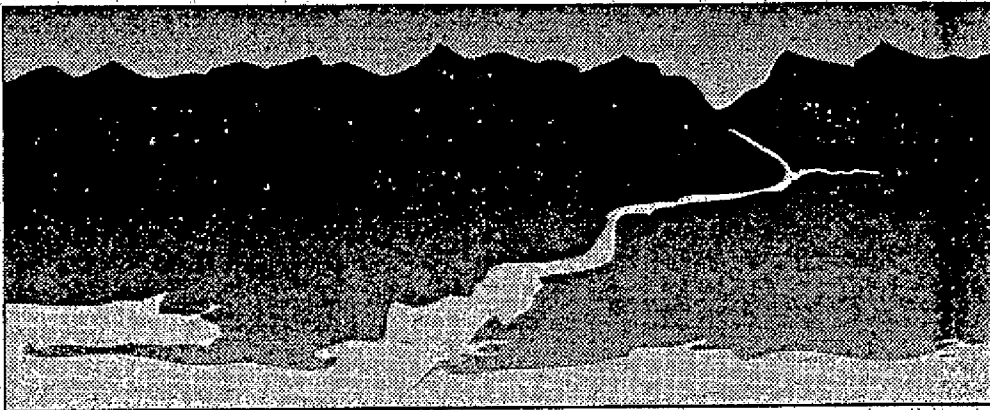

FINAL REPORT
TC 0110-03

LOWER COLUMBIA RIVER



BI-STATE PROGRAM

ASSESSING HEALTH OF FISH SPECIES AND FISH COMMUNITIES IN THE LOWER COLUMBIA RIVER

JANUARY 29, 1996

Prepared By:

TETRA TECH

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Prepared For:

**The Lower Columbia River
Bi-State Water Quality Program**

Prepared By:

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1.0 INTRODUCTION

The Oregon and Washington state legislatures created the Lower Columbia River Bi-State Water Quality Program in 1990. The Program developed a four-year plan designed to characterize water quality in the Columbia River below Bonneville Dam, identify water quality problems, determine whether beneficial uses of the river are impaired, and develop solutions to the problems identified (Bi-State Steering Committee 1990). The plan proposed a framework for evaluating water quality that consisted of: 1) an inventory of existing information; 2) reconnaissance surveys; 3) further evaluation of water quality (baseline studies); and 4) advanced studies. A number of studies have been completed, or are in progress, to help accomplish this legislative mandate. These studies have attempted to characterize historical and current contaminant levels in water, sediment, and fish and crayfish tissues; quantify amounts and identify sources of pollutants entering the river; document beneficial uses of the river; and provide recommendations for addressing concerns about potential impacts of river contaminants on fish and wildlife populations and human health. The latter studies of this Program focus on utilizing information assembled in earlier studies to design and conduct specific baseline studies (e.g., ambient monitoring of tributaries and localized contaminant investigations) and advanced studies to quantify or characterize potential risks to fish, wildlife, and humans from habitat modification and contaminants. This report addresses the health of important fish indicator species and fish assemblages in the river, measured by means of three biological assessment techniques.

1.1 HISTORICAL OVERVIEW

During 1991, the Bi-State Program completed several studies designed to inventory and characterize existing water quality data. Following this effort, a reconnaissance survey of the lower river was conducted in the fall of 1991 to collect data for a preliminary assessment of water quality that could be used to direct future studies (Tetra Tech 1993a). This survey, the most extensive collection of water quality data to date for the lower Columbia River, analyzed water, sediment, and tissue samples for a large list of chemicals of potential concern to aquatic life, wildlife, and humans. The data collected

during the reconnaissance survey showed elevated levels of certain contaminants in a number of samples. After reviewing this information, the Lower Columbia River Bi-State Program Steering Committee ranked assessing potential impacts to fish and wildlife among the top four objectives for future studies (Lower Columbia River Bi-State Program 1992).

The Fish and Wildlife Work Group (FWWG) was formed to guide the conduct of studies in this area so as to determine whether contaminant levels or habitat loss in the river might be affecting the health of resident fish and wildlife, including anadromous fish. The work group developed an extensive list of possible studies, which were then ranked according to how critical the issues they addressed were perceived to be, and how applicable the studies were to the objectives of the Bi-State Program. The ranking process also divided the possible studies into short-term, mid-term, and long-term groups. The Bi-State Steering Committee then selected 10 studies for consideration from this array, including the current study.

1.2 STUDY OBJECTIVES

The two main objectives of this study are:

- To characterize the health of fish assemblages and resident indicator fish species in the lower Columbia River
- To draw conclusions, if possible, about the impacts of water quality and/or habitat loss on fish health in the lower Columbia River.

Fish health was characterized by applying the following three biological assessment techniques:

- Fish community assessment based on the Index of Biotic Integrity (IBI) (Karr et al. 1986) and U.S. EPA Rapid Bioassessment Protocol V (RBP V) (Plafkin et al. 1989)
- Autopsy-based fish health/condition assessment of largescale sucker
- Juvenile fish skeletal abnormality assessment.

Each of these techniques is described in the following discussion. In addition to the studies listed above, National Marine Fisheries Service (NMFS) conducted another bioassessment technique in coordination with the Bi-State Program. This study utilized largescale suckers collected as part of the autopsy-based fish health/condition component; findings were published as *Assessment Of Exposure to Aromatic Compounds In Fish From The Lower Columbia River, By Use Of Appropriate Biomarkers* (Collier et al. 1995).

1.3 BIOLOGICAL ASSESSMENT TECHNIQUES

Biological assessment techniques are methods for evaluating the health of a biological community, population, or individual organisms. They provide an alternative or supplemental approach to evaluations based on detecting chemical residues in biological organisms. Previous studies by the Bi-State Program have focused on the measurement of contaminants; this study provides supplemental information on the health of fish assemblages in the lower Columbia River by examining biological indicators. The three assessment techniques used to evaluate fish health are described below.

1.3.1 Fish Community Assessment

To obtain an overall assessment of fish community structure and variation in the river, the diversity and abundance of species at sites throughout the river were quantified and evaluated using a bioassessment technique based on the U.S. EPA Rapid Bioassessment Protocol V (RBP V). The technique involves careful, standardized field collection, species identification and enumeration in the field, and community analyses using biological indices and quantification of the biomass and numbers of key species. The RBP V is based on the Index of Biotic Integrity (IBI) (Plafkin et al. 1989; Hughes and Gammon 1987; Karr et al. 1986). The IBI is a broadly-based index firmly grounded in fisheries ecology (Karr et al. 1986) that uses 12 biological metrics (e.g., number of fish species, presence of native vs exotic species, percent anomalies, species tolerance) to assess integrity based on the fish community's taxonomic and trophic composition and the abundance and condition of fish. Results of these metrics can be used to evaluate the overall health of fish communities in the river. Data provided by this technique can serve to assess beneficial use attainment, prioritize sites for further evaluation, provide a reproducible impact assessment, and assess fish community status and trends. The IBI as originally described was intended for streams and small rivers; it was modified by Hughes and Gammon (1987) for use on the Willamette

River. Tetra Tech also utilized the modified IBI in two surveys of the Willamette River (Tetra Tech 1993b; 1994a). However, this technique had not previously been used on a river the size of lower Columbia.

1.3.2 Fish Health Assessment

Fish health was assessed using the fish health/condition assessment system described in "Fish Health/Condition Assessment Procedure" (Goede 1993). This autopsy-based protocol uses a minimal amount of equipment to assess the exterior and interior tissues and organs (e.g., thymus, pseudobranch, gills, kidney, spleen, liver) by categorizing the gross appearance of these tissues. In addition, blood samples are collected by microhematocrit tube and analyzed for hematocrit, leucocrit, and plasma protein in the field. Statistical comparisons between stations are possible by assigning numerical values to the qualitative codes assigned to each organ during the autopsy. This technique was developed by the State of Utah and has been used by Oregon DEQ and Tetra Tech (1993b) on the Willamette River, and by Tetra Tech (Unpublished Data) on the upper Columbia River. It is particularly well-suited for generating data for temporal and spatial comparisons of the health of a single species.

1.3.3 Juvenile Fish Skeletal Abnormality Assessment

Evaluating skeletal abnormalities in juvenile fish provides an additional independent measure of the health of fish communities in the river and shows whether differences in the incidence of abnormalities exist among the locations where this technique was performed. Several authors have used this technique to demonstrate that increased incidence of skeletal abnormalities can be associated with stressors such as heavy metals and bleached kraft mill effluents (Bengtsson and Larsson 1986; Bengtsson 1988). Tetra Tech has utilized this technique in two recent studies of the Willamette River (Tetra Tech 1993b; 1994a).

1.4 GENERAL STUDY DESIGN

The study design and technical approach for the fish health study were developed through discussions with the FWWG and Bi-State Program Coordinators. The Task Order for this study specified that the three fish assessment techniques be employed throughout the lower Columbia River. This section discusses the rationale for dividing the river into major segments and major habitat types/land uses and the rationale for selecting sampling locations and numbers of samples for each assessment technique.

1.4.1 River Segments

The lower Columbia River was subdivided into four major segments (strata) defined by similar characteristics for the 1991 reconnaissance survey. This subdivision was useful in determining the physical processes responsible for contaminant transport. The fish health study utilized the same four subdivisions established for the reconnaissance survey:

- Segment 1 - Mouth to Tenasillahe Island (total length = 37 miles)
- Segment 2 - Tenasillahe Island to Cowlitz River (total length = 35 miles)
- Segment 3 - Cowlitz River to Willamette River (total length = 30 miles)
- Segment 4 - Willamette River to Bonneville Dam (total length = 44 miles)

Segments were defined as areas with similar flow and morphologic features. Therefore, major segment designations were based on confluences with major tributaries or the break between riverine and estuarine portions of the river. An extensive discussion of the rationale and features of each segment can be found in the Task 3 report *Review of Hydraulic, Hydrographic, Sediment Transport, and Geomorphic Characteristics of the Lower Columbia River* (Tetra Tech 1992a).

The river was further subdivided into much smaller segments, based on a pilot project designed to define an optimal sampling area for assessing fish communities (see Section 2.0). Segmentation of the entire river into standard units allowed selection of random sampling locations for each of the fish assessment techniques.

1.4.2 Major Habitat Types/Land Uses

Fish assemblages have been shown to be influenced by the riparian habitat in small streams and rivers. However, little information about fish assemblages and riparian habitat on large rivers has been documented. Therefore, to determine if riparian habitat/land use is important to fish assemblages on the lower Columbia River, it was divided into three major habitat types/land uses: backwater areas, urban/industrial areas, and main channel areas. The backwater areas were identified previously during the selection of the 1993 backwater reconnaissance survey stations (Tetra Tech 1993a). The urban/industrial areas were determined by examining aerial infrared photographs (scale 1:24,000) of the lower Columbia River taken in 1989. In addition, information on major point source discharge locations was also utilized (Tetra Tech 1992b). A standard distance of one mile downriver from a major point source established the

boundaries of urban/industrial areas around isolated point sources unless examination of aerial photos indicated a more extensive area. Areas not defined as backwater or urban/industrial were defined as main channel habitat. All urban/industrial areas identified were located along the main channel and not in backwater areas.

1.4.3 Study and Statistical Design Considerations

Independent study designs and assessment methodologies were developed for each of the three assessment techniques (Figure 1-1). However, the studies were designed so they can be related to each other. The sampling design for the fish community assessment is stratified by both major river segment and habitat type/land use, the sampling design for the fish health assessment is stratified by habitat type/land use, and the sampling design for the juvenile skeletal abnormality assessment is stratified by river segment. The study design is further discussed in the sections on the specific techniques.

1.4.4 Scientific Collection Permits

Scientific surveys which include fish sampling on the Columbia River have undergone increasing scrutiny in recent years due to the presence of endangered and threatened stocks of Pacific salmon. Prior to sampling, scientific taking permits were obtained from Oregon Department of Fish and Wildlife (ODFW), Washington Department of Fish and Wildlife (WDFW), and National Marine Fisheries Service (NMFS). Both of the state permits were issued contingent upon receipt of the Endangered Species Act (ESA) permit from NMFS. The ESA permit was prepared by the Lower Columbia Bi-State Water Quality Program and the Northwest Fisheries Science Center (NFSC) of NMFS and determined that the resident fish and human health studies were not likely to adversely affect the listed Snake River salmon. NMFS concurred with this conclusion in their November 4, 1994 consultation letter. The decision was based on the use of specific sampling gear and procedures that were outlined in the initial permit request. The permit was rigidly adhered to, which limited the possibility of adapting procedures in the field to better meet the study objectives, especially for the beach seining. The lengthy review process for this permit delayed the beginning of sampling from late summer, when fish were more accessible in shallow water, to mid-November. This delay limited the possibility of collecting juvenile fish for the skeletal deformity assessment and impacted the efficiency of the electroshocking gear used to collect the target species for the fish health assessment. The fish community assessment may also have been impacted, as many of the species may have moved out of shallow water areas. An extension of the ESA permit until March

Columbia River Fish Health Study

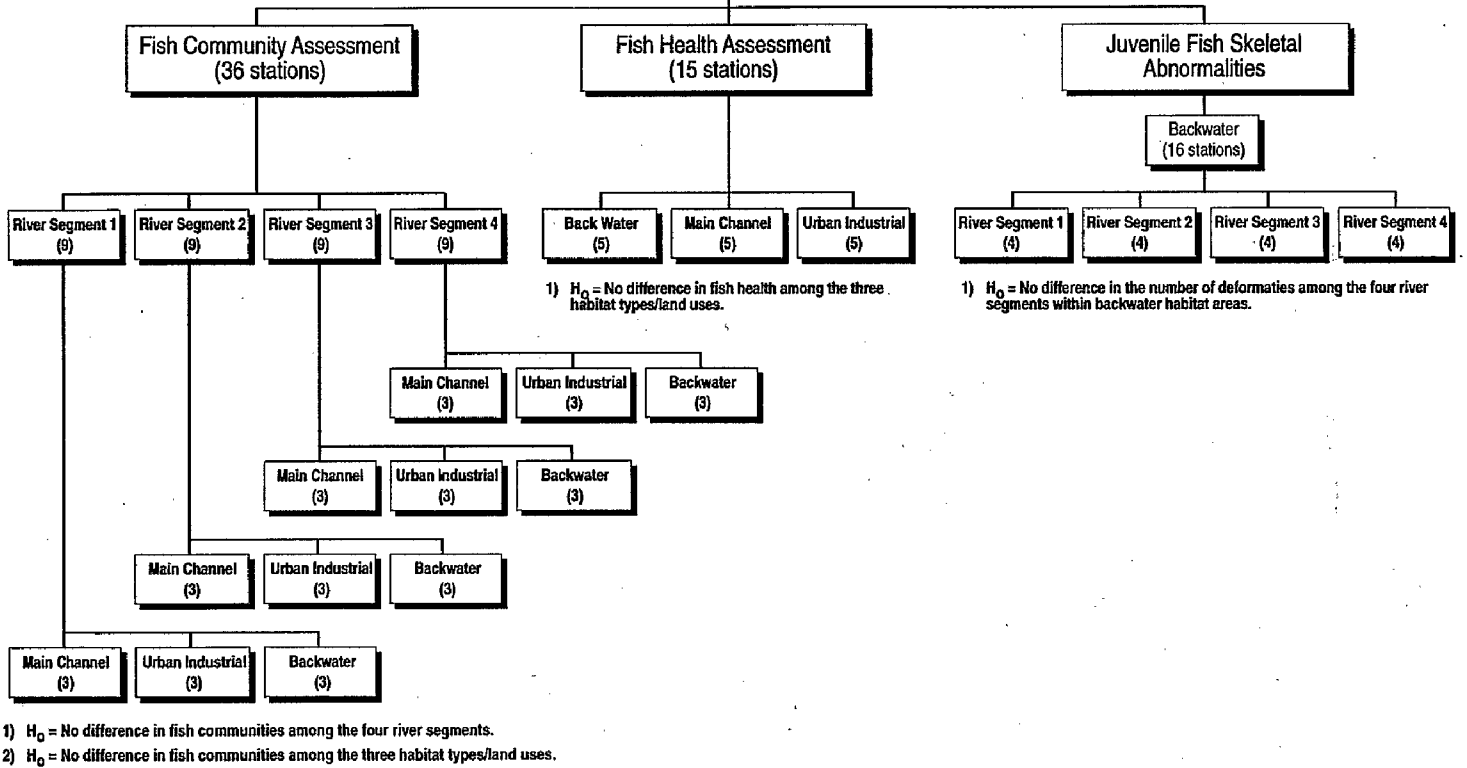


Figure 1-1. Overview of the Fish Health Study Design.

1995 to allow sampling to be completed was initiated December 15, 1994 and received January 13, 1995 from NMFS.

1.5 REPORT ORGANIZATION

The study design, field and laboratory methods, results, and discussion of each of the three assessment techniques are presented in Sections 2.0 (Fish Community), 3.0 (Fish Health), and 4.0 (Skeletal Abnormality), respectively. The results of the pilot project designed to determine the optimal sampling distance for the fish community analysis are also presented in Section 2.0. Section 5.0 compares the results of the three assessment techniques. This section also includes, where appropriate, a discussion of the results in conjunction with contaminant data collected from earlier reconnaissance surveys (Tetra Tech 1993a, 1995a).

2.0 FISH COMMUNITY ASSESSMENT

As discussed above, the fish community assessment technique used in this study has not previously been used on a river as large as the lower Columbia. This presented several unknowns when designing the study. First, the optimal size and spacing of sampling locations needed to be determined to ensure that a representative sample was collected. Therefore, a pilot project to determine this distance was conducted before the station locations were randomly selected. Details of the pilot project are discussed below in Section 2.1. Second, while fish assemblages have been shown to be influenced by the riparian habitat in small streams and rivers, it was unknown whether the distribution of fish assemblages in the lower Columbia River are so influenced. It was also unknown if fish assemblages change over the length of the river. Therefore this study was designed to test two hypotheses:

- 1) Large river fish assemblages differ according to riparian habitat types/land uses
- 2) Lower Columbia River fish assemblages differ among major sections of the river (i.e., among the four segments).

To address these hypotheses, a nested stratified random sampling design (Gilbert 1987) was used. The four major river segments as well as the three major habitat types/land uses were used in testing these hypotheses, resulting in four segments with three habitat types/land uses per segment (Figure 1-1).

2.1 PILOT STUDY

Prior to the selection of stations for fish community sampling, a pilot study was conducted to determine the optimal sampling station size. As described in Plafkin et al. (1989), typical sampling station lengths ranged from 100-200 m for small streams to 500-1,000 m in larger rivers. This study recommends that the size of a reference area be sufficient to produce 100 to 1,000 individuals and 80-90 percent of the

species expected from a 50 percent increase in sampling distance. By determining the optimal sampling distance, it was insured that each sample would be representative of the fish population in that area.

The pilot project was conducted on November 9, 1994 at Carrolls Channel near Kalama, Washington. Ideally, the pilot study would have been conducted in each of the three habitat types/land uses to provide optimal sampling distances for each one. However, due to permitting delays, the need to begin the study as soon as possible, and budget limitations, only a single habitat type/land use was selected. A 2-km long transect was established by placing marker buoys at 250-m intervals. Electroshocking was conducted (methods are given in Section 2.2.2) beginning at one end of the transect and proceeding for 250 m. All individual fish were collected, identified, and enumerated. Data for the first segment were recorded and maintained separately. Sampling continued to the second segment and so on until the entire transect has been surveyed. Results of the collections in each segment were plotted.

A total of 38 fish of 14 different species were collected from the 8 different 250-m transect segments (Table 2-1). In order to determine how many of the transect segments would constitute an optimal sampling distance, the cumulative frequency of unique species was plotted for each segment (Figure 2-1). The number of species increased with distance shocked up to a distance of 1,500 m (0.93 mi). Ninety percent of the species were collected after electroshocking a distance of approximately 1,250 m (0.78 mi). Based on this data, a standard sampling distance of 1,250 m (0.78 mi) was used to assess fish communities in the lower Columbia River. This distance was used to divide the river into segments that were then randomly sampled.

2.2 METHODS

The methods discussion is divided into separate sections for station locations, field procedures, and statistical analysis. All of the data used in the analysis were collected in the field.

2.2.1 Station Locations

A random sampling design stratified by both river segment and habitat type/land use was used to establish sampling locations for the fish community assessment (Gilbert 1987). Each of the four river segments were then divided into 1,250 m (0.78 mi) distances (i.e., the standard transect length), which were each

Table 2-1. Results of Pilot Study to Determine Optimal Sampling Distance

Segment ¹	Species	Number of Individuals	Number of Species in Segment	Number of New Species in Segment	Cumulative Number of Species
1	Starry flounder	3	2	2	2
	Mountain whitefish	1			
2	Starry flounder	2	2	1	3
	Largescale sucker	1			
3	Chinook salmon	1	3	3	6
	Largemouth bass	1			
	Banded killifish	1			
4	Smallmouth bass	2	6	5	11
	Rainbow trout	1			
	Banded killifish	1			
	Coho salmon	1			
	Speckled dace	1			
	Mottled sculpin	1			
5	Rainbow trout	2	5	1	12
	Banded killifish	3			
	Chinook salmon	2			
	Threespine stickleback	1			
	Mottled sculpin	6			
6	Reticulate sculpin	1	2	2	14
	Prickly sculpin	1			
7	Largescale sucker	1	3	0	14
	Largemouth bass	1			
	Banded killifish	2			
8	Starry flounder	1	1	0	14

¹ Each segment was 250 m in length
 Total of 38 fish of 14 different species were captured

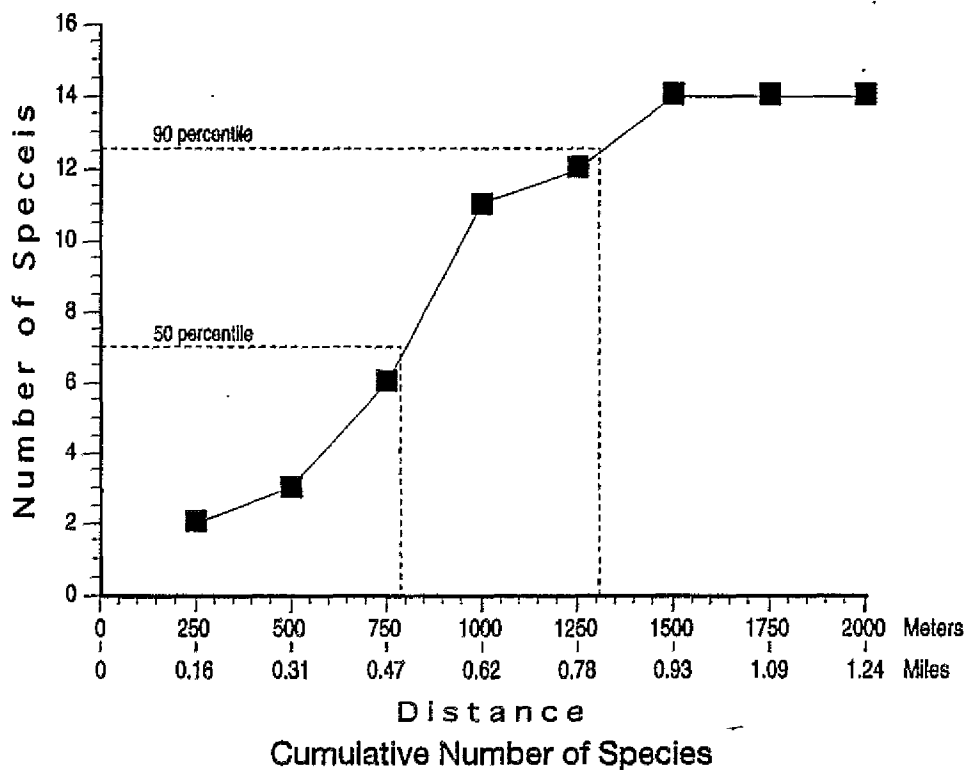
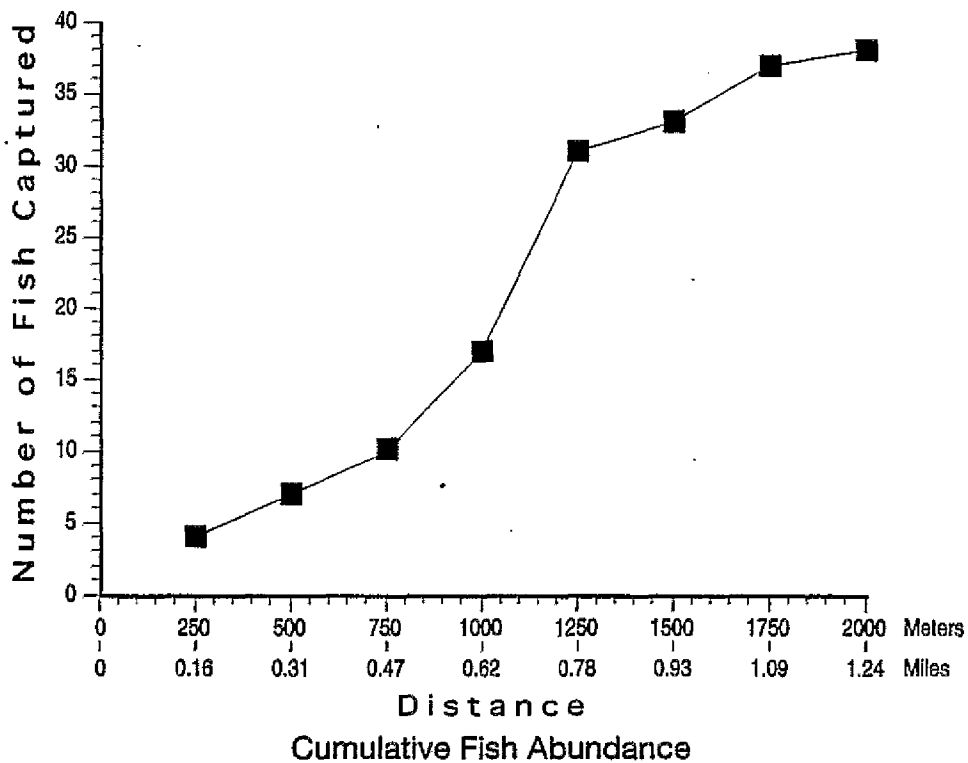


Figure 2-1. Pilot Study Data Collected in Carrolls Channel, WA.

assigned a unique identifier based on the major segment and the habitat type/land use. Three sampling locations were randomly selected from each habitat type/land use within each major segment using this classification. A total of 36 transects were sampled for fish community characteristics throughout the length of lower Columbia River, 12 in each of the three habitat types/land uses. The location of these sampling locations is given in Table 2-2 and Figures 2-2 to 2-5.

2.2.2 Field Procedures

Each of the 36 sampling sites was sampled once during the month of December 1994 (Table 2-2). A boat-mounted electroshocker (Model 7.5 GPP, Smith-Root, Vancouver, WA) was used to collect the fish along each transect. The electroshocking unit was set at approximately 300 volts which generated 3 amps DC pulsed at 120 cycles/sec. The stunned fish were collected using dip nets with a mesh size of 1 cm and maintained alive. All captured fish were identified to species in the field using the most current taxonomic keys (Page and Burr 1991, Wydoski and Whitney 1979, Oregon State University 1973). Because of the concern for endangered salmon stocks, salmon smolts were not netted, but were counted and included in the summary statistics for each station. These fish could not be identified to species. Total numbers, weights, and lengths (total) of all individuals of each species and incidence of external anomalies were recorded for each group (Plafkin et al. 1989). After measurements were made, the fish were returned to the river alive.

In addition to the fish collection efforts, two physical measurements, depth and current speed, were made at each station at 5 equidistant positions along the transect. Depth was measured using the depth transponder of the GPS unit. Current speed was measured using a Flo-MateTM Model 2000 Portable Flowmeter (Marsh-McBirney, Inc., Frederick, MD).

2.2.3 Data Analysis and Interpretation

The IBI technique yields a discrete measure of the health of the fish community. The IBI incorporates zoogeographic, ecosystem, community, population, and individual organism perspectives. The modified IBI included 7 of the 12 original metrics, five others that were modified based on guidance presented in Karr et al. (1986), and a 13th metric, total fish biomass, that was added.

Table 2-2. Fish Community Station Locations and Sampling Dates

Station	Location	Date	Starting ¹		Finishing ¹	
			Lat	Long	Lat	Long
Urban/Industrial Sites						
Segment 1						
1-1UE	Tongue Point	12/13/94	46-12.21	123-46.26	46-11.86	123-47.12
1-2UE	Astoria Boat Ramp	12/13/94	46-11.80	123-47.33	46-11.68	123-48.14
1-3UE	Maritime Museum	12/13/94	46-11.69	123-48.24	46-11.44	123-49.15
Segment 2						
2-1UE	Across from Ranier Ramp	12/9/94	45-05.86	122-55.64	45-05.88	122-56.22
2-2UE	Longview-Weyerhauser	12/10/94	46-07.08	122-58.21	46-07.50	122-58.89
2-3UE	Longview-Reynolds	12/10/94	46-07.86	122-59.60	46-08.26	123-00.27
Segment 3						
3-1UE	near St. Helens Marina	12/7/94	45-52.10	122-47.86	45-52.60	122-47.86
3-2UE	Kalama WWTP	12/9/94	45-59.88	122-50.64	46-00.55	122-50.98
3-3UE	Trojan Nuclear Plant	12/9/94	46-01.99	122-52.91	46-02.59	122-53.05
Segment 4						
4-1UE	Tomahawk Island	12/3/94	45-36.69	122-36.96	45-36.71	122-37.92
4-2UE	Hayden Island	12/3/94	45-36.80	122-40.21	45-36.42	122-39.16
4-3UE	Pearcy Island	12/3/94	45-37.55	122-43.66	45-37.09	122-42.71
Backwater Sites						
Segment 1						
1-1BE	Elochoman Slough	12/12/94	46-13.57	123-24.31	46-14.10	123-24.73
1-2BE	Welch Island	12/15/94	46-15.06	123-29.06	46-14.55	123-28.78
1-3BE	Svensen Island	12/15/94	46-10.92	123-37.90	46-10.84	123-38.67
Segment 2						
2-1BE	Carrolls Channel	12/9/94	46-03.70	122-52.11	46-04.30	122-52.08
2-2BE	Wallace Slough	12/10/94	46-08.18	123-15.07	46-08.10	123-15.80
2-3BE	Elochoman Slough	12/12/94	46-12.68	123-23.44	46-13.29	123-23.76
Segment 3						
3-1BE	Bachelor Island Slough	12/4/94	45-48.45	122-45.87	45-47.85	122-46.18
3-2BE	St. Helens	12/8/94	45-50.38	122-48.37	45-50.00	122-48.40
3-3BE	Martin Slough	12/7/94	45-56.90	122-47.24	45-57.27	122-47.82
Segment 4						
4-1BE	Flag Island	12/2/94	45-32.92	122-20.75	45-33.36	122-21.35
4-2BE	Cottonwood Point	12/2/94	45-33.56	122-19.69	45-33.63	122-18.90
4-3BE	Government Island	12/2/94	45-33.23	122-32.99	45-35.53	122-33.64
Main Channel Sites						
Segment 1						
1-1ME	Altoona	12/15/94	46-16.10	123-40.16	46-15.95	123-39.33
1-2ME	McGowan	12/14/94	46-14.57	123-53.64	46-14.74	123-54.51
1-3ME	Chinook	12/14/94	46-16.34	123-56.96	46-16.81	123-57.40
Segment 2						
2-1ME	Wallace Island	12/11/94	46-10.66	123-11.49	46-10.06	123-12.32
2-2ME	Eagle Cliff	12/11/94	46-10.54	123-12.80	n/a	n/a
2-3ME	Cathlamet Channel	12/12/94	46-10.56	123-20.64	46-10.89	123-20.96
Segment 3						
3-1ME	Shillapoo Wild. Rec. Area	12/4/94	45-42.37	122-45.79	45-41.77	122-45.94
3-2ME	Willow Bar Island	12/4/94	45-45.90	122-46.06	45-45.30	122-46.11
3-3ME	Across from Martin Slough	12/8/94	45-57.52	122-49.32	45-58.30	122-50.12
Segment 4						
4-1ME	Bridal Veil	12/1/94	45-34.35	122-09.75	45-34.75	122-08.04
4-2ME	Sand Island	12/1/94	45-33.08	122-11.65	45-33.57	122-10.71
4-3ME	Reed Island	12/1/94	45-32.15	122-18.35	45-32.37	122-19.32

¹ Each station was a 1,250 m (0.78 mi) transect along the shoreline

COLUMBIA RIVER BI-STATE WATER QUALITY PROGRAM

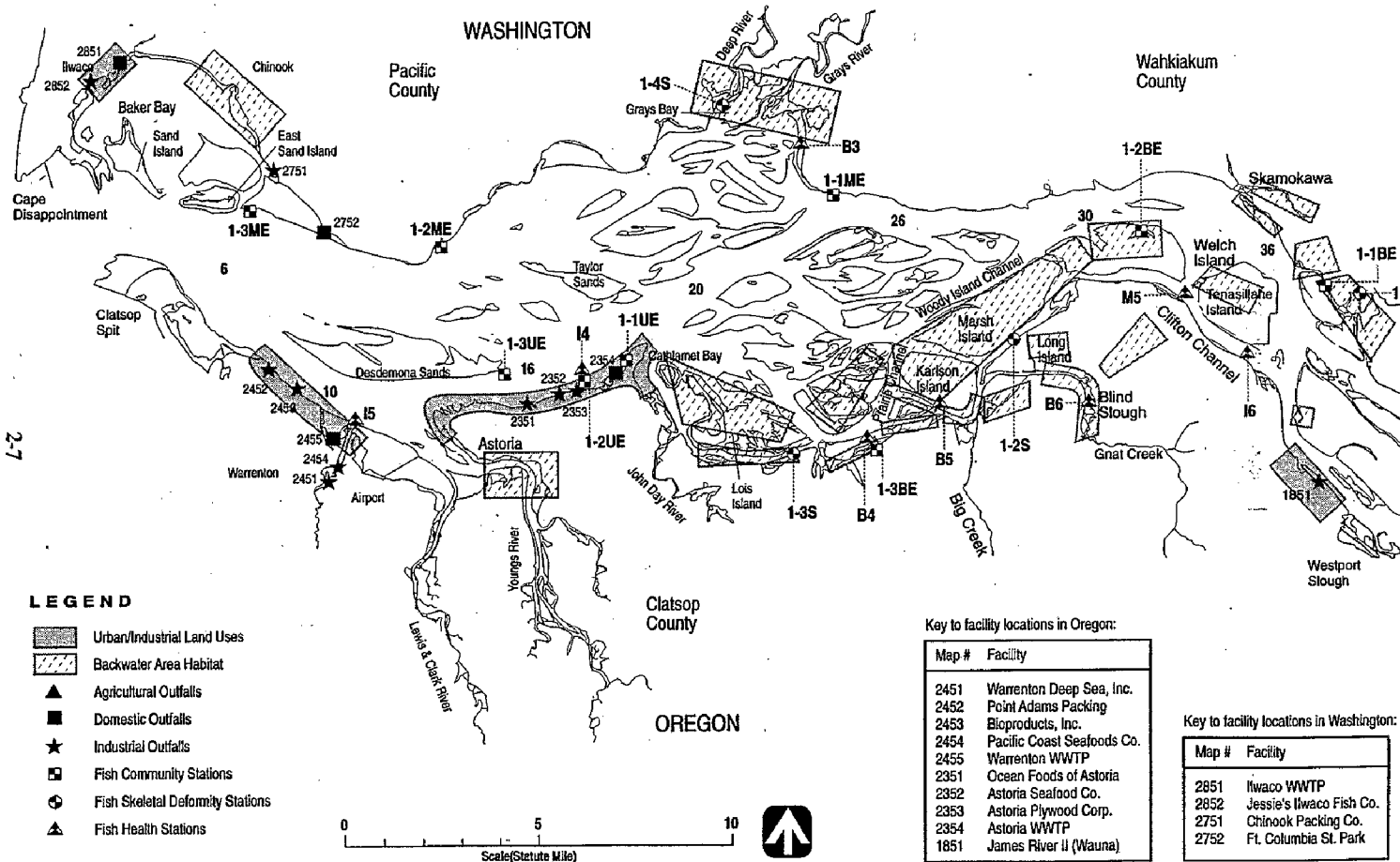


Figure 2-2. Sampling Locations - River Segment 1.

COLUMBIA RIVER BI-STATE WATER QUALITY PROGRAM

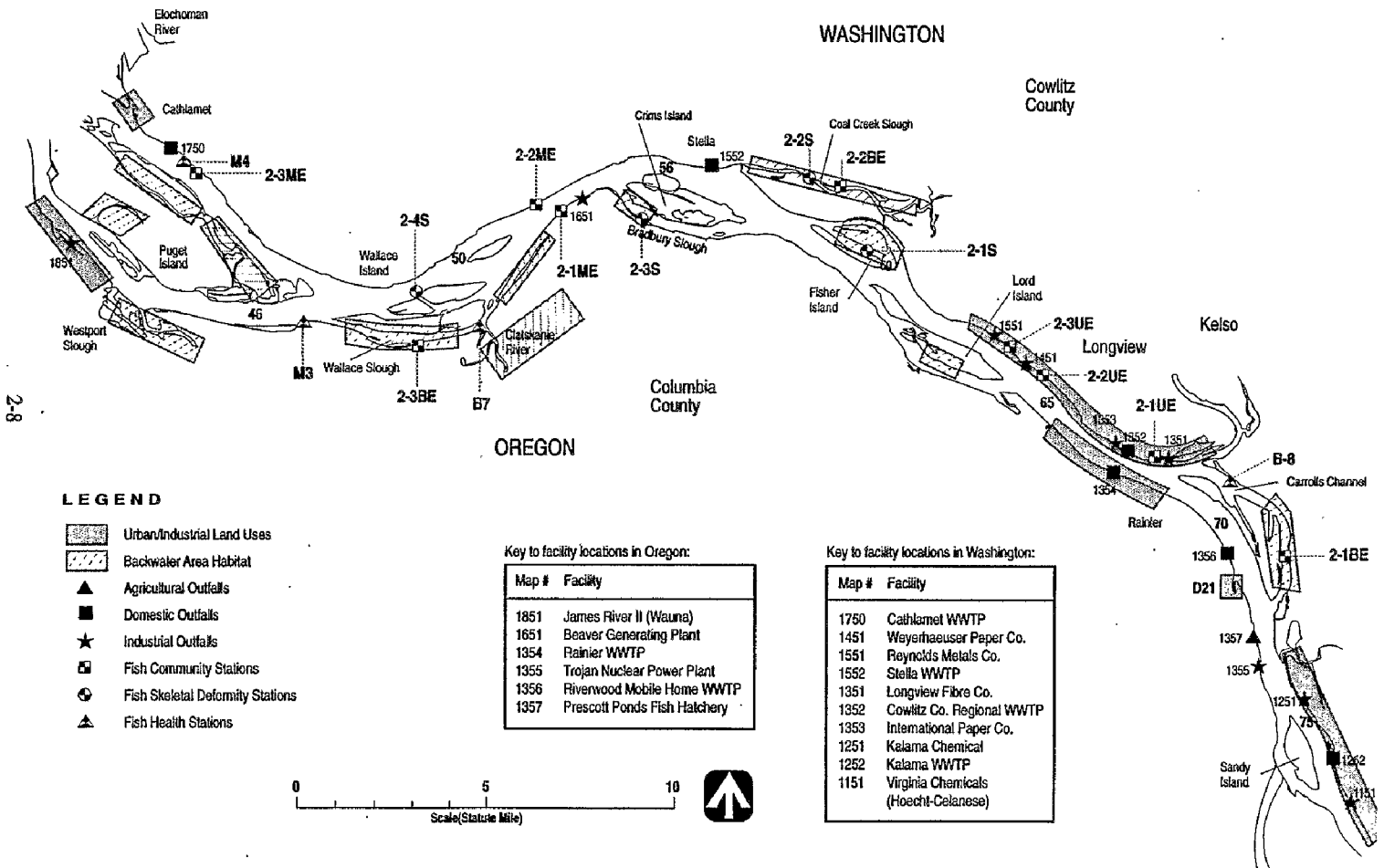


Figure 2-3. Sampling Locations - River Segment 2.

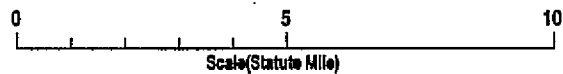
COLUMBIA RIVER BI-STATE WATER QUALITY PROGRAM

Key to facility locations in Oregon:

Map #	Facility
1051	St. Helens WWTP
1052	St. Helens Veneer Mill
1152	Chevron Chemical Co.
851	Portland WWTP

Key to facility locations in Washington:

Map #	Facility
1251	Kalama Chemical
1252	Kalama WWTP
1151	Virginia Chemicals (Hoecht-Celanese)
3151	ALCOA
3152	GATX Terminal Corp.
3153	Fort Vancouver Plywood
3154	Northwest Packing
3155	Vancouver (Westside) WWTP
3156	Great Western Milling
852	Boise Cascade Corp.
853	Ideal Basic Industries
752	Vancouver (Eastside) WWTP
951	Salmon Creek WWTP



LEGEND

- Urban/Industrial Land Uses
- Backwater Area Habitat
- Agricultural Outfalls
- Domestic Outfalls
- Industrial Outfalls
- Fish Community Stations
- Fish Skeletal Deformity Stations
- Fish Health Stations

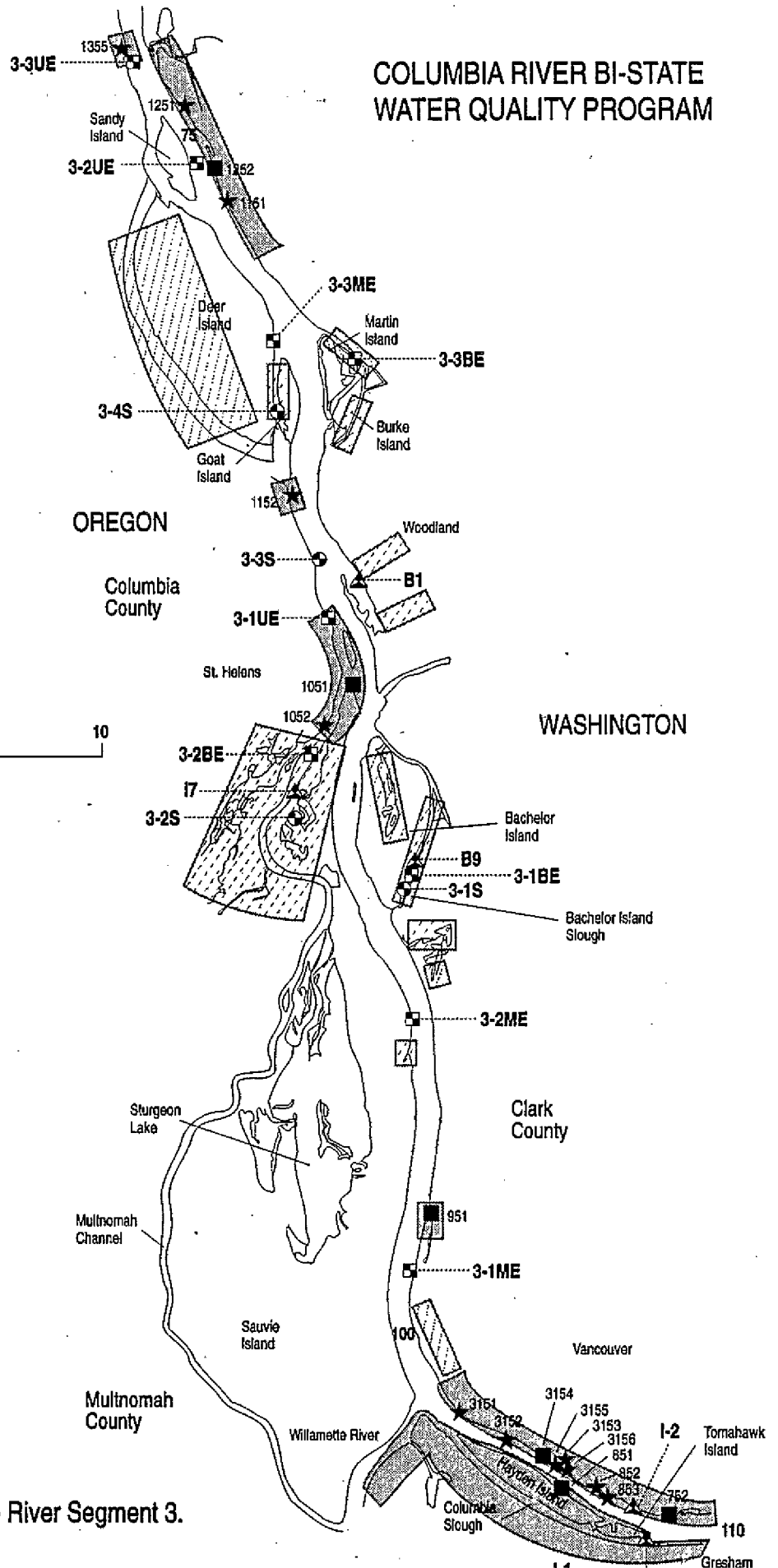


Figure 2-4. Sampling Locations - River Segment 3.

COLUMBIA RIVER BI-STATE WATER QUALITY PROGRAM

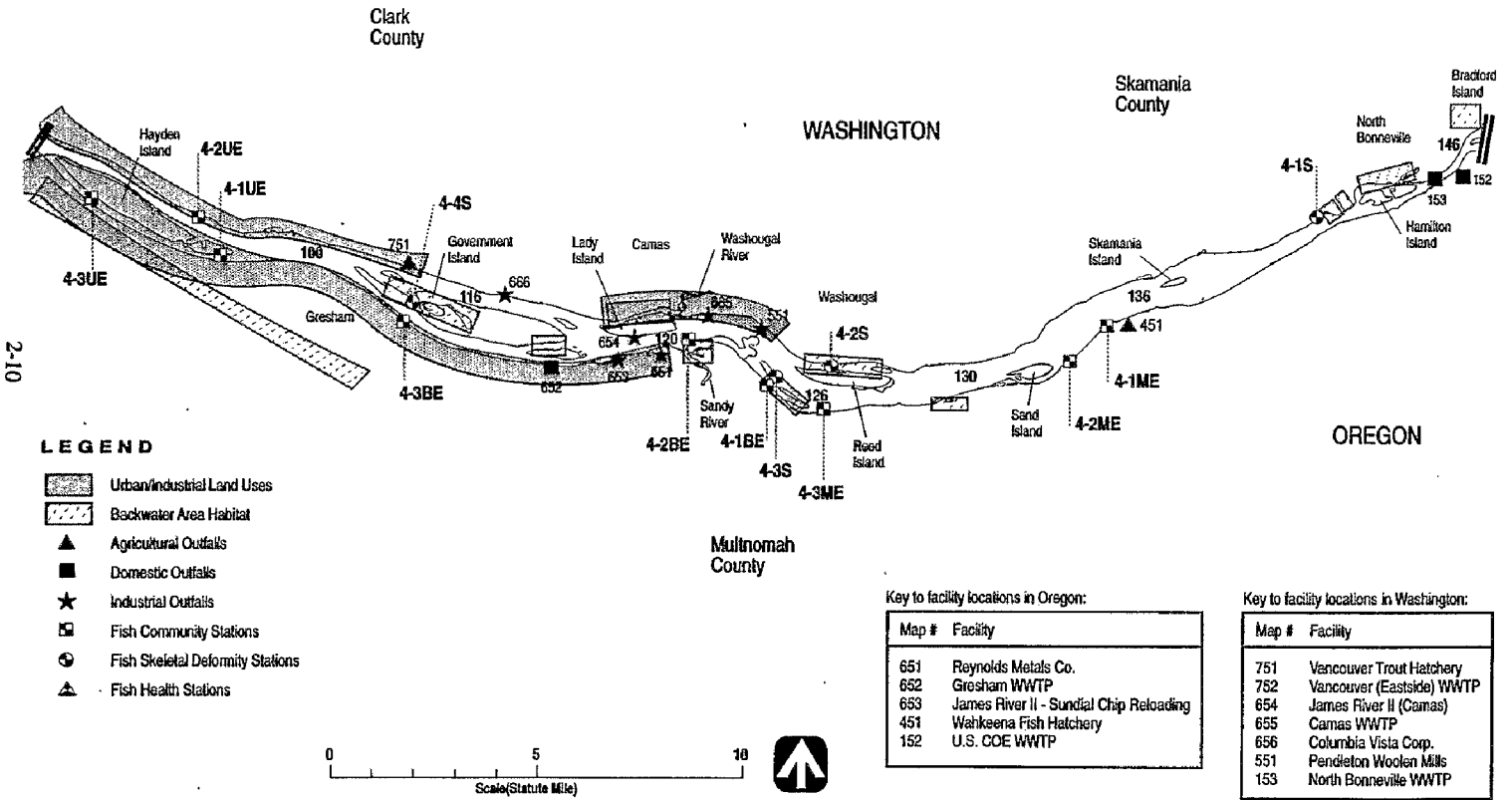


Figure 2-5. Sampling Locations - River Segment 4.

Calculation of the IBI requires that all species be assigned to a trophic guild. Trophic group assignments and tolerances for this project (Table 2-3) were based on Hughes and Gammon (1987) and appropriate field guides (Wydoski and Whitney 1979, Page and Burr 1991).

Comparisons of results from similar habitats/land uses throughout the lower river as well as comparisons within the major segments were performed by testing for normality, then using ANOVAs (Statistica v. 2.0). Multiple regression analyses (Statistica v. 2.0) were performed to determine which of the 13 metrics had the most predictive power in determining the overall IBI score. Results of the analyses from the fish assemblage sampling were evaluated with results from the other two sampling components to make an overall assessment of river health (see Section 5.0).

2.3 RESULTS

Individuals from at least 21 different species were captured (Table 2-4). To protect endangered and threatened salmon species, salmon smolts were counted without being brought aboard the boat. The smolts were either chinook or coho salmon. At 12 of the 36 stations, including all 9 of the Segment 1 stations, no fish were captured. The number of fish captured at the other 24 stations ranged from 2 (Station 3-2UE) to 57 (Station 4-1BE). Ten or more fish were captured at only 12 of these 24 stations. As many as 8 different species from 6 different families were collected at a single station. Salmon smolts were collected at the greatest number of stations (18) and were particularly abundant at the 9 Segment 4 stations. Largescale suckers were also relatively abundant at the Segment 4 stations.

The metrics and data used to calculate the modified IBI are presented in Table 2-5. Although IBI scores were calculated for all stations at which fish were captured, the scores for stations at which less than 10 fish were collected are probably not meaningful. Of the 12 stations at which more than 10 fish were captured, 3 were located in both Segments 2 and 3, while the remaining 6 stations were located in Segment 4. With respect to habitat/land use, 3 stations were located in main channel, 3 in urban/industrial areas, and 6 in backwater habitats. The IBI scores for these 12 stations ranged from 25 (Station 4-1BE) to 43 (Station 4-1ME).

TABLE 2-3. SELECTED PARAMETERS OF FISH SPECIES COLLECTED FROM THE LOWER COLUMBIA RIVER, 1994

	Species	Origin	Trophic group of adults	Relative tolerance of organic pollutants, warm water, and sediment	
Catostomidae					
	Largescale sucker	<i>Catostomus macrocheilus</i>	Native	Omnivore	Tolerant
Centrarchidae					
	Black crappie	<i>Pomoxis nigromaculatus</i>	Introduced	Insectivore	Tolerant
	Largemouth bass	<i>Micropterus salmoides</i>	Introduced	Piscivore	Tolerant
	Pumpkinseed	<i>Lepomis gibbosus</i>	Introduced	Insectivore	Tolerant
	Smallmouth bass	<i>Micropterus dolomieu</i>	Introduced	Piscivore	Intermediate
	White Crappie	<i>Pomoxis annularis</i>	Introduced	Insectivore	Tolerant
Clupeidae					
	American shad	<i>Alosa sapidissima</i>	Native	Herb./Insect.	Intermediate
Cottidae					
	Coastrange sculpin	<i>Cottus aleuticus</i>	Native	Insectivore	Intolerant
	Torrent sculpin	<i>Cottus rhotheus</i>	Native	Insectivore	Intolerant
Cyprinidae					
	Common carp	<i>Cyprinus carpio</i>	Introduced	Omnivore	Tolerant
	Goldfish	<i>Carassius auratus</i>	Introduced	Omnivore	Tolerant
	Northern squawfish	<i>Ptychocheilus oregonensis</i>	Native	Piscivore	Tolerant
	Pearmouth chub	<i>Mylocheilus caurinus</i>	Native	Insectivore	Intermediate
Fundulidae					
	Banded killifish	<i>Fundulus diaphanus</i>	Introduced	Insectivore	Tolerant
Gasterosteidae					
	Threespine stickleback	<i>Gasterosteus aculeatus</i>	Native	Insectivore	Intermediate
Percidae					
	Yellow perch	<i>Perca flavescens</i>	Introduced	Insectivore	Intermediate
Pleuronectidae					
	Starry flounder	<i>Platichthys stellatus</i>	Native	Piscivore	Tolerant
Salmonidae					
	Mountain whitefish	<i>Prosopium williamsoni</i>	Native	Insectivore	Intolerant
	Rainbow trout	<i>Oncorhynchus mykiss</i>	Native	Insectivore	Intolerant
	Steelhead trout	<i>Oncorhynchus mykiss</i>	Native	Insectivore	Intolerant

TABLE 2-4. FISH ABUNDANCE FOR STATIONS SAMPLED FOR THE LOWER COLUMBIA RIVER FISH HEALTH STUDY, 1994

Family	Common name	2-1BE	2-2BE	2-1ME	2-2ME	2-3ME	2-1UE	2-2UE	2-3UE	3-1BE	3-2BE	3-3BE	3-3ME	3-1UE	3-2UE	3-3UE	4-1BE	4-2BE	4-3BE	4-1ME	4-2ME	4-3ME	4-1UE	4-2UE	4-3UE
Catostomidae	Largescale sucker	4	5						1	5	3			13			3	4	4	6	23	2	7	2	4
Centrarchidae	Largemouth bass										2	1													
	Pumpkinseed											1		1											
	Smallmouth bass	1									1						1		1						
	White crappie									1															
	Black crappie										7														
Clupeidae	American shad		3	2			2	1	1			1	1	1	1			1	3	2	1		1		
Cottidae	Coastrange sculpin	3																							
	Torrent sculpin	6					2					1													
Cyprinidae	Common carp											1		1										1	
	Goldfish		1								1												1		
	Northern squawfish											1		1											
	Peamouth chub									4								2			1	5	2		
Pericidae	Yellow perch									3	5				1	1	1	2					1		
Fundulidae	Banded killifish	11																							
Gasterosteidae	Threespine stickleback	1	2						4							1	20	2		4	5				
Pleuronectidae	Starry flounder						1																1		
Salmonidae	Smolt	2	2	2	1	3	3	1	1		1						26	1	1	13	13	19	5	5	2
	Rainbow trout					1												1		2		5			
	Steelhead trout				2		1	2					3		1	1									
	Mountain whitefish	1					2							3			1			3	2	1			
Total # fish		29	13	4	3	4	10	3	9	13	20	9	4	21	2	3	57	11	9	31	49	32	14	7	6
# Families		6	5	2	1	1	4	2	4	4	5	5	2	6	2	3	6	5	4	5	5	4	4	2	2
# Species		8	5	2	2	2	5	3	5	4	7	7	2	7	2	3	8	6	4	7	6	8	4	2	2

Following stations omitted due to zero fish caught: 1-1ME, 1-3BE, 1-2BE, 1-2ME, 1-3ME, 1-3UE, 1-2UE, 1-1UE, 1-1BE, 2-3BE, 3-2ME, 3-1ME.

TABLE 2-5. METRICS AND DATA USED TO DETERMINE A MODIFIED INDEX OF BIOTIC INTEGRITY (IBI) FOR THE LOWER COLUMBIA RIVER, 1994

Station	# Native Species	# Cottid Species	# Native Cyprinid Species	# Native Catostomid Species	# Intolerant Species	% Carp	% Omnivores	% Insectivores	% Salmonids	# Individuals	% Introduced species	% With anomalies	Total Biomass (kg/km)	Modified IBI
2-1BE	5 (3-)	2 (3)	0 (1)	1 (3)	4 (5)	0 (5)	14 (5)	83 (5)	10 (5-)	29 (1)	25 (1)	0 (5)	0.32 (1-)	41*
2-2BE	4 (1+)	0 (1)	0 (1)	1 (3)	1 (3)	0 (5)	46 (3)	54 (5)	15 (5)	13 (1)	20 (1)	0 (5)	1.5 (1)	35+
2-1ME	2 (1)	0 (1)	0 (1)	0 (1)	1 (3)	0 (5)	0 (5)	100 (5)	50 (5)	4 (1)	0 (5)	0 (5)	0.16 (2-)	39
2-2ME	1 (1)	0 (1)	0 (1)	0 (1)	2 (3)	0 (5)	0 (5)	100 (5)	100 (5)	1 (1)	0 (5)	0 (5)	11.7 (1)	49
2-3ME	2 (1)	0 (1)	0 (1)	0 (1)	2 (3)	0 (5)	0 (5)	100 (5)	100 (5)	4 (1)	0 (5)	0 (5)	0.11 (1-)	39
2-1UE	5 (3-)	1 (1)	0 (1)	0 (1)	3 (5)	0 (5)	0 (5)	90 (5)	50 (5)	10 (1)	0 (5)	0 (5)	0.40 (1-)	42-
2-2UE	3 (1)	0 (1)	0 (1)	0 (1)	2 (3)	0 (5)	0 (3)	100 (5)	67 (5)	3 (1)	0 (5)	0 (5)	2.6 (3-)	39
2-3UE	5 (3-)	0 (1)	0 (1)	1 (3)	2 (3)	0 (5)	13 (5)	100 (5)	23 (5)	9 (1)	0 (5)	0 (5)	14.0 (1+)	43
3-1BE	2 (1)	0 (1)	1 (1)	1 (3)	0 (1)	0 (5)	39 (3)	62 (5)	0 (1)	13 (1)	50 (1)	8 (1)	1.4 (1)	25
3-2BE	2 (1)	0 (1)	0 (1)	1 (3)	1 (3)	0 (5)	20 (5)	65 (5)	5 (3)	20 (1)	72 (1)	0 (5)	0.71 (1-)	35-
3-1ME	3 (1)	1 (1)	1 (1)	0 (1)	1 (3)	0 (5)	11 (1)	11 (5)	0 (1)	9 (1)	86 (1)	0 (5)	2.9 (1)	37
3-2ME	2 (1)	0 (1)	0 (1)	0 (1)	1 (3)	0 (5)	0 (5)	100 (5)	75 (5)	4 (1)	0 (5)	0 (5)	13.8 (1)	36
3-1UE	4 (1+)	0 (1)	1 (1)	1 (3)	1 (3)	5 (3)	67 (1)	29 (3)	14 (5)	21 (1)	43 (1)	0 (5)	4.2 (1)	29+
3-2UE	2 (1)	0 (1)	0 (1)	0 (1)	1 (3)	0 (5)	0 (5)	100 (5)	90 (5)	2 (1)	0 (5)	0 (5)	7.5 (1)	39
3-3UE	2 (1)	0 (1)	0 (1)	0 (1)	1 (3)	0 (5)	0 (5)	100 (5)	11 (5)	3 (1)	33 (1)	0 (5)	5.7 (1)	35
4-1BE	6 (3)	0 (1)	2 (1+)	1 (3)	2 (3)	0 (5)	5 (5)	90 (5)	47 (5)	57 (3)	25 (1)	0 (5)	2.5 (1)	41+
4-2BE	5 (3-)	0 (1)	0 (1)	1 (3)	2 (3)	0 (5)	36 (3)	46 (5)	18 (5)	11 (1)	17 (1)	0 (5)	2.9 (1)	37-
4-3BE	3 (1)	0 (1)	0 (1)	1 (3)	1 (3)	0 (5)	36 (3)	44 (5)	11 (5)	9 (1)	25 (1)	11 (1)	4.2 (1)	31
4-1ME	7 (3)	0 (1)	1 (1)	1 (3)	3 (5)	0 (5)	20 (5)	81 (5)	58 (5)	31 (1)	0 (5)	3 (3)	5.4 (1)	43
4-2ME	6 (3)	0 (1)	1 (1)	1 (3)	2 (3)	0 (5)	47 (3)	53 (5)	30 (5)	49 (1+)	0 (5)	12 (1)	16.0 (3-)	39
4-3ME	5 (3-)	0 (1)	1 (1)	1 (3)	3 (5)	3 (3)	13 (5)	88 (5)	78 (5)	32 (1)	38 (1)	0 (5)	4.8 (1)	39-
4-1UE	4 (1+)	0 (1)	0 (1)	1 (3)	1 (3)	0 (5)	50 (1-)	43 (5)	36 (5)	14 (1)	0 (5)	0 (5)	5.5 (1)	37
4-2UE	2 (1)	0 (1)	0 (1)	1 (3)	1 (3)	0 (5)	29 (3)	71 (5)	71 (5)	7 (1)	0 (5)	0 (5)	2.2 (1)	34
4-3UE	1 (1)	0 (1)	0 (1)	1 (3)	1 (3)	0 (5)	67 (1)	33 (5)	33 (5)	6 (1)	0 (5)	0 (5)	3.4 (1)	34

Numerical criteria and values of metrics

0-4 (1)	0-1 (1)	0-2 (1)	0 (1)	0 (1)	10+ (1)	50+ (1)	0-19 (1)	0 (1)	0-49 (1)	10+ (1)	6+ (1)	0-15 (1)
5-9 (3)	2 (3)	3-5 (3)	1 (3)	1-2 (3)	1-9 (3)	25-49 (3)	20-39 (3)	1-9 (3)	50-99 (3)	2-9 (3)	2-5 (3)	16-30 (3)
10+ (5)	3+ (5)	6+ (5)	2 (5)	3+ (5)	0 (5)	0-24 (5)	40+ (5)	10+ (5)	100+ (5)	0-1 (5)	0-1 (5)	31+ (5)

Shaded rows indicate stations at which less than 10 fish were caught. IBI values calculated at these stations are probably not meaningful.

Metric values in parentheses were assigned according to the numerical criteria at the column bottoms.

* Pluses and minuses reflect marginal values. A combination of three pluses or three minuses resulted in a two-point increase or decrease in the IBI score.

Following stations omitted due to zero fish caught: 1-1ME, 1-3BE, 1-2BE, 1-2ME, 1-3ME, 1-3UE, 1-2UE, 1-1UE, 1-1BE, 2-3BE, 3-2ME, 3-1ME.

The original statistical design could not be employed because of the low number or absence of fish captured at the majority of stations. Neither the effects of habitat type/land use or river segment could be legitimately tested because of the unequal and uneven distribution of stations between these variables. For example, the habitat type/land use variable is evenly split among river segments for the backwater and urban/industrial areas, but all three of the main channel stations were from Segment 4.

To make statistical comparisons between river segment and habitat type/land use possible, the fish abundance data were pooled into 9 groups corresponding to the three river segments and the three habitat types/land uses where sufficient numbers of fish were collected (Table 2-6; Figure 2-6). With two exceptions, data from three different stations were pooled to form each group. For group 2-B (segment 2, backwater) data from only two stations were combined and for group 3-M (segment 3, main channel) fish were captured at only one station. Fish abundance for the 9 groups ranged from 4 (group 3-M) to 112 (group 4-M). As many as 15 different species from 8 different families were collected within a single group. The IBI scores for the pooled data are presented in Table 2-7. Although only 4 fish were collected in group 3-M, the IBI score for this group was used in the data analysis in order to preserve the statistical design. The IBI scores for the pooled data were more tightly clustered than were the scores from the individual stations. The scores ranged from 33 (group 3-U) to 47 (group 2-U).

Two ANOVAs were conducted using the pooled abundance data. One ANOVA compared IBI scores among habitat types/land uses (using river segments as replicates) and the other compared IBI scores among river segments (using the three habitat types/land uses as replicates) (Figure 2-6). The mean IBI scores for the three habitat types/land uses were almost identical (~40) and not statistically different from each other ($p=0.98$). The mean IBI scores for river segments 2 and 4 (44 and 42, respectively) were significantly greater ($p=0.04$) than the mean IBI score for river segment 3 (35). All three of the pooled habitat types/land uses in river segment 3 had IBI scores lower than all of the other pooled habitat types/land uses in segments 2 and 4 (Table 2-7). The station groups in river segment 3, particularly group 3-B (backwater) differed from the other station groups by having centrarchid and cyprinid species and fewer numbers of salmonids (Table 2-6).

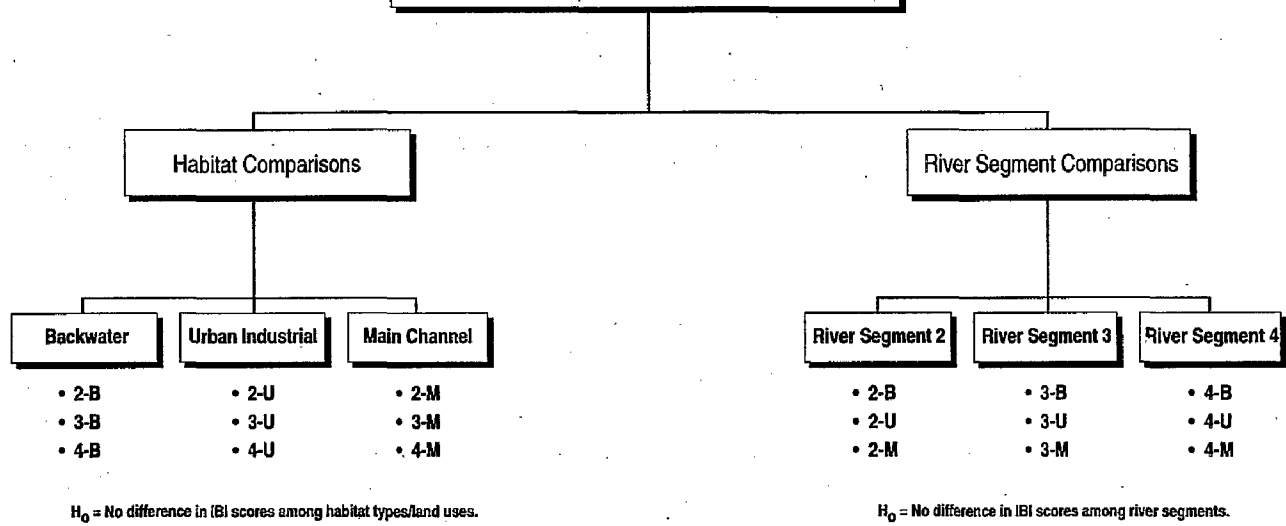
A step-wise multiple regression (Statistica v. 2.0) was performed on the pooled individual metric data to determine which metrics had the greatest effect on the IBI score. By far the best predictive metric in this survey was the number of intolerant species. Approximately two-thirds ($R^2=0.66$) of the variance in IBI

TABLE 2-6. POOLED FISH ABUNDANCE FOR STATIONS SAMPLED FOR THE LOWER COLUMBIA RIVER FISH HEALTH STUDY, 1994

Family	Common name	Segment and Habitat (B=backwater, M=main channel, U=urban/industrial)								
		2-B	2-M	2-U	3-B	3-M	3-U	4-B	4-M	4-U
Catostomidae	Largescale sucker	9		1	8		13	11	31	13
Centrarchidae	Largemouth bass				3					
	Pumpkinseed				1		1			
	Smallmouth bass	1			1			2		
	White crappie				1					
	Black crappie				7					
Clupeidae	American shad	3	2	4	1	1	2	4	3	1
Cottidae	Coastrange sculpin	3								
	Torrent sculpin	6		2	1					
Cyprinidae	Common carp				1		1		1	
	Goldfish	1			1				1	
	Northern squawfish				1		1	2		
	Peamouth chub				4			3	8	
Pericidae	Yellow perch				8		2	3	1	
Fundulidae	Banded killifish	11			3					
	Threespine stickleback	3		4			1	22	9	
Pleuronectidae	Starry flounder			1						1
Salmonidae	Smolt	4	6	5	1			28	45	12
	Rainbow trout		1					1	7	
	Steelhead trout		2	3		3	2			
	Mountain whitefish			2			3	1	6	
Total # fish		41	11	22	42	4	26	77	112	27
# Families		8	2	6	8	2	7	7	6	4
# Species		9	5	8	15	3	9	10	10	4

Note: No fish were captured in segment 1

Columbia River Fish Community Assessment



2-17

Figure 2-6. Revised Sampling Design for the Fish Community Assessment Data for the Lower Columbia River, 1994.

TABLE 2-7. METRICS AND POOLED DATA USED TO DETERMINE A MODIFIED INDEX OF BIOTIC INTEGRITY (IBI) FOR THE LOWER COLUMBIA RIVER, 1994

Segment/ Habitat ¹	# Native Species	# Cottid Species	# Native Cyprinid Species	# Native Catostomid Species	# Intolerant Species	% Carp	% Omni- vores	% Insect- ivores	% Sal- monids	# Indi- viduals	% Intro- duced species	% With anomalies	Total Biomass (kg/km)	Modified IBI
2-B	6 (3)	2 (3)	0 (1)	1 (3)	3 (5)	0 (5)	24 (5-)	66 (5)	10 (5-)	41 (1)	32 (1)	0 (5)	1.8 (1)	43--
2-M	4 (1+)	0 (1)	0 (1)	0 (1)	3 (5)	0 (5)	0 (5)	82 (5)	82 (5)	11 (1)	0 (5)	0 (5)	11.7 (1)	41+
2-U	8 (3)	1 (1)	0 (1)	1 (3)	4 (5)	0 (5)	5 (5)	73 (5)	45 (5)	22 (1)	0 (5)	0 (5)	21.6 (3)	47
3-B	6 (3)	1 (1)	2 (1+)	1 (3)	2 (3)	2 (3)	24 (5-)	62 (5)	2 (3)	42 (1)	62 (1)	2 (3+)	4.9 (1)	33+
3-M	2 (1)	0 (1)	0 (1)	0 (1)	1 (3)	0 (5)	0 (5)	75 (5)	75 (5)	4 (1)	0 (5)	0 (5)	13.5 (1)	39
3-U	6 (3)	0 (1)	1 (1)	1 (3)	2 (3)	4 (3)	54 (1)	35 (3)	19 (5)	26 (1)	15 (1)	0 (5)	17.1 (3)	33
4-B	8 (3)	0 (1)	2 (1+)	1 (3)	3 (5)	0 (5)	14 (5)	75 (5)	39 (5)	77 (3)	6 (3)	1 (5)	9.5 (1)	45+
4-M	7 (3)	0 (1)	1 (1)	1 (3)	3 (5)	1 (3+)	29 (3)	68 (5)	52 (5)	112 (5)	3 (3)	6 (1+)	25.8 (3)	41++
4-U	4 (1+)	0 (1)	0 (1)	1 (3)	1 (3)	0 (5)	48 (3-)	44 (5)	44 (5)	27 (1)	0 (5)	0 (5)	10.9 (1)	39
Numerical criteria and values of metrics														
	0-4 (1)	0-1 (1)	0-2 (1)	0 (1)	0 (1)	10+ (1)	50+ (1)	0-19 (1)	0 (1)	0-49 (1)	10+ (1)	6+ (1)	0-15 (1)	
	5-9 (3)	2 (3)	3-5 (3)	1 (3)	1-2 (3)	1-9 (3)	25-49 (3)	20-39 (3)	1-9 (3)	50-99 (3)	2-9 (3)	2-5 (3)	16-30 (3)	
	10+ (5)	3+ (5)	6+ (5)	2 (5)	3+ (5)	0 (5)	0-24 (5)	40+ (5)	10+ (5)	100+ (5)	0-1 (5)	0-1 (5)	31+ (5)	
Metric values in parentheses were assigned according to the numerical criteria at the column bottoms.														
¹ Habitat designations as follows: B=backwater, M=main channel, U=urban/industrial														
* Pluses and minuses reflect marginal values. A combination of three pluses or three minuses resulted in a two-point increase or decrease in the IBI score.														

was explained by this metric. This metric plus five others account for all of the variance in the IBI score. Stepwise R^2 values for these five additional metrics are 0.87 for percentage of carp, 0.92 for total biomass, 0.96 for percentage insectivores, 0.98 for number native species, and 1.00 for number of individuals. None of the other metrics explained any of the variance in IBI scores. The significantly lower IBI scores for river segment 3 can be explained by noting that the two most important metrics for this survey (number of intolerant species and percentage of carp) were both lower (indicating poorer water quality) for the river segment 3 station groups compared to the other station groups.

2.4 DISCUSSION

A smaller number of fish were captured during this survey than during previous surveys on large river systems which employed similar methods (Hughes and Gammon 1987; Sanders 1992; Tetra Tech 1993b, 1994a, 1995b). These other surveys were conducted in late summer or early fall, while this study was conducted in December. Although water temperature was not recorded at each station during this survey, historical data indicate that December water temperatures are at least 10°C colder than during the late summer and early fall (Hubbard et al. 1994). Except for the salmonid species, it is likely that many species present in the Columbia River are more easily captured during the warmer months when they are more active.

Several authors have used the tentative integrity classes proposed by Karr et al. (1986) to assign a qualitative label (e.g., excellent, good, fair, poor) to each IBI score (Crumby et al. 1990, Bramblett and Fausch 1991). While this approach has merit if the metrics used to create the IBI accurately reflect the biotic integrity of the system, if they do not, the labels can be misleading. Qualitative labels have not been used for this study. Additional research is needed to develop appropriate metrics for a river as large as the Columbia. Virtually all of the fish community research utilizing the IBI approach has been conducted on smaller river systems. Despite the uncertainty in the predictive power of the metrics, the IBI scores calculated in this study are useful when viewed in the context of spatial variation within the Columbia River.

Although fish community studies utilizing the IBI approach had not been conducted on the Columbia River prior to this study, a similar multi-year study was recently completed for the Willamette River

(Tetra Tech 1995b). As a tributary to the Columbia, the Willamette would be expected to share a similar species composition. All but two of the species captured in this study (banded killifish and coastrange sculpin) were also found in the Willamette River (Tetra Tech 1995b). The overall species abundance in the Willamette study was higher than in the current study. The sampling seasons of the two studies probably influenced this result. Sampling for the Willamette study (1992-1994) occurred in August-October, while the Columbia sampling did not occur until December, when the water was much colder. Many more juvenile and immature fish were captured on the Willamette River.

The IBI scores from the pooled data presented in this study were comparable to the highest scores obtained in the upper regions of the Willamette River (Tetra Tech 1995b). None of the pooled IBI scores were less than 33, while several stations located in the Portland area of the Willamette River had scores between 20-30. This difference was noted in spite of the similarities in habitat/land use between Portland Harbor and industrial areas around Vancouver and Longview.

The cause of the lower IBI scores in river segment 3 is not obvious. Each of the three river segments at which fish were captured included stations from all three of the habitat types/land uses, so variability among habitat/land uses cannot explain the observed differences. It is possible that a habitat characteristic which was not quantified or observed could explain the differences, but this hypothesis cannot be tested. The hypothesis that the lower IBI scores in river segment 3 are due to contaminant levels in water, sediment, and/or tissue is explored in greater detail in Section 5.2.1.

3.0 FISH HEALTH ASSESSMENT

Fish health was assessed using the fish health/condition assessment system described in *Fish Health/Condition Assessment Procedure* (Goede 1993). This technique was developed by the State of Utah and has been used by Oregon DEQ and Tetra Tech on the Willamette River (Tetra Tech 1993b; Haefle, R., personal communication), and by Tetra Tech on the upper Columbia River (unpublished data). It is particularly well-suited to comparing the health of a single species across time and location. This system, originally developed for salmonid fish, was used in this survey to assess largescale sucker (*Catostomus macrocheilus*). Data on largescale suckers from previous studies performed on the Willamette River (Tetra Tech 1993b; Haefle, R., personal communication) may serve as a benchmark for comparing the data collected in the present study. Largescale suckers are known from past surveys to be distributed throughout the entire length of the lower Columbia River in quantities suitable for use with this technique, so stratifying the river into segments was not part of the study design. The study was designed (see Figure 1-1) to test this hypothesis:

- Are there differences in fish health (sucker health) among the different major habitat types/land uses in the lower Columbia River?

The specific objectives of the study were to:

- Assess the health of lower Columbia River with an additional technique.
- Attempt to relate fish health data to potential contaminants of concern in the river.

3.1 METHODS

The methods discussion is divided into separate sections for study design, field procedures, and statistical analysis. All data used in the analysis were collected in the field.

3.1.1 Study Design

A random sampling design stratified by main habitat type/land use (backwater, urban/industrial, and main channel) was used to select sampling locations for the fish health assessment. The target species (largescale sucker) is found throughout the length of the river and is not restricted to a particular habitat type/land use. Prior to field sampling, the river was segmented into 1,250 m (0.78 mi) sample transect lengths which were classified by habitat type/land use. Five locations in the lower river were randomly selected from each habitat type/land use. This sampling design allows the testing of the hypothesis that there are no differences in the health of largescale suckers associated with these habitat types/land uses in the lower Columbia River. In addition to the 15 primary sampling locations, at least 10 secondary sampling locations were randomly selected to serve as backups in the event that the target species could not be obtained in sufficient numbers at the primary station. The locations of the primary stations and the alternate stations that were actually sampled are given in Table 3-1 and Figures 2-2 to 2-5.

3.1.2 Field Procedures

Largescale suckers to be used in this study were collected by electroshocking. A boat-mounted electroshocker (Smith-Root Model 7.5 GPP) was used to collect the fish at each station. The electroshocking unit was set at approximately 300 volts which generated 3 amps DC pulsed at 120 cycles/sec. The captured fish were collected using dip nets with a mesh size of 1 cm and maintained alive. Fish were handled carefully until the time of the autopsies, at which time the fish were killed with a blow to the head from a wooden club.

The target number of fish to be analyzed at each station was 20. Sampling locations were expanded both up- and downriver from the original location as needed in order to obtain the number of fish needed to conduct the assessment. In general, fish were captured within approximately one-half mile of the original transect.

The fish health assessment methods have been described in detail by Goede (1993, 1988). Field analysis of fish included:

- Sampling of blood
- Length and weight measurements
- External observations (e.g., eyes, gills, pseudobranchs, thymus)
- Internal examination (e.g., mesenteric fat, spleen, kidney, liver).

Table 3-1. Fish Health Assessment Station Locations and Sampling Dates						
Station	Location	Date	Lat	Long	Number of Fish Collected	
Urban/Industrial Sites						
I1	Tomahawk Island	1/30/95	45-36.06	122-38.33	11	
I2	Hayden Island	1/31/95	45-38.67	122-43.50	15	
I3	Shillapoo Wildlife Rec. Area		not sampled			
I4	Astoria	2/4/95	46-11.44	123-49.15	0	
I5	Skipanon waterway	2/4/95	46-11.18	123-54.29	0	
I6*	Clifton Channel	2/3/95	46-10.03	123-25.04	3	
I7*	Scappoose Bay	2/7/95	45-50.27	122-48.70	11	
Backwater Sites						
B1	Columbia City	2/1/95	45-53.51	122.47.45	2	
B2	Westport Slough		not sampled			
B3	Grays Bay	2/5/95	46-15.94	123-40.15	0	
B4	Prairie Channel	2/3/95	46-13.40	123-33.44	0	
B5	Knappa Slough	2/5/95	46-11.49	123-35.22	2	
B6*	Blind Slough	2/5/95	n/a	n/a	2	
B7*	Clatskanie River	2/2/95	46-06-45	123-12.36	11	
B8*	Carrolls Channel	2/6/95	46-03.65	122-52.15	12	
B9*	Bachelor Island Slough	2/7/95	45-49.62	122-45.62	10	
Main Channel Sites						
M1	Bachelor Island		not sampled			
M2	Deer Island		not sampled			
M3	Puget Island	2/2/95	46-08.40	123-19.16	0	
M4	Cathlamet	2/2/95	46.09.76	123.19.80	0	
M5	Clifton Channel	2/3/95	46-13.35	123-28.31	0	
* These stations were alternates selected in the field						

Observations were classified according to the autopsy classification scheme (Table 3-2) and entered into a fish autopsy worksheet.

A slight deviation from the method protocols (Goede 1993) was necessary. Blood was not collected via cardiac puncture, as is commonly done with salmonids, but via the caudal vein. This was done because of the difficulty in penetrating the membrane in the opercular cavity with the microhematocrit tubes. Blood was collected by severing the caudal peduncle and inserting a heparinized microhematocrit tube into the caudal vein. Blood was centrifuged using a Readacrit centrifuge, Model 0591 (Clay Adams, Parsippany, NJ), thereby separating the three fractions (red blood cells, white blood cells, and serum) so that the percent hematocrit (packed red cell volume) and percent leukocytes (packed white cell volume) could be easily measured. The protein (weight/volume) content of the plasma was determined using a hand-held clinical refractometer which had been zeroed with deionized water. All blood measurements were taken within 2 hours after sample collection.

Length and weight measurements were made immediately after blood samples were collected. The total fish length was determined in millimeters using a stainless steel meter stick and the weight was determined to the nearest 0.1 lbs (and later converted to grams) using a digital hanging scale.

External examinations included general remarks about fins, skin, and other external features, as well as observations of particular organs and systems such as the thymus, pseudobranch, and gills. Important conditions noted were deformities, scale loss, fin condition, external parasites, etc. All observations relating to aesthetics were included as remarks in the fish autopsy worksheet.

After external examinations were completed, fish were cut with a scalpel ventrally from the anal vent forward around the pelvic girdle and on to the pectoral girdle. Care was taken not to damage internal organs and tissues during opening. Internal examinations consisted of observations of the spleen, hindgut, kidney, liver, gall bladder, and gonads for determination of gender and state of development. Samples of liver tissue and bile from the gall bladder were collected for determination of cytochrome P450 enzyme and DNA adduct analyses. The methods and results from this component of the study, which is being conducted by the Environmental Conservation Division of National Marine Fisheries Service (Seattle, WA) will be reported elsewhere. Internal parasites were noted as either absent, present, moderately abundant, or very abundant.

Table 3-2. Description of Variables Used in the Health Assessment Index (HAI) (Page 1 of 2)

Variable	Variable condition	Field Designation	HAI Designation
Eyes	No aberrations; good "clear" eye	N	0
	Generally an opaque eye (one or both)	B	30
	Swollen, protruding eye (one or both)	E	30
	Hemorrhaging or bleeding in the eye (one or both)	H	30
	Missing one or both eyes	M	30
	Other; any aberration not fitting the above categories	OT	30
Gills	Normal; no apparent aberrations	N	0
	Frayed; erosion of tips of gill lamellae resulting in "ragged" gills	F	30
	Clubbed; swelling of the tips of the gill lamellae	C	30
	Marginate; gill with light, discolored margin along tips of the lamellae	M	30
	Pale; very light in color	P	30
	Other; any aberration not fitting the above categories	OT	30
Pseudobranchs	Normal; flat, containing no aberrations	N	0
	Swollen; convex in aspect	S	30
	Lithic; mineral deposits, white, somewhat amorphous spots	L	30
	Swollen and lithic	SL	30
	Inflamed; redness, hemorrhage, or other	I	30
	Other; any aberration not fitting the above categories	OT	30
Thymus	No hemorrhage	0	0
	Mild hemorrhage	1	10
	Moderate hemorrhage	2	20
	Severe hemorrhage	3	30
Spleen	Normal; black, very dark red, or red	B	0
	Normal; granular, rough appearance of spleen	G	0
	Nodular; containing fistulas or nodules of varying sizes	D	30
	Noticeably enlarged	E	30
	Other; gross aberrations not fitting above categories	OT	30
Hindgut	Normal; no inflammation or reddening	0	0
	Slight inflammation or reddening	1	10
	Moderate inflammation or reddening	2	20
	Severe inflammation or reddening	3	30
Bile	Yellow or straw color; bladder empty or partially full	0	*
	Yellow or straw color; bladder full, distended	1	*
	Light green to "grass" green	2	*
	Dark green to dark blue-green	3	*
Kidney	Normal; firm dark red color, lying flat along length of vertebral column	N	0
	Swollen; enlarged or swollen wholly or in part	S	30
	Mottled; gray discoloration	M	30
	Granular appearance and texture	G	30
	Urolithiasis or nephrocalcinosis; white or cream-colored mineral material in tubules	U	30
	Other; any aberrations not fitting above categories	OT	30
Liver	Normal; solid red color or light red color	A	*
	"Fatty" liver; "coffee with cream" color	C	*
	Nodules in the liver; cysts or nodules	D	*
	Focal discoloration; distinct localized color changes	E	*
	General discoloration; color change in whole liver	F	*
	Other; deviation in liver not fitting other categories	OT	*
Hematocrit ^a	Normal range	30-45%	0
	Above normal range	>45%	10
	Below normal range	19-29%	20
	Below normal range	<18%	30

Table 3-2. Description of Variables Used in the Health Assessment Index (HAI) (Page 2 of 2)

Variable	Variable condition	Field Designation	HAI Designation
Leukocrit	Range defined as normal	< 4%	0
	Outside normal range	> 4%	30
Plasma protein ^b	Normal range	3.0-6.9 mg/L	0
	Above normal range	> 7.0 mg/L	10
	Below normal range	< 30 mg/L	30
Parasites	No observed parasites	0	0
	Few observed parasites	1	10
	Moderate parasite infestation	2	20
	Numerous parasites	3	30
Fins	No active erosion	0	0
	Light active erosion	1	10
	Moderate active erosion with some hemorrhaging	2	20
	Severe active erosion with hemorrhaging	3	30
Opercles	No shortening	0	0
	Mild shortening	1	20
	Severe shortening	2	30

Source: Adams et al. (1993)

* = This variable was not used in the calculation of HAI (see text for explanation)

^a Normal ranges for centrarchid species such as largemouth bass; values for largescale sucker not available

^b Values greater than 7.0 mg/L are generally inaccurate because of factors that interfere with the protein analysis such as elevated lipids

Several steps were taken in the field to ensure that measurement bias was minimized. Since implementation of Goede's fish health/condition assessment requires some training and experience at fish autopsies, the same individual was responsible for all of the qualitative observations. Field methods for measuring blood parameters, length, weight, and other external and internal characteristics were standardized prior to data collection and followed consistently at each station. Since several fish were measured at each site, care was taken to assign a unique number to each fish. This was particularly important during the measurement of blood parameters, when the sample was no longer attached to the fish. During centrifugation, each of the tubes were placed in numbered slots in the microhematocrit centrifuge; at other times the tubes were kept in numbered positions on the tube sealant tray. The external and internal examinations of the fish were done in the same order as the centrifugation, thus ensuring that all data from one fish were correctly attributed to that fish.

3.1.3 Data Analysis and Interpretation

Information collected and recorded in the fish autopsy worksheets was summarized according to the Health Assessment Index (HAI) approach outlined in Adams et al. (1993). The HAI is calculated by assigning numerical values to each of the letter codes used in the fish health/condition assessment (Table 3-2). A high score indicates lower fish health, while lower scores indicate normal conditions. Two of the indices normally evaluated in the fish autopsy method (liver and bile) were not assigned corresponding HAI values. The normal liver of the largescale sucker does not appear to be red, which is considered normal for the salmonid species for which this method was developed. Because an alternate assessment scale for livers in largescale suckers has not been developed, this parameter was excluded from the HAI scoring. Bile color was not assigned a numerical HAI value because bile can take on differing colors depending on the feeding regime of the fish (Adams et al. 1993).

The numerical values allowed an aggregate score to be calculated for each fish; a mean score was calculated for each station at which 10 or more fish were examined. Prior to statistical tests, data were log-transformed ($x+1$) to better meet the assumptions of the parametric statistics. Levene's test for homogeneity of variances was conducted before each ANOVA. ANOVAs were used to test the null hypothesis that the fish health index score is equal among habitat types/land uses. In addition, the condition of the fish at each station, as measured by the condition factor [weight in grams $\times 10^5$ /length (mm)³], was also compared among stations and habitat types/land uses using ANOVA (Statistica v. 2.0).

3.2 RESULTS

The fish health station locations, dates of sampling, and number of fish collected at each station are given in Table 3-1. A total of 79 fish were evaluated. The target number of fish (20) could not be obtained at any of the stations. No fish were evaluated from the main channel stations. Several of the original sample locations (I3, B2, M1, and M2) were not sampled because the field crew determined that there did not appear to be suitable fish habitat at these stations. At seven other stations, no fish could be collected. At one urban/industrial station (I6) and three backwater stations (B1, B5, and B6), only two or three fish were collected. At least 10 fish were evaluated at three urban/industrial (I1, I2, and I7) and three backwater stations (B7, B8, and B9). Only data for these stations were analyzed, although a HAI score was calculated for each of the 79 fish. Data from stations at which less than 10 fish were evaluated will not be discussed further. The lack of success at the main channel sites required a change in the sampling design. Instead of testing for variations in fish health at three different habitat types/land uses, only differences between the backwater and urban/industrial sites were tested.

The fish health assessment field designations and HAI scores for each of the 79 fish are given in Table 3-3. Of the 79 fish, only 9 were males. Most of the females had ripe eggs. The precise timing of the reproductive cycle of largescale sucker on the lower Columbia River is not known, but in other areas of the Northwest, they spawn in April and May (Wydoski and Whitney 1979). The size of the fish ranged from 275-530 mm and 182-1,634 g although all but five of the fish were at least 375 mm and 363 g. The mean condition factors for the six stations at which 10 or more fish were evaluated were similar, ranging from 0.944 at Station B7 to 1.056 at Station I7.

Abnormal conditions for most of the indices were rare except for hematocrit and parasites. At least several fish at each station had apparently abnormal hematocrit levels. The definition of normal hematocrit range was taken from Adams et al. (1993) and is based on largemouth bass and redeared sunfish. Normal hematocrit values for largescale suckers are unknown. Most of the parasites seen were intestinal. At least several fish at each station had a HAI score of 0, indicating no abnormalities. The highest HAI score was 60, calculated for a fish at Station B8.

The mean HAI scores for the three urban/industrial and three backwater stations are given in Table 3-4. The mean values ranged from 4.5 at Station I7 to 17.3 at Station B7. The variability in the calculated HAI scores was high. The standard deviation equaled or exceeded the mean at all six stations.

3.3 DISCUSSION

The HAI scores calculated for the largescale suckers on the lower Columbia River were generally low, ranging from 0 to 60 for individual fish. Although a comparison between these results and results for largemouth bass and redeared sunfish collected from various regions of the Southeastern US (Adams et al. 1993) should not be emphasized due to the differences in species and regions, the mean HAI scores for the healthiest waterbodies and reference sites of the latter study were higher than the mean HAI scores calculated in this study, possibly indicating lower Columbia River largescale suckers have better health than the species in the other studies.

Two hypotheses were tested using the results of the fish autopsy procedure. The first hypothesis was that the mean condition factors at each station were not significantly different ($p=0.05$) from one another. This hypothesis was tested so that the effect of fish condition factor on all subsequent analyses could be considered. The null hypothesis stated above was accepted; no differences in fish condition factor could be detected.

The second hypothesis was that the mean HAI scores for the three backwater stations were not significantly different from the mean HAI scores at the three urban/industrial stations. The results of the statistical tests of this hypothesis are given in Table 3-4. Two preliminary ANOVAs were performed to determine if the mean HAI score at each station was significantly different from the other mean HAI scores for that habitat type/land use, before an overall mean HAI score for each triplet of stations was calculated. It was determined that for each habitat type/land use, the mean HAI scores for the three stations were not significantly different from each other (Table 3-4). The final ANOVA indicated that the overall mean HAI score for the urban/industrial stations (7.3) was significantly less than the overall mean for the backwater stations (14.2) ($p=0.05$) indicating that largescale suckers at the urban/industrial stations were in better condition than those at backwater stations.

TABLE 3-4. SUMMARY STATISTICS FOR HAI SCORES AT SIX STATIONS					
Station	Count	Mean	Standard Deviation	Log (x+1) Mean	Log (x+1) Standard Deviation
I1	11	12.7	12.7	0.75	0.72
I2	15	5.3	7.4	0.45	0.58
I7	11	4.5	10.4	0.26	0.57
B7	11	17.3	15.6	0.98	0.65
B8	12	15.0	20.2	0.77	0.72
B9	10	10.0	11.5	0.65	0.69

ANOVA - HAI SCORES AT THREE INDUSTRIAL STATIONS (I1, I2, I7)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	1.37	2	0.69	1.77	0.19
Within Groups	13.81	34	0.39		
Total	14.55	36			

ANOVA - HAI SCORES AT THREE BACKWATER STATIONS (B7, B8, B9)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	0.58	2	0.29	0.60	0.55
Within Groups	14.32	30	0.48		
Total	14.90	32			

ANOVA - HAI SCORES AT I STATIONS VS. B STATIONS					
SUMMARY					
Groups	Count	Log (x+1) Mean	Log (x+1) Standard Deviation		
ALL I Stations	37	0.48	0.64		
ALL B Stations	33	0.80	0.68		
ANOVA					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	1.78	1	1.78	4.11	0.047
Within Groups	29.45	68	0.43		
Total	31.23	69			

Although the mean HAI score was significantly lower for the urban/industrial station group, the possibility that this difference is not *biologically* significant should be considered. The HAI approach does not include a definition of what score constitutes an unhealthy population, because this value is specific to a particular species/study area combination. Prior to this study, the fish health autopsy approach had not been implemented using largescale suckers on the lower Columbia River, but it had been used for largescale suckers on the Willamette River (Tetra Tech 1993b). In the 1992 Willamette Study, the mean HAI scores for stations ranged from 38 to 65, even though several of the parameters measured in the present study (parasites, all blood indices) were not included in the scores (unpublished data). HAI scores indicate that a healthier population of largescale suckers resides in the lower Columbia River than in the Willamette.

Several characteristics of urban/industrial and backwater stations could explain the observed differences in mean HAI scores between the two habitat types/land uses. The three urban/industrial stations were generally located upriver from the three backwater stations, although Stations I7 (located near Scappoose Bay) and B9 (located in Bachelor Island Slough) were located at approximately the same river mile (Table 3-1). It is possible that the backwater stations were exposed to water with a higher degree of pollutant loading since they were located downstream of more point sources of pollutants than were the urban/industrial sites. This difference in the two station types is a natural consequence of the fact that there are few backwater sites located in or upstream of the industrial areas of Portland/Vancouver, St. Helens, or Longview. Another difference between the two habitat types/land uses is that the flow at the backwater stations was generally slower than at the urban/industrial sites, which were generally located along the main channel of the river. The slower flowing water at the backwater stations promotes the deposition of fine sediments, which are thought to be more frequently associated with contamination (Tetra Tech 1995a). The hypothesis that the observed differences are due to variation in contaminant concentrations in water, sediment, and/or tissue is discussed in greater detail in Section 5.2.2.

4.0 JUVENILE FISH SKELETAL ABNORMALITIES

Juvenile fish skeletal abnormalities were assessed as a third independent measurement of fish health in the lower Columbia River. The specific hypothesis addressed in this study design is:

- Are there differences in the number of skeletal abnormalities in juvenile fish (sucker/peamouth/squawfish) among different segments of the river?

Several authors have used this technique to demonstrate that increased incidence of skeletal abnormalities can be associated with many stressors including heavy metals and bleached kraft mill effluents (Bengtsson and Larsson 1986; Bengtsson 1988). Tetra Tech has utilized this technique in three recent studies of the Willamette River (Tetra Tech 1993b; 1994a; 1995b).

4.1 METHODS

The methods discussion is divided into separate sections for study design, field procedures, laboratory procedures, and statistical analysis.

4.1.1 Sampling Design

This assessment utilized random sampling design stratified across the four major river segments discussed in Section 1.4.1. Within each segment, three habitat types/land uses were identified (i.e., backwater, urban/industrial, and main channel). Only areas identified as backwater habitat were targeted for sampling sites for the juvenile skeletal abnormality assessments. Because the juvenile skeletal abnormality technique had not been used on the Columbia River before, there were no data on where juveniles are typically found. Backwater areas were selected because of the higher probability of finding appropriately-sized individuals of the target species due to lower current speeds and higher food availability.

Juvenile largescale suckers were the primary target species for this study. This species was selected because it is a primary prey item for bald eagles and because it is also being used in the fish health assessment study. However, all fish captured were preserved and a determination was made regarding which species to analyze after collection efforts were completed. Secondary target species include peamouth and northern squawfish.

Four sampling locations, based on the transect length identified in the pilot study (1,250 m), were randomly selected from the backwater habitats identified in each major river segment. Juvenile fish were collected at a total of 16 backwater sampling locations throughout the river. This study design allows testing the hypothesis that there are no differences in the number of skeletal abnormalities in juvenile fish among different segments of the river. In addition to the 16 primary sampling locations, at least 12 secondary sampling locations were randomly selected to serve as backups in the event that the juvenile fish could not be obtained in sufficient numbers at the primary stations. The locations of the primary and alternate stations that were actually sampled are given in Table 4-1 and Figures 2-2 to 2-5.

4.1.2 Field Collection Methods

The actual sampling location for each station was determined in the field. Juvenile fish were collected by seining in shallow water areas. Each sampling location had to contain enough relatively flat beach area to allow the deployment of the net. A 50-m beach seine (variable mesh size ranging from 9.5 to 19 mm) was used to crowd fish into shallow water for capture. The net was anchored on the shoreline, dragged through the water using a small boat, and returned to the shoreline at a point upstream of the original point. All fish captured in the seine, with the exception of salmon smolts, were collected and preserved in 10 percent buffered formalin. Salmon smolts were not handled, but were allowed to escape over the top of the seine corkline. To comply precisely with ESA permit requirements, the net was only deployed two times at each station. Typically, the upstream end of the first deployment served as the downstream end of the second deployment.

4.1.3 Laboratory Methods

Fish tissue was cleared and cartilage and bone stained using methods similar to those reported by Taylor (1967) and Potthoff (1984). The fish samples were first neutralized to prevent bone calcium loss by placing the fish into a saturated sodium borate solution for at least 12 hours. Next, body pigmentation was removed by placing the samples in a bleaching solution consisting of 10 parts 3% hydrogen peroxide

Table 4-1. Juvenile Fish Skeletal Deformity Station Locations and Sampling Dates					
Station	Location	Date	Time	Latitude	Longitude
River Segment 1					
1-1S	Elochoman Slough	11/21/94	1000	46-14.56	123-25.69
1-2S	Marsh Island	11/20/94	0830	46-13.12	123-34.24
1-3S*	Elochoman Slough	11/21/94	0800	46-13.42	123-23.80
1-4S	Gray's Bay	11/20/94	1200	46-18.03	123-42.83
River Segment 2					
2-1S	Fisher Island Slough	11/22/94	0800	46-09.81	123-02.92
2-2S	Coal Creek Slough	11/22/94	0930	46-11.39	123-06.91
2-3S*	Bradbury Slough	11/22/94	1045	46-10.16	123-07.95
2-4S	Wallace Island Slough	11/21/94	1230	45-08.44	123-16.98
River Segment 3					
3-1S	Bachelor Island Slough	11/19/94	1100	45-48.10	122-46.03
3-2S	Scappoose Bay	11/19/94	0930	45-49.72	122-50.07
3-3S	Across from Columbia City	11/19/94	1230	45-53.27	122-47.16
3-4S	Goat Island	11/19/94	1300	45-56.41	122-49.34
River Segment 4					
4-1S*	Beacon Rock	11/18/94	1415	n/a	n/a
4-2S	Reed Island	11/17/94	1615	45-33.50	122-18.22
4-3S	Gary/Flag Island	11/18/94	0930	45-32.92	122-20.72
4-4S	Government Island	11/18/94	1100	45-35.48	122-33.78
* These stations were alternates selected in the field					

solution with 90 parts 1% potassium hydroxide solution. Fish tissue was then cleared by trypsin digestion: the fish were held in a trypsin enzyme buffer solution (30 parts of saturated sodium borate solution supernatant, 75 parts distilled water, and trypsin powder) until the tissue was visibly clear. The enzyme buffer solution was changed approximately weekly during this step. To assist in the identification of skeletal deformities, the bone was then stained red by placing the samples in a 1% potassium hydroxide solution containing alizarin red S dye for several days. The fish were then placed back in a trypsin buffer solution until the fish tissue was cleared of the dye. The cleared and stained fish were then placed in a glycerin solution.

Each fish was measured (total length) and examined under 12X magnification with a dissecting microscope for skeletal deformities. A sample that displayed curvature of the spine (scoliosis), fused vertebrae, or deformed vertebrae was classified as exhibiting skeletal deformities.

4.1.4 Data Analysis and Interpretation

The percent incidence of skeletal abnormalities was reported for each site. The specific types and incidence of each deformity was recorded. Qualitative comparisons among the four stations within each major segment were conducted and reported. No statistical comparisons were made because of the low number of fish captured at some of the sites. An assessment of fish health at these sites was made based on the incidence of skeletal abnormalities.

4.2 RESULTS

The number and species of fish collected from each station is given in Table 4-2. This table also includes the overall mean length (mm) for each species. A total of 596 fish were collected at all 16 stations. Very few of the original target species (largescale sucker, peamouth, and Northern squawfish) were obtained. Over 90 percent of the fish captured were three-spined sticklebacks (72 percent) or banded killifish (18 percent). Although fish were collected at every station, less than 35 fish were collected at all but 3 stations (1-2S, 1-4S, and 2-4S). No individual fish species was collected at all 16 stations, although three-spined stickleback and banded killifish were collected at 14 and 12 stations, respectively.

Table 4-2. Number of Fish Captured for Juvenile Fish Skeletal Deformity Study

Species	Station																Total for Each species	Mean Length (mm)
	1-1s	1-2s	1-3s	1-4s	2-1s	2-2s	2-3s	2-4s	3-1s	3-2s	3-3s	3-4s	4-1s	4-2s	4-3s	4-4s		
Three-spined stickleback	17	44	4	89	16		18	196	2	4		1	13	2	12	14	432	45.2
Banded killifish		47	4		14	2	1	3	5	3	1	21		3		5	109	50.6
Peamouth		1	2					5		1				2	3		14	58.7
Bluegill					2					1		5		2	3		13	36.4
Largescale sucker					1	1						2	1	1			6	65.7
Starry flounder		1				1	1				2						5	131.6
Largemouth bass		1															1	na
Prickly sculpin									4			2		1			7	77
American shad				1												1	2	72.5
Smelt				1													1	na
Black crappie																1	1	na
Mountain whitefish											1						1	na
Unknown killifish		1						1									2	83.5
Speckled dace															2		2	68
Total # of Fish	17	95	10	91	33	4	20	205	11	9	4	31	14	11	20	21	596	

Five species of fish were analyzed for skeletal deformities: three-spined stickleback, banded killifish, bluegill, peamouth, and largescale sucker. The other species of fish were not analyzed because they were too large (e.g., starry flounder and prickly sculpin) or were obtained in very small numbers (i.e., 1 or 2 individuals). Table 4-3 gives the results of the analyses. Skeletal deformities were observed in 8 of the 573 individual fish examined (1.4 percent): 6 three-spined sticklebacks, 1 banded killifish and 1 peamouth. Approximately 75 percent of the individuals examined were three-spined sticklebacks. The overall incidence of skeletal abnormality for this species was identical (1.4 percent) to the percentage for all species.

The percentage of deformed fish observed in each of the four river segments (pooling data from each station) ranged from zero (segment 4) to 2.2 percent (segment 3). It should be noted that the percentages for segments 3 and 4 are based on 46 and 60 fish, respectively, as compared to segments 1 and 2, which each consisted of more than 200 individuals. Because the occurrence of skeletal abnormalities in the fish examined in this study was very low, the percent abnormality values for segments 3 and 4 were considered estimates due to the smaller sample sizes. The target sample size for each station was originally 200 fish. No statistical comparisons among river segments were performed due to the uncertainty caused by low sample size. However, from a qualitative standpoint, it is clear from Table 4-3 that each species and river segment had a very low (<2.3 percent) incidence of skeletal deformities.

4.3 DISCUSSION

The incidence of skeletal deformities observed in the lower Columbia River is within the range of 2-5 percent reported for unstressed natural fish populations and laboratory stocks (Gill and Fisk 1966, Wells and Cowan 1982). Conclusions about the health of fish populations on the lower Columbia River are probably premature due to the species sampled and the time of year sampling took place.

The percent abnormality values observed for the lower Columbia River were similar to or lower than those seen for juvenile Northern squawfish in the Willamette River (Tetra Tech 1993b; 1994a; 1995b). In the Willamette study, the incidence of skeletal abnormalities was as high as 75 percent, although it was less than 5 percent at sampling locations near the mouth (i.e., Portland) and in the upstream section (e.g.,

Table 4-3. Number of Deformed and Undeformed Fish

Species	Station														Total for Each species		
	1-1s	1-2s	1-3s	1-4s	2-1s	2-2s	2-3s	2-4s	3-1s	3-2s	3-3s	3-4s	4-1s	4-2s		4-3s	4-4s
Three-spined stickleback																	
undeformed	17	43	4	87	15		18	195	1	4		1	13	2	12	14	426
deformed	0	1	0	2	1		0	1	1	0		0	0	0	0	0	6
Banded killifish																	
undeformed		46	4		14	2	1	3	5	3	1	21		3		5	108
deformed		1	0		0	0	0	0	0	0	0	0		0		0	1
Peamouth																	
undeformed		1	2					4		1				2	3		13
deformed		0	0					1		0				0	0		1
Bluegill																	
undeformed					2					1		5		2	3		13
deformed					0					0		0		0	0		0
Largescale sucker																	
undeformed					1	1						2	1	1			6
deformed					0	0						0	0	0			0
Total # of fish analyzed	17	92	10	89	33	3	19	204	7	9	1	29	14	10	18	18	573
Total # undeformed	17	90	10	87	32	3	19	202	6	9	1	29	14	10	18	18	565
Total # deformed	0	2	0	2	1	0	0	2	1	0	0	0	0	0	0	0	8
Percentage deformed	0	2.2	0.0	2.2	3.0	0.0	0.0	1.0	14.3	0	0	0	0	0	0	0	1.4
Percentage deformed for entire river segment	1.9				1.2				2.2				0.0				

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Corvallis to Eugene) of the main stem. Comparison should be made with caution, however, because the two datasets measured different species that were collected at different times of the year.

The two primary species examined during this survey (three-spine stickleback and banded killifish) reach a maximum size of approximately 100 mm in length (Page and Burr 1991). Although samples (e.g., otoliths or scales) were not taken from these species for the purposes of aging the fish, an estimate of age can be made using fish length. The mean total length of the sticklebacks was 45.2 mm (Table 4-2). These fish may live to an age of three years, but in Washington, it appears that approximately 90 percent of these fish live for only one year, with the remaining 10 percent surviving a second year (Wydoski and Whitney 1979). These fish typically spawn in May or June (Wydoski and Whitney 1979). From this information, it can be deduced that most of the sticklebacks examined were young-of-year (hatched in 1994) and a small proportion were one-year olds (hatched in 1993). For banded killifish, the limited available age-length data suggest that 50 mm fish (the mean length as given in Table 4-2) are also either young-of-year or one-year olds (Scott and Crossman 1973). Banded killifish also appear to spawn in May or June (Scott and Crossman 1973).

The original study design called for the collection of juvenile (young-of-year) fish approximately 25-35 mm in length (Tetra Tech 1994b), because a population of larger fish might not include as many individuals with skeletal abnormalities because of the reduced fitness and subsequent higher mortality rate imparted by these deformities. Almost all of the fish examined in this study were larger than 25-35 mm, and appeared to be mostly older juveniles and sub-adults. It is possible that the low incidence of skeletal deformities observed in this study reflects the age of the fish as much as or more than the potential stressors to which they were exposed.

The hypothesis that larger (i.e., older) fish may have a lower incidence of skeletal abnormalities was explored statistically using the three-spined stickleback data. First, a simple linear regression of size class (2 mm increments) versus proportion of skeletal abnormalities was performed. The regression line was not significantly different from zero ($p=0.72$), indicating that there was not a significant relationship between size class and abnormalities. Given the very low incidence of skeletal abnormalities in this dataset, the relationship between size and abnormalities can also be examined by pooling data. The incidence of skeletal abnormalities was compared for two groups of sticklebacks, one less than or equal to 35 mm and one greater than 35 mm. In the smaller group, 4 of 146 fish had abnormalities, while in the larger group 2 of 286 fish had abnormalities. A 2x2 chi-square test indicated that this distribution

of size and proportion of abnormalities were not significantly related ($p = 0.085$). However, given the limited number of fish in the dataset, definite conclusions about the relationship between size and incidence of abnormalities cannot be made.

5.0 SYNTHESIS OF THE THREE BIOASSESSMENT TECHNIQUES

The study designs for these three bioassessment techniques are independent, but designed to relate to each other. Because of the results of the field collections, each of the three techniques ended up focusing on a different species (e.g., largescale sucker vs. three-spine stickleback) or level of organization (individual vs. community). For this reason, the results from these studies may not be expected to agree. This section compares, contrasts, and summarizes the results from the three techniques.

5.1 COMPARISON OF RESULTS

Each bioassessment study was designed to test a different hypothesis. The fish community assessment technique tested the effects of habitat/land use and river segment on the health of fish communities, as measured by the IBI. The fish autopsy technique tested the effects of habitat/land use on the health of largescale sucker populations, as measured by the HAI. The juvenile skeletal abnormality technique tested the effects of river segment on the incidence of skeletal abnormalities. The necessity of sampling in winter rather than late summer and early fall caused by delays in the permitting process, resulted in sample sizes that were insufficient to test any of the hypotheses completely. However, pooling the available data allows partial testing of each hypothesis.

For the fish community assessment, the effects of habitat/land use could not be tested by station because not enough fish were caught in some habitats/land uses to calculate a meaningful IBI value. In addition, habitat type/land use stations where enough fish were caught were unevenly distributed among the river segments. It was possible, however, to test the effects of river segment and habitat/land use by pooling data from several stations (see Figure 2-6). No fish were collected from river segment 1, so the effects of this segment could not be tested. The results of ANOVA tests on the pooled data indicated that there was no significant effect of habitat/land use on IBI scores, and that the IBI scores from river segment 3 were significantly lower than the IBI scores from river segments 2 and 4.

For the fish autopsy assessment, it was not possible to test the effects of all three habitat types/land uses because an insufficient number of largescale suckers were captured at main channel stations. The HAI scores for the urban/industrial stations were significantly lower (i.e., better condition) than the HAI scores for backwater stations, although all mean HAI scores from this study showed better condition than at sites known to be associated with chemical contamination (Adams et al. 1993).

For the juvenile fish skeletal abnormality assessment, the effects of river segment on the incidence of skeletal abnormalities could not be tested for a single species due to the small number of fish captured at stations in river segments 3 and 4. A qualitative comparison indicated that the proportion of skeletal abnormalities was very low (<2.3 percent) for all species and river segments. There did not appear to be any meaningful relationship between river segment and incidence of abnormalities.

As might be expected, the results from the three bioassessment techniques do not yield consistent results. River segment appears to influence fish health for the fish community technique, but not for the skeletal abnormality technique. Land use/habitat type appears to influence fish health for the fish autopsy technique, but not for the fish community assessment technique. This lack of agreement among the three techniques was also observed on the Willamette River, where each technique identified a different segment of the river with the poorest fish health (Tetra Tech 1993b). The lack of agreement among the techniques, rather than discouraging their simultaneous use, highlights the fact that sublethal effects of stressors on fish health can be manifested in many different ways which a single technique might be unable to detect.

None of the three bioassessment techniques appears to be more sensitive than the others for this particular sampling effort. Although significant effects of habitat/land use and river segment were noted for the fish autopsy and fish community techniques, respectively, the absolute differences in scores (HAI for fish autopsy and IBI for fish community) were relatively small and may not be biologically meaningful. These results contrast with the results from the use of these three techniques on the Willamette River (Tetra Tech 1993b, 1994a, 1995b). In these studies, the juvenile fish skeletal abnormality technique appeared to be the most sensitive of the three techniques. Dramatic differences in the proportion of skeletal abnormalities were noted for the Newberg Pool area compared to other parts of the Willamette River main stem (Tetra Tech 1995b). However, the sensitivity of this technique for the lower Columbia River

cannot be fairly compared to its sensitivity for the Willamette River until the same target species (i.e., Northern squawfish) can be evaluated on the lower Columbia River.

5.2 RELATIONSHIP OF BIOASSESSMENT RESULTS TO KNOWN CONTAMINANT CONCENTRATIONS

There are many possible explanations for the variability of results for the three fish health assessment techniques described for this survey, some of which have been discussed above. One possible explanation which frequently receives a great deal of attention by both investigators and the public is chemical contamination. The hypothesis that the observed differences in the results for the different assessment techniques are due to variations in chemical contaminant concentrations in water, sediment, and/or fish is discussed below in separate sections for each technique.

5.2.1 Fish Community Assessment

The results of the fish community assessment indicated that the pooled data from river segment 3 stations has a significantly lower mean IBI score than the pooled data from river segments 2 or 4. The pooled data from these three segments came from 27 different stations (Table 2-4). Many of the stations sampled for contaminant concentrations in water, sediment, and tissue in two previous reconnaissance surveys (Tetra Tech 1993a, 1995a) were located near the fish community assessment stations. Rather than examine contaminant concentrations at each station individually, which would yield a large body of information that would be difficult to summarize, the reconnaissance survey data were evaluated on a river segment-wide basis to determine if contaminant concentrations in river segment 3 were different than contaminant concentrations in river segments 2 and 4. This type of general comparison is appropriate in light of the fact that although fish communities were evaluated over relatively short lengths of river (1,250 m) during this study, the fish that make up these communities may travel many miles from these points depending on environmental factors such as season, river stage and flow, and time of day. Thus, an evaluation of contaminant levels over the entire river segment gives a good indication of the magnitude of chemical stressors to which these fish may have been exposed.

Contaminant data from river segments 2, 3, and 4 were compared to each other and available reference levels (e.g., standards, guidelines, action levels, or criteria; Tetra Tech 1995a). Table 5-1 presents the

Table 5-1. Frequency of Exceedance of Available Reference Levels for Water, Sediment, and Tissue Samples Collected During Lower Columbia River Reconnaissance Surveys

Water	Number of Exceedances/Number of Stations			
	Conventionals	Bacteria	Metals	Semi-volatiles
Region 2 (13 stations)	0.5	0.1	1.5	0.0
Region 3 (18 stations)	0.7	0.2	1.4	0.1
Region 4 (11 stations)	0.4	0.3	1.1	0.0

Sediment	Number of Exceedances/Number of Stations			
	Metals	Dioxins/furans	Semi-volatiles	Pesticides/PCBs
Region 2 (13 stations)	0.8	0.3	0.3	0.4
Region 3 (18 stations)	1.6	0.2	0.2	0.9
Region 4 (18 stations)	0.8	0.2	0.1	0.2

Tissue	Number of Exceedances/Number of Stations			
	Dioxins/furans	Semi-volatiles	Pesticides/PCBs	
Region 2 (7 stations)	0.4	0.6	0.0	
Region 3 (14 stations)	0.4	0.7	0.1	
Region 4 (10 stations)	0.3	0.4	0.0	

Source: Tetra Tech (1995a)

frequency of exceedances of water, sediment, and tissue reference levels for the stations in each of the three river segments measured in the 1991 and 1993 reconnaissance surveys (Tetra Tech 1993a, 1995a). An exceedance was a measured value not in keeping with applicable reference levels (Tetra Tech 1995a). For many stations, more than one exceedance was noted. For six of the analytical group/river segment combinations, the number of exceedances per station was highest in river segment 3 (Table 5-1). This trend was most pronounced for sediment metals and pesticides/PCBs, for which the number of exceedances per station in segment 3 was double that in either segments 2 or 4. The higher proportion of reference level exceedances in segment 3 may help explain the lower IBI scores calculated for this segment in this study.

5.2.2 Fish Health Assessment

The analysis of the largescale sucker autopsy data indicated that the three urban/industrial stations had a significantly lower mean HAI score than did the three backwater stations. Several measurements of sediment and water pollutant concentrations have been reported in the vicinity of some of the fish health assessment stations (Tetra Tech 1993a, 1995a). Water, sediment, and biota samples were collected from at least one station in the vicinity of each of the six fish health stations, with the exception of Station I2, near which no fish contaminant analyses have been made, and Station I1, near which no water contaminant analyses have been made. A summary of the contaminant analyses at these stations is given in Table 5-2. This summary is not intended to be sufficient for a quantitative comparison between the fish health stations, but should allow a qualitative discussion. No significant contamination (defined as exceedance of available reference values) was noted at any of the water stations located near the fish health stations. For sediment samples, problem chemicals have been identified near two of the three stations for both backwater and urban/industrial habitat types. Problem chemicals have also been identified at all of the fish tissue stations located near the fish health stations. This brief examination of recent contaminant concentrations near the locations of the fish health stations does not indicate that either habitat type is associated with a higher degree of contamination in the lower Columbia River.

This result is further confirmed by the results of the NMFS biomarker study (Collier et al. 1995) that was conducted using the same largescale suckers that were used in this fish health assessment. That study used two methods to assess exposure of largescale suckers to aromatic compounds: levels of biliary FACs, and hepatic AHH activities (induction of P4501A enzymes). There were no significant between-site differences for either of these measures, and no significant differences between industrial/urban sites

Table 5-2. Lower Columbia River Reconnaissance Survey Results From Stations Near Fish Health Assessment Locations						
	Fish Health Assessment Stations					
	I1	I2	I7	B7	B8	B9
Water	none	W38 (91 ^a)-NSC ^b	W32 (91)-NSC	W18 (91)-NSC	W25 (91)-NSC	W31 (91)-NSC W33 (91)-NSC
Sediment	D31 (91)-NSC	E11 (91)-18th out of 54 ^c	10 (93 ^d)-4 metals ^e	D15 (91)-NSC	D20 (91)-15th out of 54 ^c	D25 (91)-16th out of 54
					8 (93)-4 metals ^e	11 (93)-2 metals ^e
Fish	D31 (91)- pesticides and PCBs ^f not in top 8 out of 20 ^g	none	10 (93)-PCBs ^f	D15 (91)- pesticides ^f not in top 8 out of 20 ^g	D20 (91)-not in top 8 out of 20 ^g	11 (93)-PCBs ^f
					8 (93)-PCBs ^f	

^a 91 refers to 1991 Lower Columbia River Reconnaissance Survey (Tetra Tech 1993b)
^b NSC (no significant contamination) indicates that measured values at this station did not generally exceed any available reference guidelines
^c Indicates overall contaminant ranking among the 54 sediment stations sampled
^d 93 refers to the 1993 Lower Columbia River Backwater Reconnaissance Survey (Tetra Tech 1994a)
^e Indicates the number of metals that were detected at concentrations above reference guidelines
^f Indicates that compounds within these analytical groups were detected at concentrations above reference guidelines
^g Station overall contaminant ranking was not among the top 8 of the 20 stations sampled

and backwater areas. Overall mean levels of biliary FACs in largescale sucker were comparable to levels previously measured in other fish species (e.g. white sturgeon) from moderately contaminated areas. However, due to the lack of a dose-response relationship for largescale suckers and the lack of between-site differences, the study could not conclude that the FAC data showed evidence of exposure. The hepatic AHH activities in largescale suckers were also considerably lower than previously reported for other fish species from moderately and severely contaminated sites and do not indicate substantial exposure of the fish sampled in this study to aromatic compounds.

5.2.3 Skeletal Abnormality Assessment

The analysis of skeletal abnormalities in five different species of fish indicated that the incidence of abnormalities was not associated with a particular species or segment in the river. The absence of differences as a function of river segment occurred in spite of the observed variability in contaminant concentrations in different segments of the river (see Table 5-1 and Tetra Tech 1995a). The lack of a meaningful relationship between contaminant concentrations and skeletal abnormalities could be due to several factors including: 1) the overall low incidence of skeletal abnormalities found in all samples; 2) the timing of sampling; 3) the use of species (e.g., three-spine stickleback) where the response to stressors is unknown; and 4) the larger size of the fish examined in this study compared to the range for which this assessment technique has proved the most useful (Tetra Tech 1993b, 1995b).

5.3 RECOMMENDATIONS FOR FUTURE RESEARCH

The primary reason each of the hypotheses for the three bioassessment techniques could not be completely tested was the inconsistent success of capturing fish at the sampling locations. At many stations, no fish were captured. A possible explanation for these results that could be remedied for future sampling efforts is the season in which sampling took place. Originally, the study was to be conducted in the late summer. However, due to the extra time required to obtain the ESA permit, the entire study was delayed until the winter season. Field sampling began immediately after the ESA permit was obtained in November. The implications of this delay are discussed below.

Prior to this study, the three bioassessment techniques described in this document had not been used on the lower Columbia River. These techniques were used on the Willamette River during the summer

months (Tetra Tech 1993b, 1994a, 1995b) and resulted in a much larger and consistent catch of fish compared to the catch for the present study. In addition, in the 1991 and 1993 reconnaissance surveys, where collection of fish for tissue analyses was important, it was found that fish (e.g., largescale sucker) were more common and more easily caught in summer. In winter, many of the fish species that inhabit the lower Columbia and Willamette Rivers are found in deeper water than in summer (Wydoski and Whitney 1979). Consequently, these fish are harder to capture using the electrofishing gear employed for this study, which can only be used effectively in water less than 3 m deep. For the juvenile skeletal deformity study, results are additionally compromised by the late collection. It can be assumed that by winter, many of the young-of-year with skeletal deformities may have died from the deformity or become prey. The utility of these three techniques for the assessment of fish health on the lower Columbia River cannot be fairly assessed until the sampling can be repeated during summer when the target species are more likely to be easily captured.

In addition to the sampling season, another possible reason for the inconsistent fish collections may be the size of the standardized transects (1,250 m). Prior to sample selection, a pilot project for the fish community assessment technique was performed to define the optimal sampling distance. This resulted in segmenting the entire lower river into 1,250 m (0.78 mi) segments. Sampling locations for this project were then randomly selected within each habitat type/land use or major river segment to maximize the statistical power of the sampling design. This random selection assumes that within each habitat type/land use and/or river segment, the likelihood of catching fish of the target species is relatively uniform. This appears to be a good assumption for the fish community technique, but may not be as appropriate for the fish health and skeletal deformity techniques.

Although station locations were randomly selected for the present study, the actual sampling locations were selected in the field using the pre-determined coordinates as a starting point. The fish autopsy and juvenile fish skeletal abnormality sampling locations were generally within 2-3 km of the pre-determined coordinates. These locations were more widely spaced from the original coordinates due to the necessity of including suitable largescale sucker habitat (for fish autopsy) and suitable beach habitat for the beach seining (skeletal abnormality). For future studies using these assessment techniques, either separate standardized sampling transect distances should be established or additional discretion given to field personnel to select sampling locations within a broader area to minimize sampling efforts in unsuitable locations.

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APPENDIX A

RESPONSE TO REVIEWER'S COMMENTS

REVIEWER: Don Yon, ODEQ

No specific comments on this report.

REVIEWER: Bill Young, ODEQ

No specific comments on this report.

REVIEWER: Bruce McCain, NOAA/NMFS

No specific comments on this report.

REVIEWER: Brian Offord, Ecology

No specific comments on this report.

REVIEWER: Avis Newell, ODEQ

No specific comments on this report.

REVIEWER: Charles Simenstad, UW School of Fisheries

No specific comments on this report.

REVIEWER: Jon Graves, CREST

No specific comments on this report.

REVIEWER: Jean Cameron, Oregon Environmental Council

No specific comments on this report.

REVIEWER: Lawrence Curtis, East Tennessee State University

No specific comments on this report.

REVIEWER: Raymond Pierotti, University of Kansas

No specific comments on this report.

REVIEWER: Richard D. Olsen - Argonne National Lab

Comment: *This report details the results of attempts to assess fish health in the LCR using three methods. However, the study results and conclusions from the three methods were not consistent with each other nor with similar studies on the Willamette River. This makes interpretation and application to overall program goals difficult and may invalidate the studies. While I am not a fisheries biologist, I have reviewed this report from the*

perspective of the scientific logic employed in the study design. This report should be reviewed by qualified fishery and statistical experts. My specific review comments and suggestions are listed below.

Response:

Comment: *The fish community assessment method involved application of a modified IBI technique which had previously been used on the Willamette River. This technique was chosen for use on the Columbia River despite the fact that it was not designed for such a situation and had never been tested or previously employed on a large river. Additionally, when the three fish health assessment methods were used on the Willamette River they yielded inconsistent conclusions in that each technique identified a different river segment as having poorest fish health. Logic would suggest to me that based on the fact that the lack of validation of the biological assessment method for large rivers and the fact that the Willamette River study yielded inconsistent and conflicting conclusions for the three techniques, the applicability of the methods to the Columbia River would be suspect. I suspect there are more standardized or accepted methods for assessing fish health for large rivers. The authors seem to be aware of potential problems with the technique and indicate on page 2-1 the existence of several issues (although only two are identified).*

Response: The specific assessment methodologies utilized for these studies were specified in the Task Order issued by the Bi-State Program. It was recognized from the beginning that these methodologies had not been used on rivers as large as the Columbia, but the original intent of the studies was to try these methods and see if they were useful or could be modified for the larger river. Unfortunately, because of the delay in sampling, this original intent could not be addressed by the data collected.

Comment: *The definition and selection of major habitat types is not completely clear to me. First of all, while "backwater" and "main channel" are clearly fish habitat types, "urban/industrial" does not seem appropriate as a habitat type. It would appear to me that "urban/industrial" areas could exist in combination with either backwater or main channel areas and that they are more of an indication of land use and perturbation potential than fishery habitat. It would also seem to me that if one of the objectives was to assess effects of contaminants on fish health, it would have been more logical to select known areas of pollution (e.g., downstream of known contaminant discharges) and to then compare these sites with reference sites not having contaminant sources. Some of the urban/industrial sites were evidently chosen in relation to discharges. The lack of clearly defined reference sites or "controls" is a possible problem in the study design.*

Response: Classification of urban/industrial habitat has been changed to urban/industrial land uses. Urban/industrial sites were a subset of the main channel habitat and did not include any backwater locations. The objectives for the fish community assessment did not include the assessment of contaminant effects on fish. As discussed above, the use of the three methodologies used in the study were intended as a preliminary assessment of the methods. No previous fish community data had been collected to identify reference areas, so none could be selected *a priori*. Therefore, a random sampling design was selected. This design was presented, discussed, and approved by the LCR Fish and Wildlife Work Group.

Comment: *In relation to the comment above, I am also a bit concerned about the use of randomly*

selected sampling locations. This is probably appropriate if the only objective is to test for differences between habitat types. However, given that pollution sources and mixing areas associated with them are explicitly located, it seems more logical to establish preset sampling locations in relation to areas of discharges (e.g., a given distance upstream and downstream from the discharge) rather than establish sites randomly within the general area. Again, specifically how these sites are chosen is related to the hypothesis being tested, but the study design must include "controls". It appears that the current study attempted to test two hypotheses with the same sampling design, when it may have been better to utilize two different designs for the two hypotheses. At any rate, I believe the design employed had a lower probability of correlating fish health with contaminant sources.

Response: The objective of the study was not to correlate fish health with contaminant sources. It was to test for differences among habitat types/land uses and among major river segments.

Comment: *A pilot study was undertaken to determine the appropriate length for sampling transects (page 2-1). The pilot study was undertaken in an area where it was believed fish abundance was high. It seems to me that if one were attempting to determine correct transect length so that all sampling sites would yield sufficient numbers of fish, transect length should be determined in an area with low fish populations. This would assure that final transect length would provide appropriate fish numbers in areas with low populations as well as areas with high populations. Testing transect length in areas of high population would only assure appropriate capture numbers for high population areas and would likely under sample low population areas. This may at least partly explain why too few fish and species numbers were collected for many of the sampling sites. This may have been a serious flaw in the study design.*

Response: Additional text was added to the document to explain that ideally the pilot study would have been conducted in all habitat types/land uses to provide optimal sampling distances for each one. However, due to permitting delays, the need to begin the study as soon as possible, and budget limitations, only a single habitat type was selected for the pilot study in consultation with the Bi-State Coordinators.

Comment: *It is unfortunate that fish collections had to be done during mid-Winter. This in combination with the potential problem noted above for transect length apparently prevented collection of adequate numbers of fish at most sites. The low capture numbers prevented use of the original study design and may also have caused problems with the statistical analyses employed because these statistics assume normally distributed populations in the sample sized tested, something which may not have been true in this situation. The pooling of samples within habitat types probably also resulted in some "averaging" of individual numbers and would of course have masked actual among site differences. This could explain in part the fact that IBI scores for the three habitat types were nearly identical (page 2-15).*

Response: Prior to performing the statistical tests, the data were tested for normality. If the distributions were not normally distributed, log (x+1) transformations of the data were performed and the tests repeated. All tests described in the text met the assumptions of the parametric tests used. Clarifying text was added to the document.

Comment: *The fish health assessment study focused on one species (Largescale sucker) although other*

fish taken in the sampling were preserved for possible later analysis. The fish health autopsy approach used had not previously been employed using Largescale suckers within the LCR. This study did not stratify the river into segments as was done for the fish community study. This may have hampered identification of spatial contaminant effects. While the Largescale sucker is one of the target species for the bi-state LCR studies, I question whether it is appropriate to focus on only one species and whether this particular species is the best choice. In general suckers tend to be much more tolerant of pollution than are salmonids or other groups, and may therefore not be as sensitive an indicator of contaminant effects as other families. I strongly recommend that the current fish health study be expanded to evaluate all fish captured during the 1994 sampling.

Response: No other fish species were collected for later analyses. The largescale sucker was selected as the target species because it is a resident species and a bottom feeder; both of which make it a species that would more likely be exposed to contaminants and one that would likely exhibit effects of exposure.

Comment: *Data analyses were limited for the fish health study because too few fish were captured at most sampling locations and no fish were taken from the main channel and seven other sites. Although ANOVA was performed on the data, I believe there is some question as to whether the populations analyzed were normally distributed and appropriate for application of parametric statistics. The low numbers of fish taken certainly raise questions about conclusions based on this study. The fact that some of the analytical results were difficult to explain bears this out (e.g., the fact that Largescale suckers at urban/industrial sites were in better condition than those from backwater sites).*

Response: See discussion above on statistical issues.

Comment: *A third bioassessment study examined juvenile fish skeletal abnormalities within different river segments. As was the case with the other fish community and health studies, this technique had not previously been employed on the Columbia River. While this study did look for differences among river segments, it did so only for backwater habitat sites because it was believed higher numbers of juveniles for the target species would be found at those sites. However, very few of the target species were collected during the study. In the discussion on page 4-6, results are compared to those for juvenile Northern Squawfish in the Willamette River and it is concluded that abnormalities were similar or lower in frequency. I would argue that based on the small numbers of target species examined, the fact that only backwater areas were sampled, and the obvious difference in target species and river basin between the two studies that a comparison of results from the separate studies is not valid.*

Response: The frequency of abnormalities found on the lower Columbia River were generally similar to the frequency of abnormalities representing background conditions (i.e., 2-5 percent) as identified by Gill and Fisk (1966) and Wells and Cowan (1982) for unstressed natural fish populations and laboratory stocks. The Willamette River is the largest tributary to the lower Columbia River, thus, while some differences exist between the basins, the Willamette River would be expected to be the most similar and is appropriate for use in comparisons.

Comment: *Section 5.0 beginning on page 5-1 discusses the three bioassessment studies. This section acknowledges that most of the original study design objectives could not be addressed*

because too few fish were collected. I would suggest that for reasons noted above, none of the bioassessment study results should be used for synthesis of conclusions regarding habitat and contamination effects for fish in the LCR. The application of techniques that had not previously been used on a system as large and complex as the Columbia River, the problems with capture of too few fish, the fact that all three studies had different sampling and analytical regimes, and the lack of appropriate "control" or reference sites in my opinion makes any conclusions suspect. Again, I strongly suggest that qualified fishery biologists and statisticians review the study design and data analysis to determine if anything can be salvaged from this work.

Response:

The discussions of results from these studies relating to contaminant effects are highly qualified and are obviously not intended for conclusions about habitat and contaminant effects on fish for the LCR. This report was reviewed by multiple reviewers fisheries and statistical expertise. No other comments were recieved.