATPase activity, thyroid hormone levels, and cortisol in the Humptulips and Chehalis River basins, March - May 1988. (R=Residents, M=Migrants)

Site/Parameter	3/21-22		4/18-20		4/28-30		5/5/-9		5/13-14[1]		5/27-28	
	R	M	R	M	R	M	R	M	R	M	R	M
Stevens Creek												
ATPase	15	-	15	7	15	15	15	15	15	15	0	15
T3,T4	14	-	-	15	15	-	15	15	15	15	15	15
Cortisol	-	-	-	-	-	10	-	14	-	15	-	-
Lower Humptulips												
ATPase	-	-	-	-	-	21	-	18	-	19	-	13
T3,T4	-	-	-	-	-	-	-	-	-	-	-	-
Cortisol	-	-	-	-	-	-	-	-	-	-	-	-
Bingham Creek												
ATPase	15	-	15	15	15	15	15	15	15	15	-	15
T3,T4	15	-	-	15	15	-	15	15	15	15	15	15
Cortisol	-	-	-	-	-	15	-	15	-	15	-	-
Stillman Creek[2]												
ATPase	15	-	15	15	15	15	12	15	15	15	-	15
T3,T4	-	-	-	15	15	-	15	15	15	15	15	-
Cortisol	-	-	-	-	-	-	-	-	-	-	-	-
Scoop Trap												
ATPase	-	-	-	13	-	15	-	15	-	13	-	-
T3,T4	-	-	-	15	-	15	-	15	-	15	-	-
Cortisol	-	-	-	-	-	14	-	15	-	30	-	-
Lower Chehalis												
ATPase	-	-	-	13	-	19	-	6	-	26	-	-
T3,T4	-	-	-	-	-	-	-	-	-	-	-	-
Cortisol	-	-	-	-	-	-	-	-	-	-	-	-

1 Cortisol samples were collected on May 16, 17.
2 Samples were obtained from several locations in the Stillman Creek drainage.

Appendix Table 1E. Numbers of wild coho measured for morphometrics, color pattern changes, condition factor and growth, and hematocrits, March - May 1988. (R=Residents, M=Migrants)

Site/Parameter	3/21-22		4/18-20		4/28-30		5/5/-9		5/13-14		5/27-28	
	R	M	R	M	R	M	R	M	R	M	R	M
Stevens Creek												
Morphometrics	14	-	-	15	-	15	-	15	15	-	-	-
Color Pattern	19	-	14	7	15	15	17	14	15	15	-	15
Condition Factor, Growth	19	-	14	7	15	15	17	15	15	15	-	15
Hematocrits	19	-	14	7	15	15	0	15	15	15	-	15
Lower Humptulips												
Morphometrics	-	-	-	-	-	-	-	-	-	-	-	-
Color Pattern	-	-	-	-	-	21	-	18	-	19	-	13
Condition Factor, Growth	-	-	-	-	-	21	-	18	-	19	-	13
Hematocrits	-	-	-	-	-	-	-	-	-	-	-	-
Bingham Creek												
Morphometrics	15	-	15	15	15	15	15	15	15	15	15	-
Color Pattern	16	-	15	15	15	15	15	15	15	15	-	15
Condition Factor, Growth	16	-	15	15	15	15	15	15	15	15	-	15
Hematocrits	16	-	15	15	15	15	15	15	15	15	-	14
Stillman Creek[1]												
Morphometrics	-	-	-	-	-	-	-	-	-	-	-	-
Color Pattern	15	-	15	14	15	15	12	15	15	15	-	15
Condition Factor, Growth	15	-	15	14	15	15	12	15	15	15	-	15
Hematocrits	15	-	15	13	15	15	12	15	15	15	-	15
Scoop Trap												
Morphometrics	-	-	-	-	-	-	-	-	-	-	-	-
Color Pattern	-	-	-	13	-	15	-	15	-	13	-	-
Condition Factor, Growth	-	-	-	13	-	15	-	15	-	13	-	-
Hematocrits	-	-	-	13	-	15	-	15	-	13	-	-
Lower Chehalis												
Morphometrics	-	-	-	-	-	-	-	-	-	-	-	-
Color Pattern	-	-	-	-	13	-	5	-	12	-	-	-
Condition Factor, Growth	-	-	-	-	13	-	6	-	26	-	-	-
Hematocrits	-	-	-	-	13	-	0	-	11	-	-	-

[1] Samples obtained from several locations within the Stillman Creek drainage.

Appendix Table 1F. Numbers of wild coho used to determine the general health and *Nanophyetus* loadings in the Humptulips and Chehalis River basins, March - May 1988. (R = Resident, M = Migrants)

Site/Parameter	3/21-22		4/18-20		4/28-30		5/5/-9		5/13-14		5/27-28	
	R	M	R	M	R	M	R	M	R	M	R	M
Stevens Creek												
General Health	19	-	17	7	15	14	17	15	17	15	-	-
Histology	-	-	-	-	-	-	-	-	17	15	-	-
Nanophyetus	19	-	15	7	15	14	17	15	17	15	-	-
Lower Humptulips												
General Health	-	-	-	-	-	19	-	18	-	-	-	-
Histology	-	-	-	-	-	-	-	-	-	-	-	-
Nanophyetus	-	-	-	-	-	19	-	18	-	-	-	-
Bingham Creek												
General Health	16	-	15	15	14	13	15	15	15	15	-	-
Histology	-	-	-	-	-	-	-	-	15	15	-	-
Nanophyetus	16	-	15	15	14	13	15	15	15	15	-	-
Stillman Creek[1]												
General Health	15	-	15	15	15	15	11	15	15	15	-	-
Histology	-	-	-	-	-	-	-	-	15	15	-	-
Nanophyetus	15	-	15	15	15	15	11	15	15	15	-	-
Scoop Trap												
General Health	-	-	-	13	-	15	-	15	-	13	-	-
Histology	-	-	-	-	-	-	-	-	-	13	-	
Nanophyetus	-	-	-	13	-	15	-	15	-	13	-	-
Lower Chehalis												
General Health	-	-	-	10	-	16	-	6	-	14	-	-
Histology	-	-	-	-	-	-	-	-	-	14	-	-
Nanophyetus	-	-	-	10	-	16	-	6	-	14	-	-

[1] Samples were obtained from several locations in the Stillman Creek drainage.

Appendix Table 1G. Numbers of wild coho salmon used to evaluate response to a secondary stress and immunocomptence in the Humptulips and Chehalis River basins, March - May 1988. (R=Resident, M=Migrants)

Site/Parameter	3/22		4/18-21		4/28-5/1		5/5-6		5/13-14[2]		5/27-28	
	R	M	R	M	R	M	R	M	R	M	R	M
Stevens Creek												
Secondary Stress	-	-	-	-	-	15,10	-	-	-	-	-	-
Immune Response	-	-	-	14	-	-	-	14	-	-	-	-
Lower Humptulips												
Secondary Stress	-	-	-	-	-	-	-	-	-	-	-	-
Immune Response	-	-	-	-	-	-	-	-	-	19	-	-
Bingham Creek												
Secondary Stress	-	-	-	-	-	15,10	-	-	-	-	-	-
Immune Response	-	-	-	17	-	-	-	20	-	-	-	-
Stillman Creek[1]												
Secondary Stress	-	-	-	-	-	-	-	-	-	-	-	-
Immune Response	-	-	-	-	-	-	-	-	-	-	-	-
Scoop Trap												
Secondary Stress	-	-	-	-	-	15,10	-	-	-	-	-	-
Immune Response	-	-	-	-	-	-	-	20	-	-	-	-
Lower Chehalis												
Secondary Stress	-	-	-	-	-	-	-	-	-	-	-	-
Immune Response	-	-	-	-	-	-	-	-	-	-	-	-

[1] Samples were obtained from several locations in the Stillman Creek drainage.
[2] Cortisol collected on May 16, 17.

Appendix Table 1H. Numbers of cultured coho salmon examined for morphometrics color pattern changes, growth and condition factor, hematocrits, ATPase activity, and thyroid hormones, March-June 1987.

Site Parameter	Date							
	Mar 12-13	Mar 25-26	Apr 8-9	Apr 22-23	May 3-5	May 13-15	May 27-28	June 8
Humptulips, Pd. 17								
Morphometrics	-	-	-	-	-	-	-	-
Color Patterns	113	130	130	131	130	130	130	130
Growth, Condition	113	130	130	131	130	130	130	130
Hematocrits	15	15	15	15	15	15	15	15
ATPase	15	15	15	15	15	15	15	15
T3, T4	-	15	15	-	-	15	-	-
Humptulips, Pd. 18								
Morphometrics	15	15	15	15	15	15	15	-
Color Patterns	136	130	131	131	130	130	130	130
Growth, Condition	136	130	131	131	130	130	130	130
Hematocrits	15	15	15	15	15	15	15	15
ATPase	15	15	15	15	15	15	15	15
T3, T4	-	15	15	-	-	15	-	-
Simpson								
Morphometrics	15	15	15	15	15	15	15	-
Color Patterns	136	130	130	130	130	130	130	130
Growth, Condition	136	130	130	130	130	130	130	130
Hematocrits	15	15	15	15	15	15	15	15
ATPase	15	15	15	15	15	15	15	15
T3, T4	-	15	15	-	-	15	-	-

Appendix Table 1I. Numbers of cultured salmon used to determine general health and *Nanophyetus* loadings, March-June 1987.

Site/ Parameter	Date							
	Mar 12-13	Mar 25-26	Apr 8-9	Apr 22-23	May 3-5	May 13-15	May 27-28	June 8
Humptulips, Pd. 17								
General Health	15	15	15	15	15	14	15	15
Histology	-	-	-	15	-	-	-	-
Nanophyetus	15	15	15	15	15	14	15	15
Humptulips, Pd. 18								
General Health	15	15	15	15	15	15	15	15
Histology	-	-	-	15	-	-	-	-
Nanophyetus	15	15	15	15	15	15	15	15
Simpson								
General Health	15	15	15	14	15	15	13	15
Histology	-	-	-	-	15	-	-	-
Nanophyetus	15	15	15	14	15	15	13	15

Appendix 1J. Numbers of cultured coho salmon examined for color pattern changes, growth and condition factor, morphometric changes, hematocrits, ATPase, thyroid hormones, and cortisol, February - May 1988. Thyroid hormones were also sampled in earlyJune in the sequestered populations.)

Site/Parameter	2/16	3/9-10	3/21	4/4	4/18	4/28	5/5	5/13	5/26
Humptulips Production									
Morphometrics	15	15	15	15	-	15	-	-	-
Color	100	100	100	100	100	100	-	-	-
Growth, Condition	100	100	100	100	100	100	-	-	-
Hematocrits	15	14	15	15	15	15	-	-	-
ATPase	15	15	15	15	-	15	15	-	-
T_3, T_4	15	15	15	15	15	15	-	-	-
Cortisol	15	15	15	15	15	15	-	-	-
Humptulips Sequestered									
Morphometrics	-	-	-	-	-	-	15	15	-
Color	-	100	100	100	100	100	100	100	-
Growth, Condition	-	100	100	100	100	100	100	100	-
Hematocrits	-	15	15	15	15	14	15	-	-
ATPase	-	15	15	15	15	15	15	15	15
T_3, T_4	-	15	15	15	15	15	15	15	-
Cortisol	-	15	15	15	15	15	15	15	-
Simpson Production									
Morphometrics	15	15	15	15	-	15	15	15	-
Color	100	100	100	100	100	100	100	100	-
Growth, Condition	100	100	100	100	100	100	100	100	-
Hematocrits	15	15	15	15	14	15	15	15	-
ATPase	15	15	15	15	15	15	15	15	-
T_3, T_4	15	15	15	15	15	15	15	15	-
Cortisol	15	15	15	15	15	15	15	15	-
Simpson Sequestered									
Morphometrics	-	-	-	-	-	-	-	-	-
Color	-	100	100	100	100	100	100	100	-
Growth, Condition	-	100	100	100	100	100	100	100	-
Hematocrits	-	15	15	15	13	14	14	15	-
ATPase	-	15	15	15	15	15	14	15	15
T_3, T_4	-	15	15	15	15	15	15	15	-
Cortisol	-	15	15	15	15	15	15	15	-

Appendix Table 1K. Numbers of cultured coho salmon used to determine general health and *Nanophyetus* loadings, February to May 1988.

Site/Parameter	2/16	3/9	3/21	4/4	4/18	4/28	5/5	5/13
Humptulips Production								
General Health	15	15	15	15	15	15	-	15
Histology	-	-	-	-	-	-	-	-
Nanophyetus	15	15	15	15	15	15	15	-
Humptulips Sequestered								
General Health	-	15	15	15	15	15	15	-
Histology	-	-	-	-	-	-	15	-
Nanophyetus	-	15	15	15	15	15	15	15
Simpson Production								
General Health	15	15	15	15	13	15	15	-
Histology	-	-	-	-	-	-	15	-
Nanophyetus	15	15	15	15	13	15	15	15
Simpson Sequestered								
General Health	-	15	15	15	15	15	14	-
Histology	-	-	-	-	-	-	14	-
Nanophyetus	-	15	15	15	15	15	14	15

Appendix Table 1L. Numbers of cultured coho salmon used to evaluate response to a secondary stress and immunocompetence, February - May 1988.

Site/Parameter	2/16	3/9	3/21	4/4	4/18	4/28[1]	5/5	5/13	5/26
Humptulips Production									
Secondary Stress	-	-	-	-	-	15,10	-	-	-
Immune System	-	-	-	-	20	20	-	-	-
Humptulips Sequestered									
Secondary Stress	-	-	-	-	-	-	-	-	-
Immune System	-	-	-	-	-	-	-	-	-
Simpson Production									
Secondary Stress	-	-	-	-	-	15,10	-	-	-
Immune System	-	-	-	-	19	20	-	-	-
Simpson Sequestered									
Secondary Stress	-	-	-	-	-	-	-	-	-
Immune System	-	-	-	-	-	-	-	-	-

1 Immune assay was conducted on May 29th. For the secondary stress test, the first number is number evaluated for the resting cortisol level while second number is number assayed after the secondary stress.

Appendix Table 1M. Number of coho salmon assayed for ATPase activity in the inner harbor and North Bay, May 1987.

Site	Date		
	May 3	May 12	May 25-26
Inner Grays Harbor			
Cow Point	9	10	2
Rennie Island	1	13	6
Moon Island	6	12	3
Buoy 30	5	10	1
S. Channel W.	8	11	4
North Bay			
Campbell, Outer	-	-	3
Campbell, Mid.	-	-	5
Campbell, Inner	-	-	2

Appendix Table 1N. Numbers of coho used to determine the ATPase activity, immune response, and *Nanophyetus* loadings in coho captured in the Chehalis River estuary and North Bay, spring 1988.

Site/Parameter	4/30-5/1	5/7-8	5/15-16	5/29-31
CHEHALIS RIVER ESTUARY				
San Island	0	11	0	0
Immune Response	-	-	-	-
Nanophyetus	-	11	-	-
Cow Point				
ATPase	19	20	26	16
Immune Response	-	18	20	-
Nanophyetus	17	-	-	15
Weyco Outfall				
ATPase	0	3	20	14
Immune Response	-	3	19	-
Nanophyetus	-	-	-	11
Moon Island				
ATPase	10	15	20	8
Immune Response	-	13	20	-
Nanophyetus	10	-	-	7
Buoy 30				
ATPase	0	22	16	18
Immune Response	-	19	-	-
Nanophyetus	-	-	-	18
S. Channel West				
ATPase	13	20	17	11
Immune Response	-	-	-	-
Nanophyetus	13	15	15	10
NORTH BAY				
Outer Campbell Slough				
ATPase	0	8	15	8
Immune Response	-	-	-	-
Nanophyetus	-	8	15	8
Mid-Campbell Slough				
ATPase	0	20	21	13
Immune Response	-	16	19	-
Nanophyetus	-	-	-	13
Upper Campbell Slough				
ATPase	17	15	10	18
Immune Response	-	-	-	-
Nanophyetus	15	15	10	15

Appendix Table 10. Numbers of fish used to evaluate ATPase, cortisol, response to a secondary stress, immune system response, and long-term growth and mortality (long-term holding) during the barging assay, 1988. (H = Hatchery, W = Wild)

	BARGE DAY											
	0		1		2		3		4		5	
	H	W	H	W	H	W	H	W	H	W	H	W
Inner Harbor[1]												
ATPase	15	14	16	13	14	13	14	15	13	15	15	15
Cortisol	15	15	-	-	-	-	15	15	-	-	15	15
Secondary Stress[3]	15	-	-	-	-	-	-	-	-	-	15	-
Immune Response	20	20	-	-	-	-	20	-	-	-	20	20
Long-term Holding	92	-	109	-	109	-	83	-	97	-	82	-
North Bay[2]												
ATPase	15	15	13	16	15	13	15	15	13	14	15	15
Cortisol	15	15	-	-	-	-	-	-	-	-	15	15
Secondary Stress[3]	15	-	-	-	-	-	-	-	-	-	15	-
Immune Response	20	20	-	-	-	-	-	-	-	-	20	20
Long-term Holding	102	-	-	-	-	-	-	-	-	-	100	-

[1] Sampled between May 31 (Day 0) and June 4, 1988
[2] Sampled between June 3 (Day 0) and June 7, 1988.
[3] Sample sizes for cortisol: resting levels were N=15 and after the secondary stress were N=9-10

Appendix Table 1P. Numbers of coho salmon used to evaluate ATPase activity, cortisol, immune system response and long-term growth and mortality (long-term holding) during the live box assay in 1989. Live box experiment 1 occurred 4/27-5/8 and experiment 2 was conducted 5/17-5/28.

Live Box	Day 5[1]				Day 9				Day 14			
Experiment 1	**ATPase**	**Cortisol**	**Immune System**	**Long-term Holding**	**ATPase**	**Cortisol**	**Immune System**	**Long-term Holding**	**ATPase**	**Cortisol**	**Immune System**	**Long-term Holding**
Montesano	15	-	-	100	15	15	-	79	15	15	19	77
Elliot Slough	15	-	-	93	15	15	-	92	15	15	19	54
Wishkah	15	-	-	103	15	15	-	89	0	-	-	-
South Channel	15	-	-	103	15	15	-	85	15	15	17	95
North Channel	15	-	-	93	0	15	-	101	0	-	-	-
North Bay 1[2]	15	-	-	95	15	15	-	-	15	15	18	54
North Bay 2	-	-	-	-	-	-	-	91	-	-	-	68
Experiment 2												
Montesano	15	-	-	103	15	15	-	70	15	15	19	137
Elliot Slough	15	-	-	112	15	15	-	78	15	15	18	108
Wishkah	15	-	-	92	15	14	-	76	15	15	20	75
South Channel	15	-	-	81	15	15	-	104	15	15	20	86
North Channel	15	-	-	92	15	15	-	102	15	15	19	128
North Bay[1]	15	-	-	91	15	15	-	29	15	15	20	108
North Bay[3]	15	-	-	103	15	15	-	-	0	-	-	-

[1] Baseline data obtained 4/21/89 AND 5/12/89 at the Hatchery (15 ATPase, 15-cortisol, 20-Immune Assay)
[2] Fish from both North Bay boxes were pooled for some parameters.
[3] Pooled sample on Day 5 & 14 for some parameters.

Appendix Table 1Q. Assays used, numbers of fish used per effluent treatment, and dates assays were performed during the period fish were held at Marrowstone Field Station to evaluate the effects of exposing coho to various effluents. Fish were exposed to effluents at the Aberdeen Sewage Treatment Plant from 5/26 - 5/31, 1988.

	5/31 -6/1	6/1-2			6/7	6/9	6/15 -16	6/29 -30	7/7-8	7/27-28		8/25	9/1 -2	11/16-18		12/ 29
Treatment	MFO[1]	FHA	SWC	HISTO	FHA	VIBRIO	FHA	FHA	HISTO	FHA	HISTO	HISTO	FHA	FHA	HISTO	FHA
Weyco 3%	10	10	10	5	10	10	10	10	5	10	5	5	10	10	5	10
Weyco 3%	10	10	10	5	10	10	10	10	5	10	5	5	10	10	5	10
Weyco 10%	10	10	10	5	10	10	10	10	5	10	5	5	10	10	5	10
Weyco 10%	10	10	10	5	10	10	10	10	5	10	5	5	10	10	5	10
Weyco 30%	10	10	10	5	10	10	10	10	5	10	5	5	10	10	5	10
Weyco 30%	10	10	10	5	10	10	10	10	5	10	5	5	10	10	5	10
ITT 3%	10	10	10	5	10	10	10	10	5	10	5	5	10	10	5	10
ITT 3%	10	10	10	5	10	10	10	10	5	10	5	5	10	10	5	10
ITT 10%	10	10	10	5	10	10	10	10	5	10	5	5	10	10	5	10
ITT 10%	10	10	10	5	10	10	10	10	5	10	5	5	10	10	5	10
ITT 30%	10	10	10	5	10	10	10	10	5	10	5	5	10	10	5	10
ITT 30%	10	10	10	5	10	10	10	10	5	10	5	5	10	10	5	10
Aberdeen STP	10	10	10	5	10	10	10	10	5	10	5	5	10	10	5	10
Aberdeen STP	10	10	10	5	10	10	10	10	5	10	5	5	10	10	5	10
Aberdeen STP	10	10	10	5	10	10	10	10	5	10	5	5	10	10	5	10
Aberdeen STP	10	10	10	5	10	10	10	10	5	10	5	5	10	10	5	10
CU+	10	10	10	5	10	10	10	10	5	10	5	5	10	10	5	10
CU+	10	10	10	5	10	10	10	10	5	10	5	5	10	10	5	10
Chehalis R.	10	10	10	5	10	10	10	10	5	10	5	5	10	10	5	10
Chehalis R.	10	10	10	5	10	10	10	10	5	10	5	5	10	10	5	10
Control	10	10	10	5	10	10	10	10	5	10	5	5	10	10	5	10
Control	10	10	10	5	10	10	10	10	5	10	5	5	10	10	5	10
Control	10	10	10	5	10	10	10	10	5	10	5	5	10	10	5	10

1 Symbols represent the following assays: MFO = Mixed Function Oxygenase; FHA = Fish Health Assessment; SWC = Salt Water Challenge; HISTO = Histology; VIBRO = Vibrio challenge.

Appendix Table 1R. Number of coho remaining over time at the Marrowstone Field Station following exposure of fish to effluents at the Aberdeen Sewage Treatment, 5/26-5/31, 1988. On each date, the fish remaining were used to assess growth of each treatment group.

Treatment	Initial # at Marrowstone	Date (Number Remaining)						
		6/13-14	7/13	8/16	9/13	10/13	11/15	12/4
WEYCO 3%	211	163	132	92	66	56	51	32
WEYCO 3%	211	165	131	93	62	51	44	25
WEYCO 10%	211	163	132	100	60	54	51	31
WEYCO 10%	211	165	135	88	58	54	42	25
WEYCO 30%	204	132	100	64	37	31	24	8
WEYCO 30%	212	165	153	94	94	59	51	14
ITT 3%	212	166	134	101	62	52	48	31
ITT 3%	211	163	131	97	69	61	55	36
ITT 10%	210	162	131	85	57	50	49	30
ITT 10%	211	165	132	95	68	64	54	36
ITT 30%	212	161	131	90	54	41	25	10
ITT 30%	210	161	127	89	59	52	43	27
Aberdeen STP	210	165	133	86	66	61	57	40
Aberdeen STP	211	162	133	97	64	46	12	7
Hoquiam STP	211	162	134	103	77	70	65	46
Hoquiam STP	212	163	135	98	69	61	53	35
Cu+	210	164	134	98	65	55	52	36
Cu+	205	146	114	83	58	53	42	25
Chehalis R.	212	167	135	103	61	56	52	33
Chehalis R.	212	165	132	91	66	58	53	36
Control	212	161	134	85	57	47	42	26
Control	212	164	132	89	68	59	53	36
Control	212	166	135	99	60	49	40	25

Appendix Table 1S. Sample sizes used for tests of the effects of exposing fish to effluents at the Aberdeen Sewage Treatment Plant in 1989. All evaluations were conducted at the Marrowstone Field Station.

Treatment	# Days Exposed	Date Transported to Marrowstone	# Transferred	Date and Type of Evaluation Conducted[1]					
				4/27 SWC	5/8-9 WT.	6/7-8 WT.	8/14-15 WT.	10/11-12 WT.	12/4 WT.
ITT 30%	5	4/26	109	10	109	109	80	43	33
ITT 30%	5	4/26	118	10	108	107	78	50	33
ITT 5%	5	4/26	108	10	98	95	68	36	29
ITT 5%	5	4/26	110	10	99	99	72	34	25
WEYCO 30%	5	4/26	115	10	103	100	48	29	24
WEYCO 30%	5	4/26	116	10	106	103	55	36	31
WEYCO 5%	5	4/26	112	10	101	100	69	38	31
WEYCO 5%	5	4/26	110	10	98	95	64	38	27
Control 1	5	4/26	121	10	110	109	58	34	20
Control 1	5	4/26	111	10	99	99	57	22	-
Control 1	5	4/26	91	10	81	79	55	40	32
				5/6 SWC	5/17-18 WT.	6/15-16 WT.	8/16-17 WT.	10/19-20 WT.	12/15 WT.
MIX 30%	5	5/5	117	10	106	106	80	44	32
MIX 30%	5	5/5	124	10	114	112	67	38	31
MIX 5%	5	5/5	108	10	98	97	68	43	24
MIX 5%	5	5/5	110	10	99	97	66	32	18
MIX 5%	14	5/5	109	10	93	93	64	41	31
MIX 5%	14	5/5	107	10	97	96	65	46	38
ITT 5%	14	5/5	108	10	98	98	70	44	36
ITT 5%	14	5/5	105	10	94	94	73	45	34
WEYCO 5%	14	5/5	110	10	99	98	60	24	9
WEYCO 5%	14	5/5	108	10	97	96	59	38	29
Control 2	5	5/5	112	10	101	98	71	29	21
Control 2	5	5/5	119	10	107	106	80	33	21
Control 3	14	5/5	107	10	96	95	58	42	38
Control 3	14	5/5	104	10	94	91	43	26	15

[1] SWC = Salt Water Challenge; WT = Growth Evaluation