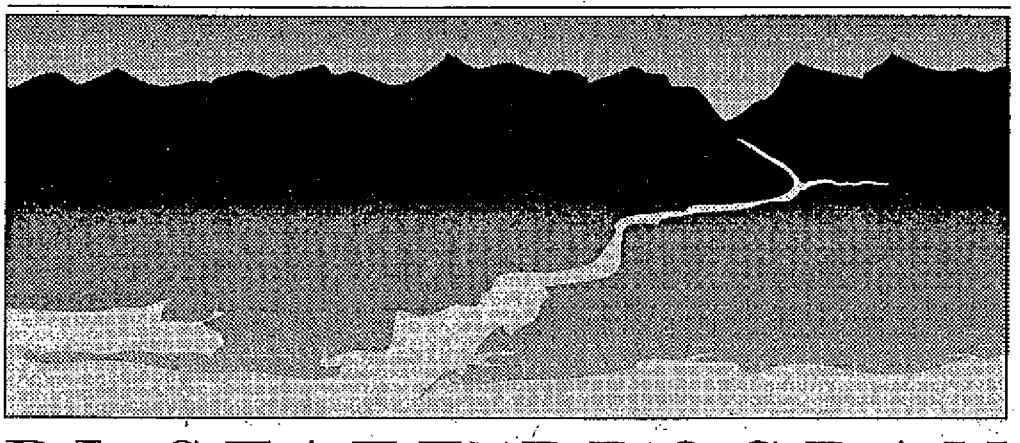
FINAL REPORT
TC 0110-01

LOWER COLUMBIA RIVER



BI-STATE PROGRAM

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

Sampling Plan

SEPTEMBER 16, 1994

Prepared By:
TETRA TECH

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The Lower Columbia River
Bi-State Water Quality Program

Prepared By:

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FIGURES

ACKNOWLEDGMENTS

This report was prepared by Tetra Tech, Inc. of Redmond, Washington for the Lower Columbia River Bi-State Water Quality Program under Contract Nos. C0091228 (WDOE) and 78-91 (ODEQ). The Project Coordinators for the Bi-State Program were Mr. Don Yon of Oregon Department of Environmental Quality and Mr. Brain Offord of Washington Department of Ecology. Tetra Tech's Project Manager for the Lower Columbia River Bi-State Water Quality Program was Dr. Steven Ellis. The authors of this report were Dr. Ellis, Mr. Tad Deshler, and Mr. Gary Braun. Word processing was provided by Ms. Lisa Fosse. Graphical support was provided by Ms. Kim Tapia.

1.0 INTRODUCTION

The Oregon and Washington state legislatures directed the formation of 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 lower Columbia River, identify water quality problems, determine whether beneficial uses of the river are impaired, and develop solutions to problems identified in the river below Bonneville Dam (Bi-State Steering Committee 1990). The four-year plan proposed a framework and precedence for conducting studies to evaluate water quality that consisted of: 1) inventory of existing information; 2) reconnaissance surveys; 3) further evaluation of water quality (baseline studies); and 4) advanced studies. Since the inception of the Bi-State Program, a number of studies have been completed, or are in progress, to help accomplish the legislative mandate for the Bi-State Program. These studies have attempted to characterize historical and current contaminant levels in water, sediment, and a small number of fish species and crayfish throughout the river; quantify the amount and 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. As the Bi-State Program approaches its final year of existence, attention has been focused on utilizing the information that has been assembled in earlier data inventory and reconnaissance studies to design and accomplish specific baseline studies (e.g., ambient monitoring of tributaries, localized contaminant investigations) and advanced studies that attempt to quantify, or characterize, the potential risks to fish, wildlife, and humans from habitat modification and contaminant levels in the lower Columbia River. This sampling plan describes a scope of work that will apply three biological assessment techniques to characterize the "health" of important fish indicator species and fish assemblages within the lower Columbia River.

1.1 HISTORICAL OVERVIEW

Prior to describing the study approach for this scope of work, it may be helpful to provide a historical overview of activities within the Bi-State Program that contributed to the development of the study

described in this document. 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 that would enable a preliminary assessment of water quality to be made and could be used to direct future studies (Tetra Tech 1993a). This survey, which represents 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 water, fish tissue, and sediment samples. After reviewing the information obtained in these initial studies, the Lower Columbia River Bi-State Program Steering Committee met on October 20, 1992 to review and prioritize future study objectives for the Program during 1993. The implementation of studies to assess potential impacts to fish and wildlife was ranked among the top four study objectives by the Bi-State Steering Committee members (Lower Columbia River Bi-State Program 1992). Subsequently, the Lower Columbia River Bi-State Program convened a work group to provide specific recommendations regarding how these fish and wildlife studies should be conducted to determine whether contaminant levels or habitat loss in the river may be affecting the health of resident fish and wildlife.

The Fish and Wildlife Work Group (FWWG) was convened by the Bi-State Committee to decide what studies might best address concerns relating to these beneficial uses and to provide the Committee with guidance on studies that might be conducted as part of the Bi-State Program. The work group proposed a long list of recommended fish and wildlife studies related to water quality on the lower Columbia River. To narrow the list of studies to those perceived to be the most critical, and those that have the greatest applicability to the objectives of the Bi-State Program, the fish and wildlife work group members ranked the studies in terms of short-term, mid-term, and long-term priorities. Based on the rankings, 10 studies were selected for consideration by the Bi-State Steering Committee. The study described in this sampling plan was among the ten studies recommended to the Bi-State Committee by the FWWG. Other studies recommended by the FWWG will be performed by other organizations and will be described elsewhere.

1.2 STUDY OBJECTIVES

Two main objectives have been established for this study. They 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 will be characterized by applying three biological assessment techniques. Biological assessment techniques are methods that may evaluate a biological community, population, or individual organism health. They provide an alternative or supplemental approach to evaluating affects based on chemical residues found in biological organisms. The three assessment techniques to be used for this project are:

- Fish community assessment technique based on the U.S. EPA Rapid Bioassessment Protocol V (RBP V)

- Autopsy-based fish health/condition assessment of largescale sucker

- Juvenile fish skeletal abnormality assessment.

Each of these methods will be described in detail in Section 2.0.

In addition to the studies listed above, National Marine Fisheries Service (NMFS) will be conducting another bioassessment technique in coordination with the Bi-State Program. This study entitled, *Assessment Of Exposure to Aromatic Compounds In Fish From The Lower Columbia River, By Use Of Appropriate Biomarkers*, will utilize largescale suckers collected as part of the autopsy-based fish health/condition component. The NMFS project will not be described further in this document.

2.0 TECHNICAL APPROACH

This section provides the technical approach for the fish health study to be conducted on the lower Columbia River. Section 2.1 provides a description and rationale for the general study design for the project. The following three sections (2.2-2.4) provide the study design details and the specific methodologies for each individual technique. Finally, Section 2.5 discusses the precautions that will be taken to prevent adverse impacts to endangered species of salmon.

2.1 GENERAL STUDY DESIGN

The study design and technical approach for the fish health study were developed through discussions with the FWWG, Bi-State Program Coordinators, and Tetra Tech. The Task Order for this study specified that three fish assessment techniques were to be employed (i.e., fish community assessment using the IBI, fish autopsy/condition assessment, and skeletal abnormalities) throughout the lower Columbia River. Sampling locations for each of these techniques will be divided into river segments and habitat types, as described below.

2.1.1 River Segments

For this fish health study, the 146 mile stretch of the lower Columbia River was subdivided into four major segments.

- 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)

Areas with similar flow and morphologic features were grouped into the same segment. 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). To be consistent with the earlier Bi-State Program studies, the same four major river segments will be used in this study.

The river will also be further subdivided into much smaller segments, based on a pilot project to define an optimal sampling distance for assessing fish communities (see Section 2.2.1). Segmentation of the entire river into standard units will allow selection of random sampling locations for each of the fish assessment techniques.

2.1.2 Major Habitat Types

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 type is important to fish assemblages on large rivers, the lower Columbia River was divided into three major habitat types. These include backwater areas, urban/industrial areas, and main channel areas. These three major habitat types are depicted in Figures 1-4. The backwater areas were identified previously during the selection of the 1993 backwater reconnaissance survey stations (Tetra Tech 1993c). The classification of riparian areas as urban/industrial was made 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 1992c). A standard distance of one mile downriver from a major point source was used to establish the boundaries around isolated point sources unless examination of areal photos indicated that a more extensive area was impacted. Areas not defined as backwater or urban/industrial are considered main channel habitat.

2.1.3 Study and Statistical Design Considerations

The study objectives will be addressed through a series of three independent measures of fish health on resident species collected from the lower Columbia River. These assessment measures include a fish community assessment using the Index of Biotic Integrity (IBI) (Plafkin et al. 1989; Hughes and Gammon 1987; Karr et al. 1986); the Fish Health/Condition Assessment Procedure (Goede 1993); and assessment

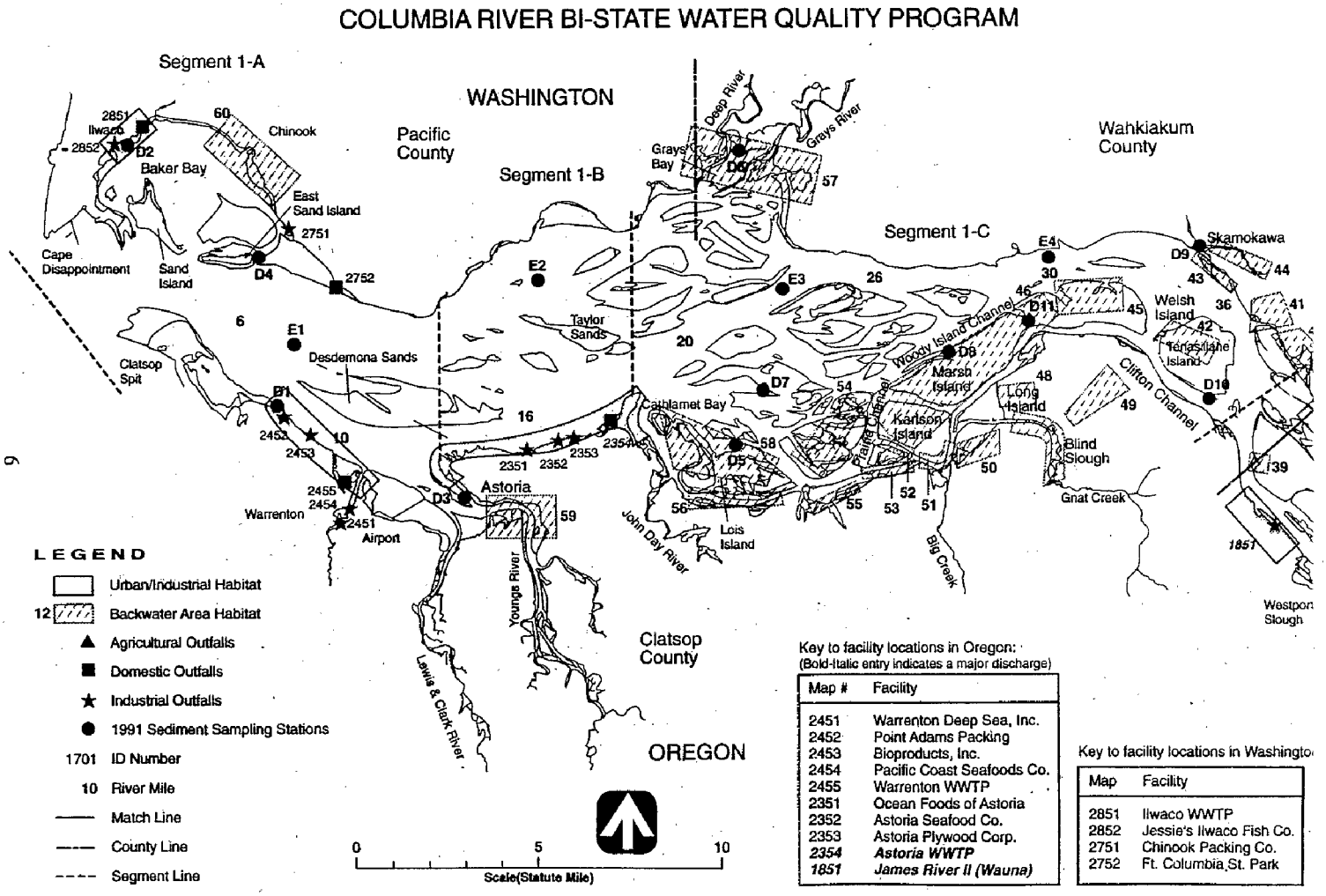
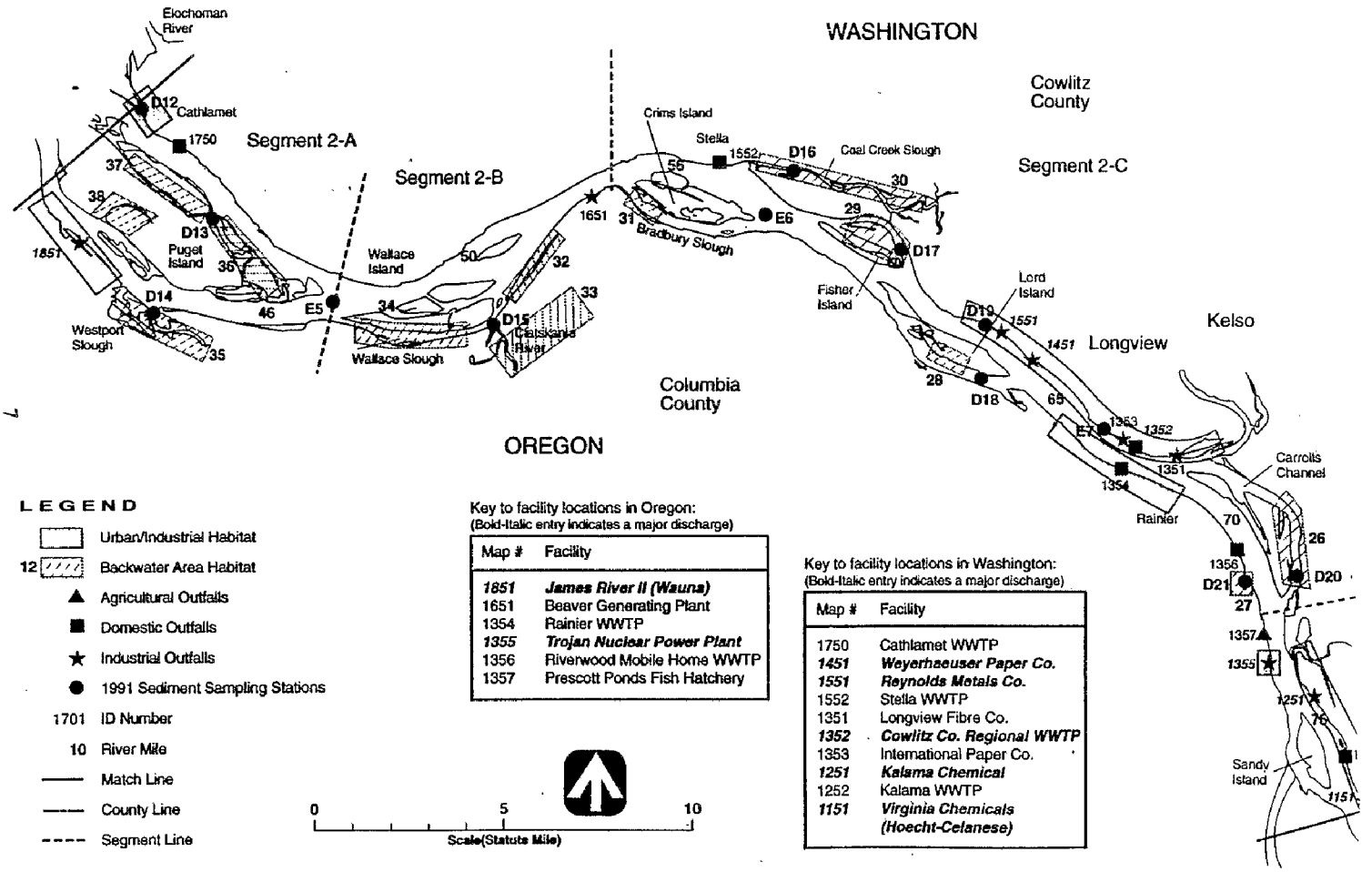


Figure 1. Habitat Classifications For Sampling Locations - River Segment 1.

COLUMBIA RIVER BI-STATE WATER QUALITY PROGRAM



LEGEND

- Urban/Industrial Habitat
- 12 Backwater Area Habitat
- Agricultural Outfalls
- Domestic Outfalls
- Industrial Outfalls
- 1991 Sediment Sampling Stations
- 1701 ID Number
- 10 River Mile
- Match Line
- County Line
- Segment Line

Key to facility locations in Oregon:
(Bold-Italic entry indicates a major discharge)

Map #	Facility
1851	***James River II (Wauna)***
1651	Beaver Generating Plant
1354	Rainier WWTP
1355	***Trojan Nuclear Power Plant***
1356	Riverwood Mobile Home WWTP
1357	Prescott Ponds Fish Hatchery

Key to facility locations in Washington:
(Bold-Italic entry indicates a major discharge)

Map #	Facility
1750	Cathlamet WWTP
1451	***Weyerhaeuser Paper Co.***
1551	***Reynolds Metals Co.***
1552	Stella WWTP
1351	Longview Fibre Co.
1352	***Cowlitz Co. Regional WWTP***
1353	International Paper Co.
1251	***Kalama Chemical***
1252	Kalama WWTP
1151	***Virginia Chemicals (Hoecht-Celanese)***

0 5 10
Scale(Statute Mile)

Figure 2. Habitat Classifications For Sampling Locations - River Segment 2.

Key to facility locations in Oregon:
(Bold-Italic entry indicates a major discharge)

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

Key to facility locations in Washington:
(Bold-Italic entry indicates a major discharge)

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 Malting
852	***Boise Cascade Corp.***
853	Ideal Basic Industries
752	***Vancouver (Eastside) WWTP***
951	***Salmon Creek WWTP***

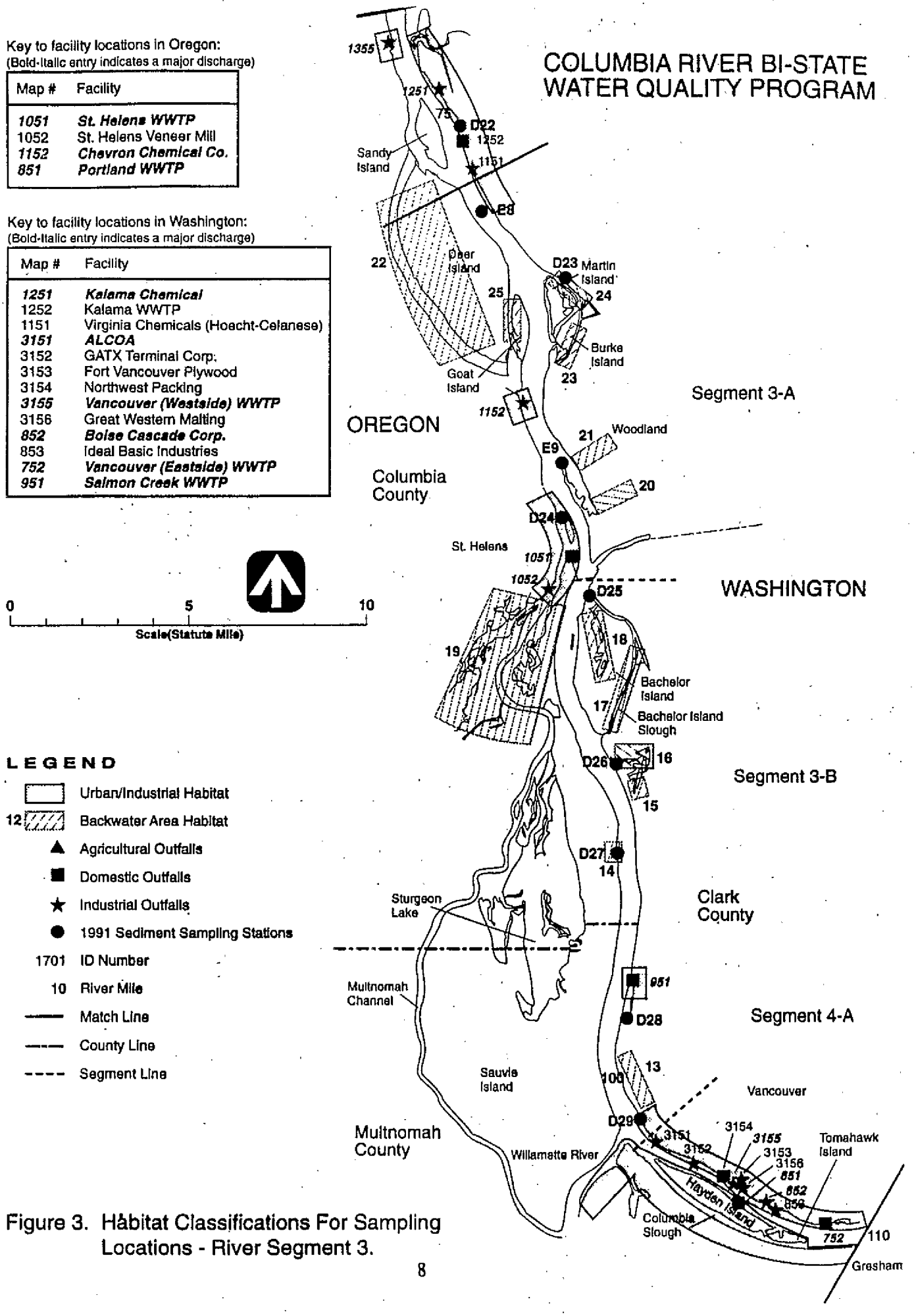
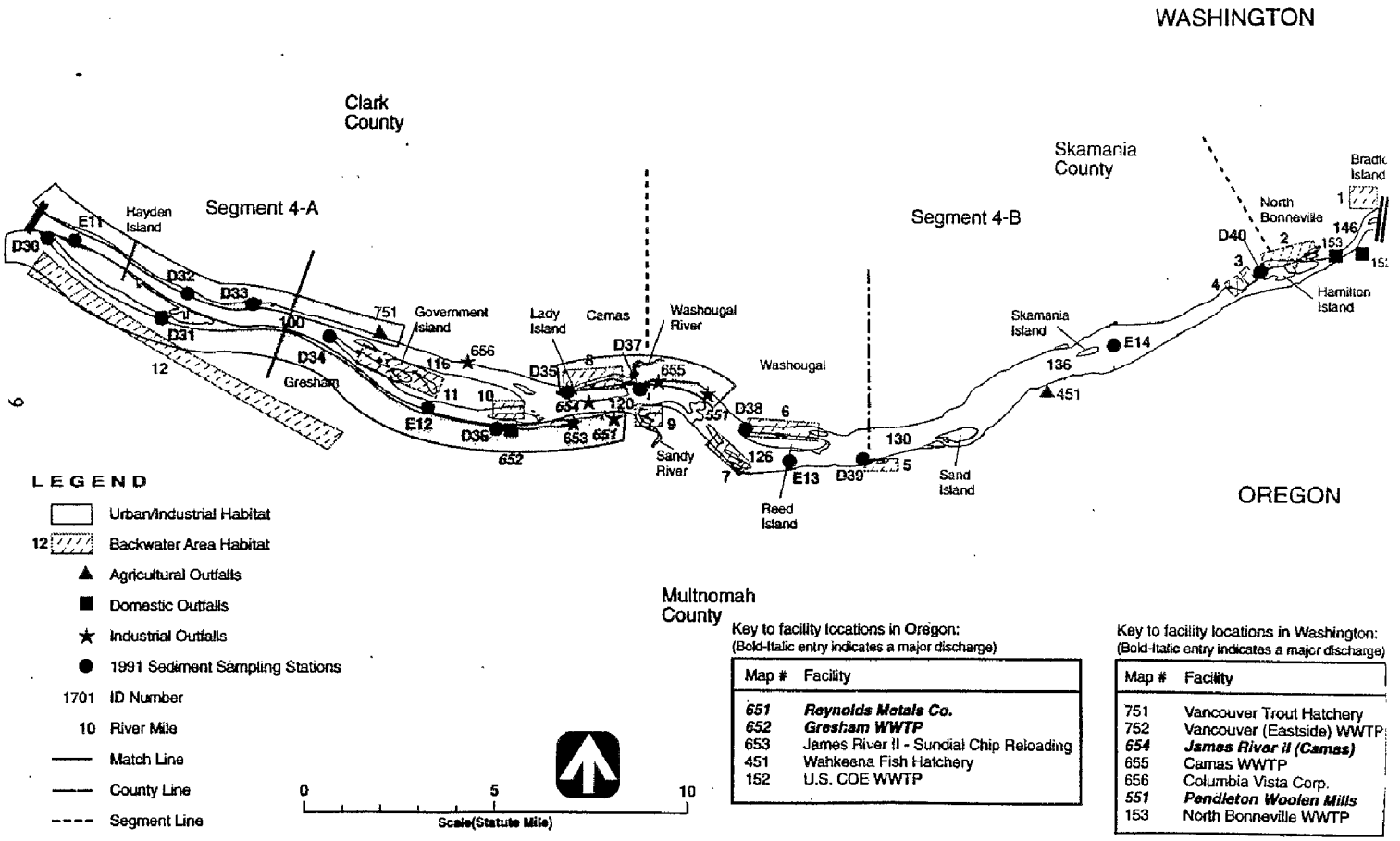


Figure 3. Habitat Classifications For Sampling Locations - River Segment 3.

COLUMBIA RIVER BI-STATE WATER QUALITY PROGRAM



Key to facility locations in Oregon:
(Bold-Italic entry indicates a major discharge)

Map #	Facility
651	***Reynolds Metals Co.***
652	***Gresham WWTP***
653	James River II - Sundial Chip Reloading
451	Wahkeena Fish Hatchery
152	U.S. COE WWTP

Key to facility locations in Washington:
(Bold-Italic entry indicates a major discharge)

Map #	Facility
751	Vancouver Trout Hatchery
752	Vancouver (Eastside) WWTP
654	***James River II (Camas)***
655	Camas WWTP
656	Columbia Vista Corp.
551	***Pendleton Woolen Mills***
153	North Bonneville WWTP

Figure 4. Habitat Classifications For Sampling Locations - River Segment 4.

of skeletal abnormalities in juvenile fish. The overall goal of the entire study is to assess the health/condition of the resident fish assemblages in the lower Columbia River. The study designs and assessment methodologies for each assessment technique are independent. However, the studies presented below have been designed such that they can be related to each other, within the constraints of the Task Order. Briefly, the main design features of the three studies include division of the lower river into four major segments and division of river habitats into three broad groups - backwater, urban/industrial, and main channel. The fish community assessment utilizes the major segments and the habitat types, resulting in a stratified random sampling design (stratified by both major river segment and habitat type). The fish health assessment utilizes the three habitat types throughout the river, resulting in a stratified random sampling design (stratified by habitat type). Finally, the juvenile skeletal abnormality assessment utilizes the four major river segments and a single habitat type (i.e., backwater), also resulting in a stratified random sampling design (stratified by river segment). Greater detail about the study design is given below in separate sections for each technique.

2.2 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 will be quantified and evaluated using a bioassessment technique based on the RBP V. The technique involves careful, standardized field collection, species identification and enumeration in the field, and community analyses using biological indices or quantification of the biomass and numbers of key species. The RBP V is based primarily 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). The IBI uses 13 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 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; however, 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 has not been used on a river the size of the lower Columbia River.

2.2.1 Pilot Study to Determine an Optimal Sampling Distance

As described in U.S. EPA's RBP V, typical sampling station lengths ranged from 100-200 m for small streams to 500-1,000 m in larger rivers. According to the RBP V, it is recommended that the size of a reference area should 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. A pilot study will be conducted to determine the optimal sampling distance for the lower Columbia River. This distance needs to be determined to ensure that each sample is representative of the fish population in that area.

The pilot project will be conducted at single location, most likely a backwater area where fish abundance is expected to be high. Initially, a 2-km long transect will be established by placing marker buoys at each end. Then, additional markers will be placed at 250 m intervals along the transect. After the marked transect is established, electroshocking will be conducted beginning at one end of the transect and proceeding for a 250 m. All individuals will be collected, identified, and enumerated. Data for that first segment will be recorded and maintained separately. Sampling will then continue to the second segment and so on until the entire transect has been surveyed. Results of the collections in each segment will be plotted. The cumulative distance at which the addition of new species approaches an asymptote will be defined as the standard transect distance and be used to subdivide the river. If the entire 2-km transect is not sufficient to reach an asymptote, this initial transect will be extended until that point is reached. Once a standard transect length is determined, that distance will be used to partition the entire river into small segments.

2.2.2 Stratified Random Sampling Design

A stratified random sampling design will be used to establish sampling locations for the fish community assessment (Gilbert 1987). The four major river segments discussed above will be used. Within each of these major segments, three habitat types have been identified, as discussed above (i.e., backwater, urban/industrial, and main channel) (Figures 1-4). Each small segment (i.e., the standard transect length) will be assigned a unique identifier based on the major segment and the habitat type. Three sampling locations will be randomly selected from each habitat type within each major segment using this classification. A total of 36 transects will be sampled for fish community characteristics throughout the

length of lower Columbia River; 12 in each of the three habitat types. Specific sampling locations will be determined after the standard transect length has been established by the pilot study. However, potential sampling locations are depicted in Figures 1-4.

This sampling design allows assessment of these hypotheses:

1. There are no differences among fish assemblages associated with different riparian habitats in the lower Columbia River.

2. There are no differences among fish assemblages associated with different major river segments in the lower Columbia River; segregated by habitat type and by pooled samples within a major segment.

2.2.3 Field Collection Methods and Laboratory Analyses

A total of 36 sampling transect locations will be randomly selected to meet the sampling design discussed above. Within each major segment, three samples from each of the three habitat types will be sampled. Each sampling site will be sampled once during the month of October 1994. The sample transect length will be determined by the pilot study, but is anticipated to be between 0.5 and 2.0 km.

A boat-mounted electroshocker (Model 7.5 GPP) will be used to collect the fish along each transect. The electroshocker is considered to be the most applicable gear for sampling fishes in large rivers because it is easily standardized and less selective than alternative gears. This method has also been used successfully on the Columbia (Hjort et al. 1981, Tetra Tech 1993a) and Willamette (Hughes and Gammon 1987; Tetra Tech 1993b, 1994a) Rivers. Therefore, fish will be sampled with a boat-mounted electroshocker that generates approximately 3 amps DC pulsed at 120 cycles/sec while moving downriver. The captured fish will be collected using dip nets with a mesh size of 1 cm and maintained alive. All captured fish will be identified to species in the field using the most current taxonomic keys (Page and Burr 1991, Wydoski and Whitney 1979, Oregon State University 1973). Individual fish whose identification is questionable or tentative, will be preserved in 10 percent buffered formalin and returned to the laboratory for positive identification. Individuals from each species will be separated into adults and juveniles based on size and coloration. Total numbers, weights, and lengths (total) of up to 30 individuals of each species and incidence of external anomalies will be recorded for each group (Plafkin

et al. 1989). It is anticipated that most fish will be identified in the field and returned to the river alive.

In addition to the fish collection efforts, two physical measurements, depth and current speed, will be made at each station at 100 m intervals along the transect. The position of each transect will be recorded using a GPS unit attached to the boat.

All, or almost all, of the data collected in support of the fish assemblage sampling will be collected in the field. Individuals that can not be positively identified will be returned to the laboratory. Thus, little laboratory analyses will be performed.

2.2.4 Quality Assurance/Quality Control (QA/QC) for Field and Laboratory

Prior to each electroshocking event, the electroshocker will be tested to ensure that it is generating the necessary amperage and working properly. The scale used for weighing fish samples will be calibrated daily. The most current taxonomic keys will be used for all identifications.

2.2.5 Data Analysis and Interpretation

The RBP V is based primarily on the Index of Biotic Integrity (IBI)(U.S. EPA 1989). This technique yields a discrete measure of the health of the fish community. Fish data will be analyzed through the use of the modified IBI (and modified IBI metrics), cluster analysis, detrended correspondence analysis, ANOVA, and a site-by-species table, as utilized by Hughes and Gammon (1987). The IBI incorporates zoogeographic, ecosystem, community, population, and individual organism perspectives. It has been modified by Hughes and Gammon (1987) for the Willamette River and has been used by Tetra Tech (1993b; 1994a) in two surveys of the Willamette River. The modified IBI included 7 of the 12 original metrics, four others that were modified based on guidance presented in Karr et al. (1986), and a 13th metric, total fish biomass that was added.

Calculation of the IBI requires that all species be assigned to a trophic guild. Trophic group assignments and tolerances will be assigned for the fish data collected for this project based on those assigned by Hughes and Gammon (1987).

Results of these metrics will be used to evaluate the overall health of the river. Comparisons of results from similar habitats throughout the lower river as well as comparisons within the major segments will

be performed using ANOVAs. Results of the analyses from the fish assemblage sampling will be evaluated with results from the other two sampling components to make an overall assessment of the river health.

2.3 FISH HEALTH ASSESSMENT

Fish health will be 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 on the Willamette River, and by Tetra Tech 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. This system was originally developed for salmonid fish, but it can be used for other fish species as well. In this survey, largescale sucker (*Catastomus macrocheilus*) will be examined. Salmonid species might be obtained in sufficient quantity on the lower Columbia River, but a worst case scenario is more likely to be obtained using a resident species. Largescale sucker was used in previous studies performed on the Willamette River (Tetra Tech 1993b; Haefle, R., personal communication). The data collected in these studies may serve as a benchmark with which to compare the data collected in the present study. The specific objectives of the study are to:

- Utilize an additional assessment technique to assess the health of lower Columbia River
- Collect additional data on fish health in the river and attempt to relate the results to potential contaminants of concern in the river.

2.3.1 Stratified Random Sampling Design

A stratified random sampling design similar to the fish assessment design will be used to determine sampling locations for the fish health assessment. The major difference in the two designs is that the river

will not be divided into four major segments. The target species (i.e., largescale sucker) is found throughout the length of the river and is not restricted to a particular habitat type. Thus, the sampling locations will be stratified by the three habitat types discussed above (i.e., backwater, urban/industrial, and main channel). Although a sampling design similar to that for the fish community sampling (i.e., river segmentation and habitat type) could have been proposed for this component as well, there are insufficient resources available to accomplish this goal.

As discussed above, the river will be segmented into sample transect lengths by habitat type. Five random sampling locations throughout the entire lower river will be selected from each of the habitat types. A total of 15 fish health assessment sampling locations will be assessed. This sampling design allows the testing of the hypothesis that there are no differences in the health of largescale suckers associated with three different riparian habitats in the lower Columbia River. In addition to the 15 primary sampling locations, at least 10 secondary sampling locations will be randomly selected to serve as backups in the event that the target species can not be obtained in sufficient numbers at the primary station.

Ideally, 20 individuals of the target species should be captured to conduct the assessment. Therefore a sampling location, although based on the transect length, will likely be expanded both up- and downriver from the original location, in order to obtain the number of fish needed to conduct the assessment.

2.3.2 Field Collection Methods and Laboratory Analyses

Largescale suckers to be used in the fish autopsy/condition assessment technique (20 fish at each sampling location) will be collected by electroshocking, as described in Section 2.2.3. Fish will be collected alive, and handled carefully until the time of the autopsies, at which time the fish are killed with a blow to the head from a wooden club.

The fish health assessment methods have been described in detail in Goede (1993, 1988) and the WRBWQS Field Sampling Plan (Tetra Tech 1992b). Field analysis of fish will include:

- Sampling of blood
- Length and weight measurements

- External observations (e.g., eyes, gills, pseudobranchs, thymus)

- Internal examination (e.g., mesenteric fat, spleen, kidney, liver).

Observations will be classified according to the autopsy classification scheme outlined in Attachment A. Data will be entered into the fish autopsy worksheet shown in Attachment B.

A slight deviation from the method protocols (Goede 1993) will be necessary. Cardiac puncture on the suckers will not be performed through the opercular cavity, as is commonly done with salmonids, but directly into the heart after the fish are opened ventrally. This will be done because of the difficulty encountered in penetrating the membrane in the opercular cavity with the microhematocrit tubes. Blood will be collected by cardiac puncture using a heparinized microhematocrit tube. Blood will be centrifuged, 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) can be easily measured on a plastic reader card containing a nomograph. The protein (weight/volume) content of the plasma will be determined using a hand-held clinical refractometer which has been zeroed with deionized water. All blood measurements will be taken no more than 2 hours after sample collection. This information will be recorded in the fish autopsy worksheets (Attachment B).

Length and weight measurements will be made immediately after blood samples have been collected. The total fish length will be determined in millimeters and the weight in grams. This information will be recorded in the fish autopsy worksheets.

External examinations will include general remarks about fins, skin, and other external features, as well as, observations of particular organs and systems. Important conditions to note are deformities, scale loss, fin condition, external parasites, etc. All observations relating to aesthetics will be included as remarks in the fish autopsy worksheet.

After external examination has been completed, fish will be cut with a scalpel ventrally from the anal vent forward around the pelvic girdle and on to the pectoral girdle. Care will be taken not to damage internal organs and tissues during opening. Once all of the fish have been opened, examination will begin keeping in mind the differences in the appearance of organs and tissue resulting from circumstances of collection

(e.g., method and time since collection, whether fish were collected live or dead).

Further external examination of "opened" fish will be done by checking for normalcy or abnormalcy (e.g., exopthalmia, hemorrhages, blindness, missing eyes, other). The gills will be examined and classified as normal, frayed, clubbed, marginate, pale, or other. Pseudobranchs will then be examined and classified as abnormal, swollen, lithic, swollen and lithic, inflamed, or other. The thymus will then be examined to determine whether there is no hemorrhage, mild hemorrhage, or severe hemorrhage. The classification system developed by Goede (1993) will be used to rank tissue condition (Attachment A). Observations will be coded according to this classification scheme and entered into the fish autopsy worksheets.

After this external examination of the fish has been completed, an internal examination of the fish will be conducted on the opened fish beginning from the mesenteric fat depot, through the spleen and hindgut, back up through the kidney, liver, and gall bladder, to the gonads for determination of gender and state of development. The mesentery tissue will be observed for hemorrhage and inflammation. Deviations from normalcy will be recorded as remarks. Mesenteric fat will be ranked from zero to four using Goede's (1993) ranking system developed for salmonids. The spleen will then be examined and classified as black, red, granular, nodular, enlarged, or other. The hindgut will be opened to examine the inner lining or mucosa. Contents of the hindgut will be lightly scraped out to observe the relative reddening or inflammation (e.g., none, slight, or severe). The appearance of the kidney will then be classified as normal, swollen, mottled, granular, urolithiasic, or other. The liver will be examined, keeping in mind the conditions of sampling which can affect the liver's appearance, and its color/condition will be noted. The bile will be observed indirectly by observing the color of the gall bladder. The ranking scheme considers fullness of the bladder and degree of green pigmentation of the bile inside. The sex of the fish shall be determined lastly and their spawning condition noted. Any other abnormal appearance will be noted and the mesenteric tissue in the visceral cavity will be checked for hemorrhage and inflammation.

All of the data collected in support of the Fish Health task will be collected in the field. There will not be any lab analyses performed.

2.3.3 QA/QC for Field and Laboratory Analyses

Most of the data collected will be descriptive in nature and will not be amenable to traditional QA/QC procedures. However, several steps will be taken in the field to ensure that measurement bias is

minimized. Since implementation of Goede's fish health/condition assessment requires some training and experience at fish autopsies, the same two-person team will be responsible for all data collection. Field methods for measuring blood parameters, length, weight, and other external and internal characteristics will be standardized prior to the cruise and followed consistently at each station.

Since 20 fish will be measured in one batch, care will be taken to assign a unique number to each fish in the batch. This is particularly important during the measurement of blood parameters, when the "sample" will no longer be attached to the fish. During centrifugation, each of the tubes will be placed in numbered slots in the microhematocrit centrifuge, while at other times, the tubes will be placed in a numbered position on the tube sealant tray. The external and internal examination of the fish will be done in the same order as the centrifugation, thus ensuring that all data from one fish are correctly attributed to that fish.

The only analytical equipment (other than the centrifuge) employed in the field is a refractometer. This instrument will be calibrated using deionized water.

2.3.4 Data Analysis and Interpretation

Information collected and recorded in the fish autopsy worksheets will be summarized according to Goede (1993). The mean, standard deviation, and coefficient of variation will be calculated for the length, weight, ktl (condition factor), hematocrit, leucocrit, and plasma protein. The percent of the total sample falling into each category for each classification parameter will be determined (e.g., number of fish with normal eyes divided by the total number of fish sampled). A summary of the normal organs and tissue for the appropriate categories will be recorded in the autopsy sheet as described in Goede (1993, 1988). The means will be summarized for categories in which the relative degree of normalcy was observed (e.g., thymus, mesenteric fat depot, hindgut, and bile). The relative proportion of gender will be noted and any general remarks will be recorded.

The computer program (AUSUM), developed by Goede and Houghton (1987), accompanying this assessment procedure provides a template for managing this database and facilitates standard reporting. All data from this study will be entered into a database using this computer program template.

Heading information will also be included in this database to permit future recall, manipulation, and

comparisons with similar data sets. This information will include location of study, quality control, species, strain, autopsy date, sample size, age, markings, water temperature, water body sampled, names of investigators, and any additional remarks.

In addition to the computer program AUSUM, which produces summary statistics that are not amenable to statistical tests of significance (e.g., ANOVA), the fish health/condition assessment data will be used to calculate a Health Assessment Index (HAI) for each station (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. In this way, an aggregate score can be calculated for each fish and a mean score can be calculated for each station. These mean HAIs and their associated variances can then be used in statistical tests such as ANOVAs to test the hypothesis that fish health is equal between the three habitat types.

2.4 JUVENILE FISH SKELETAL ABNORMALITY ASSESSMENT

Assessment of juvenile fish skeletal abnormalities is a third assessment technique that will be used to evaluate fish health in the lower Columbia River. The purpose of evaluating skeletal abnormalities in juvenile fish is to provide an additional independent measure of the health of fish communities in the river and to determine if differences in the incidence of abnormalities exist among the locations where this technique will be performed. 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 two recent studies of the Willamette River (Tetra Tech 1993b; 1994a).

2.4.1 Stratified Random Sampling Design

A stratified random sampling design will be used to establish sampling locations for the fish community assessment. The four major river segments discussed in Section 2.1.1 will be used to stratify the sampling locations for this technique. Within each of these major segments, three habitat types have been identified (i.e., backwater, urban/industrial, and main channel) (Figures 1-4). Only areas identified as backwater habitat will be targeted for sampling sites for the juvenile skeletal abnormality assessments. Because the juvenile skeletal abnormality technique has not been used on the Columbia River before, no data exist on where the juveniles will be found. Tetra Tech has selected backwater areas because of the

higher probability of finding appropriately-sized individuals of the target species due to low current speeds and higher food availability.

Juvenile largescale suckers are 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, we will collect multiple species and determine which species will be used for analysis after collection efforts are completed. This will be done because difficulties are expected in collecting appropriately-sized fish due to the timing of the sampling. Other possible species that may be used in the analyses include peamouth and northern squawfish.

Four sampling locations, based on the transect length identified in the pilot study, will be randomly selected from the backwater habitats identified in each major river segment. Juvenile fish will be collected at a total of 16 sampling locations throughout the river. This study design will allow the hypothesis that there are not differences in the number of skeletal abnormalities in juvenile fish (sucker/peamouth/squawfish) among different segments of the river to be tested. In addition to the 16 primary sampling locations, at least 12 secondary sampling locations will be randomly selected to serve as backups in the event that the juvenile fish can not be obtained in sufficient numbers at the primary station.

2.4.2 Field Collection Methods and Laboratory Analyses

Skeletal abnormalities in juvenile fish will be performed at 16 locations as discussed above. The position of each station will be recorded using a GPS unit attached to the boat. Juvenile fish will be collected by seining in shallow water areas. A 50-m beach seine (variable mesh size ranging from 9.5 to 19 mm) will be used to crowd schools of juvenile fish into shallow water for capture. The net will be staked on the shoreline and dragged through the water using a small boat, before being returned to the shoreline at a point upstream of the original point. At this time, the target species include largescale suckers, peamouth, and northern squawfish. Final selection of the species will be made after fish have been collected at all sites and an assessment of abundance has been made. This flexibility is incorporated due to the anticipated timing of the sampling during the month of October, when it may be difficult to capture appropriately-sized juvenile fish of the target species.

The net will be deployed one or two times at each station as necessary to capture approximately 200 juvenile fish (e.g., 8-12 cm in length). This limit on the level of effort at each station is necessary

because juvenile fish of the desired size range may not be abundant during the time of year proposed for sampling (October/November). After the fish are captured, they will be immediately fixed in 10-15 percent marble chip-buffered formalin and returned to the laboratory for further processing.

Once in the laboratory, the fish species will be selected and the clearing and staining process will begin. Clearing and staining techniques will follow the enzyme procedure of Potthoff (1984). First, fish are dehydrated in absolute ethanol, then the cartilage will be stained with alcian blue, followed by bleaching with hydrogen peroxide. The bone material will be stained with alizarin red and then destained with sodium borate and trypsin. Once the fish are cleared, they will be preserved in glycerin and thymol. (Note: If largescale suckers are selected as the species to analyze, each individual will have to be skinned prior to the clearing and staining process.)

After clearing and staining, the fish will be microscopically examined under low power (6.4X to 16X). The stains will render the cartilage a dark blue and the bone a brilliant red. The color contrast of these structures will allow for observations of the minutest detail. The juvenile fish skeletons are then examined for various skeletal abnormalities (e.g., deformation, closure failure of the hemal and neural arch, deformation of the centrum).

2.4.3 QA/QC for Field and Laboratory

The field collection methods are nonquantitative and therefore have no QA requirements. However, when collecting the juvenile fish, we will attempt to remain in the immediate vicinity of the randomly selected transect location. Clearing and staining procedures will strictly follow the methods specified by Potthoff (1984). Ten percent of the fish specimens examined will be re-examined to verify the accuracy of the initial assessment.

2.4.4 Data Analysis and Interpretation

The percent incidence of skeletal abnormalities will be reported for each site. The specific types, and the incidence of each one, will also be reported. Qualitative comparisons among the four stations within a major segment will be conducted and reported. Quantitative comparisons will also be made among the four major segments using ANOVAs. Based on the incidence of skeletal abnormality, an assessment of fish health at these sites will be made.

Results of the analyses from the skeletal abnormality sampling component will be evaluated along with results from the other ecological sampling components to make overall assessments of the river health and to evaluate the most efficient sampling techniques for evaluating the river.

2.5 ELECTROFISHING FIELD PROCEDURES TO PROTECT ENDANGERED SALMON SPECIES

During the time in which these studies will take place (October), threatened and endangered salmon species could be present in the study area. This section is intended to describe the precautions that will be taken during the electrofishing conducted for both the fish community assessment and fish autopsy/ condition assessment techniques.

The following characteristics of and modifications to the typical electrofishing procedures have been proposed to ensure that endangered salmon species are not adversely affected by electrofishing.

- Pulsed DC (direct current), rather than DC or AC (alternating current) will be used. Pulsed DC, at a frequency of 40-120 cycles/sec, has been shown to produce the least amount of physiological damage of any of the three current regimes (Smith 1989).

- Pulsed DC will be transmitted in only 10 second bursts, rather than continuously. In this manner, galvanotaxis, the tendency for a fish to be attracted to the anode, will be less likely to proceed to galvanonarcosis, whereby a fish is stunned by the current. Galvanotaxis rarely produces any permanent physiological damage, but does still allow fish to be captured (Smith 1989).

- During the fish community assessment, any fish that can be reached by the dipnets is typically brought aboard and measured. For this study, if a salmon is encountered, the fish will not be brought aboard using dipnets, but will instead simply be observed, taking note of the approximate size. This modification will not compromise the data collection efforts for the fish community assessment.

In addition to these field procedures, a fundamental characteristic of salmonid fish tissue, its high conductivity, makes it unlikely that endangered salmon species will be adversely affected. The maximum current, and hence the maximum galvanotaxis/galvanonarcosis effect, is applied to a fish whose conductivity closely matches the surrounding water (Smith 1989). The average conductivity of Columbia River water above the estuary is approximately 100 µMhos/cm (Tetra Tech 1993a), while the conductivity of freshwater fish ranges from about 500-1,500 µMhos/cm (Smith 1989). Salmonid fish are at the higher end of that range (approximately 1,250 µMhos/cm), making them harder to stun than many other freshwater fish.

3.0 DELIVERABLES AND SCHEDULE

Three products will be submitted as part of this Task Order:

- **Work/Sampling Plan.** A final work and sampling plan (this document) that identifies the rationale, study design, and QA/QC measures to be followed. The final will be submitted by September 19, 1994.

- **Lower Columbia River Fish Health Study Report.** This report will discuss the results of the analyses from each of the three assessment techniques described above. This report will provide a brief summary of the results and identify relevant data qualifiers if necessary. The latitude and longitude of each fish sampling site will also be reported. The report will summarize the study results in tabular and graphical form and compare the data among habitat types and major river segments. Based on these comparisons, an assessment of the potential adverse effects will be made. Where possible, the results of the study will be compared to existing and historical data on the lower Columbia River, including data from earlier reconnaissance surveys performed for the Bi-State Program (Tetra Tech 1993a, 1994b). Finally, suggestions about future sampling that should be performed will be made. The data from all three techniques will be evaluated together and an overall assessment of fish condition will be made. The draft report will be submitted by March 24, 1995. The draft report will be revised and a final will be submitted by April 24, 1995 or within two weeks of receiving consolidated comments from the Bi-State Program Contract Officers.

- **Fish Health Assessment Data - ARC INFO Format.** All appropriate fish health assessment data collected in the study with identified sampling location and other pertinent data will be saved in digital format suitable for input into the Arc Info system being developed by the Bi-State Program. The data will be submitted in electronic format by March 24, 1995.

4.0 STAFFING

The following key personnel will carry out this work assignment.

Name	Description	Activities
Dr. Steve Ellis	Project Manager	Supervision, financial issues, report preparation and review, technical support, and work group presentations
Mr. Tad Deshler	Field Team Leader	Autopsy-based fish health sampling, report preparation
Ms. Kim Stark	Field Team Leader	Fish community sampling, report preparation
Mr. Dick Miller Taxon Aquatic Monitoring	Field Team Leader	Skeletal abnormalities, report preparation
Ms. Lisa Fosse	Clerical	Word processing
Ms. Kim Tapia	Graphics	Illustration, presentation support

5.0 COST

The studies described in this plan will be performed under a fixed-price contract for a total of $147,500.

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ATTACHMENT A

AUTOPSY CLASSIFICATION SCHEME

Length: Total length in millimeters

Weight: Weight in grams

Ktl: $= \frac{W \times 10^5}{L^3}$

Eyes: Normal (N), Exopthalmia (E1, E2), Hemorrhagic (H1, H2), Blind (B1, B2), Missing (M1, M2), Other (OT)

Gills: Normal (N), Frayed (F), Clubbed (C), Marginate (M), Pale (P), Other (OT)

Pseudobranch: Normal (N), Swollen (S), Lithic (L), Swollen and Lithic (S&L), Inflamed (I), Other (OT)

Thymus: No Hemorrhage (0), Mild Hemorrhage (1), Severe Hemorrhage (2)

Fins: No active erosion or previous erosion healed over (0), Mild active erosion with no bleeding (1), Severe active erosion with hemorrhage and/or secondary infection (2)

Opercles: No shortening (0), Mild shortening (1), Severe shortening (2)

Mesentery Fat: Internal body fat expressed with regard to amount present:

0 - None
1 - Little, where less than 50% of each cecum is covered
2 - 50% of each cecum is covered
3 - More than 50% of each cecum is covered
4 - Ceca are completely covered by large amount of fat

Spleen: Black (B), Red (R), Granular (G), Nodular (NO), Enlarged (E), Other (OT)

Hind Gut: No inflammation (0), Mild inflammation (1), Severe inflammation (2)

Kidney: Normal (N), Swollen (S), Mottled (M), Granular (G), Urolithic (U), Other (OT)

Liver: Red (A), Light red (B), "Fatty" liver; "coffee with cream" color (C), Nodules in liver (D), Focal discoloration (E), General discoloration (F), Other (OT)

Bile:

0 - Yellow or straw color; bladder empty or partially full
1 - Yellow or straw color; bladder full, distended
2 - Light green to "grass" green
3 - Dark green to dark blue-green

Blood:

Hematocrit - Volume of red blood cells (erythrocytes) expressed as percent of total blood volume. Centrifuged 5 minutes.

Leucocrit - Volume of white blood cells (leucocytes) expressed as percent of total blood volume. "Buffy" zone of the packed cell column.

Plasma Protein - Amount of protein plasma, expressed as gram percent (grams per 100 ml).

ATTACHMENT B

FISH AUTOPSY WORKSHEET

FISH AUTOPSIES

Wildlife Resources
2/91 FES-25

Date _____ Unit _____ Strain _____ Quality Control # _____
Location _____ Fish Source _____ Age _____ Case History # _____
Investigator(s) _____ Hatch Date _____ Tissue Collection # _____
Reason for Autopsy _____ Remarks _____

Smp no	Lgth mm	Wght gm	Kid	Eye	Gill	Pebr	Thy	Fat	Spl	Hind Gut	Kid	Liv	Bile	Sex	Hem	Leu	Pl. Pro	Fin	Opl	Remarks
1																				
2																				
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GENERAL REMARKS

Fins _____ Gonads _____

Skin _____ Other _____