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SURVIVAL OF PINK SALMON (ONCORHYNCHUS GORBUSCHA) TO ADULT FOLLOWING 10-D EXPOSURE OF FRY TO THE WATER SOLUBLE FRACTION OF NORTH SLOPE CRUDE OIL

by

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PREFACE

The potential for accidental release of large quantities of crude oil into the nearshore marine environment off the British Columbian coast, Canada, prompted an examination of the potential consequences of such events to local ecosystems. The Department of Fisheries and Oceans, with funding from the Panel on Energy Research and Development, undertook this investigation to assess the long term influence on pink salmon fry of a short term (10 d) exposure to the water soluble fraction of crude oil. The crude oil exposure period and dose represented concentrations and durations of exposure to be expected in semi-protected waters following an offshore oil well blowout.

Pink salmon fry from the Quinsam River Salmon Hatchery near Campbell River, B.C. were selected because of their economic status, the known sensitivity of this species to oil, and their importance in nearshore food webs. The field component of the work was conducted between 1990 and 1992.

The study examined the effect of exposure of pink salmon fry to the water soluble fraction of crude oil and their long term ocean survival to harvest and escapement . In addition, the influence of oil exposure on growth, hydrocarbon avoidance, and liver, kidney, and gill cellular morphology, was assessed during the exposure period. The following report is one of a series which describe the results of this investigation.

TABLE OF CONTENTS

ABSTRACT

Birtwell, I.K., R. Fink, R. Alexander, W. Bengeyfield, and C.D. McAllister. 1996. Survival of pink salmon (Oncorhynchus gorbuscha) to adult following 10-d exposure of fry to the water soluble fraction of North Slope crude oil. Can. Tech. Rep. Fish. Aquat. Sci. 2095: 49 p.

Salt water acclimated, coded-wire tagged and adipose-clipped Quinsam River pink salmon fry (Oncorhynchus gorbuscha) were exposed to 25-54 or 178-349 $\mu g \cdot L^{-1}$ of the water soluble fraction (WSF) of North Slope crude oil. Approximately 30,000 fry in each control, low dose and high dose treatment group were exposed for 10 d then released to Discovery Passage, British Columbia, to complete their two year life cycle in the Pacific Ocean. The experiment was replicated three times in consecutive years (1990, 1991, and 1992). Adipose-clipped adult pink salmon were captured in the commercial fishery, and in their natal Quinsam River. Subsequently their tag codes were analyzed and assigned to the appropriate hydrocarbon treatment or control group.

Although exposure of pink salmon fry to the water soluble fraction of crude oil resulted in significant effects on short- and long-term growth, hydrocarbon avoidance, and on the cellular morphology of liver, kidney, and gill tissue, it did not result in detectable differential mortality to adulthood.

The number of adult pink salmon with legible coded-wire tags recovered from fisheries or the natal stream were 169, 317, and 172. The total number of adult pink salmon recovered was 303, 295, and 293 for the control, low and high dose groups respectively.

To our knowledge this investigation represents the first replicated oil-life cycle study with a species of Pacific salmon.

Key words: crude oil, water soluble fraction, toxicity, pink salmon, life cycle, growth, ocean survival.

RÉSUMÉ

Birtwell, I.K., R. Fink, R. Alexander, W. Bengeyfield, and C.D. McAllister. 1996. Survival of pink salmon (Oncorhynchus gorbuscha) to adult following 10-d exposure of fry to the water soluble fraction of North Slope crude oil. Can. Tech. Rep. Fish. Aquat. Sci. 2095: 49 p.

Des alevins de saumon rose (Oncorhynchus gorbuscha) de la rivière Quinsam acclimatés à l'eau salée après implantation d'une micromarque codée et ablation de la nageoire adipeuse ont été exposés à des concentrations hydrosolubles de 25-24 ou 178-349 $\mu g\text{-}L^{-1}$ de pétrole brut North Slope. Après une période d'exposition de dix jours, environ 30 000 alevins faisant partie de chacun des groupes de traitement témoins, faible dose et forte dose, ont été relâchés dans le passage Discovery (Colombie-Britannique), pur compléter leur cycle biologique de deux ans dans l'océan Pacifique. L'expérience a été répétée pendant trois années consécutives (1990, 1991 et 1992). Des saumons adultes ayant subi l'ablation de la nageoire adipeuse ont été capturés au cours des pêches commerciales et dans leur cours d'eau d'origine, la rivière Quinsam. Par la suite leurs micromarques codées ont été analysées au groupe approprié, soit le groupe soumis aux hydrocarbures ou le groupe témoin.

Bien que l'exposition des alevins de saumon rose à des concentrations hydrosolubles de pétrole brut ait eu des effets importants à la fois sur la croissance à long terme, l'évitement d'hydrocarbures et la morphologie cellulaire des tissus de foie, des reins et des branchies, on n'a décelé aucune différence dans le taux de mortalité avant l'âge adulte.

Le nombre d'adultes portant des micromarques codées lisibles qui ont été pêchés ou qui ont réussi à revenir dans leur cours d'eau d'origine était de 169, 317 et 172 respectivement pour les années de récupération. Le nombre total de saumons roses adultes récupérés était de 303, 295 et 293 respectivement pour les groupes témoins, faible dose et forte dose.

A notre connaissance, cette investigation est la première étude répétée pétrole-cycle biologique chez une espèce de saumon du Pacifique.

Mots clés: pétrole brut, fraction hydrosoluble, toxicité, saumon rose, cycle biologique, croissance, survie en mer.

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

In 1989, the subject of oil spills in the coastal waters of British Columbia received increased attention as a result of two accidental spills, the *Nestucca* bunker fuel spill off Grays Harbour, Washington, (Duval et al. 1989), and the much larger *Exxon Valdez* crude oil spill in Prince William Sound, Alaska in 1989 (Galt et al. 1991). In many respects the *Exxon Valdez* spill demonstrated the inadequacy of oil containment and recovery operations under natural conditions. Despite a massive cleanup effort mounted several days after the spill, using the best technology available at the time, and with very significant short term funding, the greater part of the spilled oil was never recovered. During the initial three days after the spill when calm conditions prevailed, only 3000 barrels of the estimated 250,000 barrels spilled were actually recovered (Kelso and Kendziorek, 1991).

Widespread contamination from the *Exxon Valdez* was followed by the loss of 100,000-300,000 seabirds and several thousand marine mammals (Kelso and Kendziorek, 1991). Approximately 1200 km of shore were oiled (Maki, 1991) with full removal of oil from some areas expected to take one or more decades (Kelso and Kendziorek, 1991). Oil contamination of intertidal spawning beds was still affecting pink salmon and Pacific herring populations three years after the spill (Schneider, 1993).

In response to the continued presence of oil tanker traffic along the British Columbian coast, together with the possibility of future oil and gas exploration and production (Chevron 1982) several government initiatives were undertaken to examine the potential consequences to Pacific marine ecosystems following a major marine oil spill (FEARO 1986; States/BC Task Force, 1990). The Canadian Department of Fisheries and Oceans (DFO) recommended two areas of research including "(1) toxicological studies with juvenile salmon and herring in a stratified water column; and (2) research on the survival of oil-exposed salmonids released to the marine environment using mark-recapture techniques" (cited in Duval et al. 1990). Both these research proposals were submitted to the Panel on Energy Research and Development (PERD) Committee 6.7 for funding. This investigation was conducted in response to the second of these priority research areas.

The primary objective of the study was to determine whether exposure of saltwater acclimated pink salmon fry to sublethal concentrations of dissolved petroleum hydrocarbons had a significant effect on their subsequent ocean survival to adult (Figure 1). The experimental protocol required simultaneous 10-d exposures of three treatment groups: a control, a low hydrocarbon concentration (low dose), and a higher hydrocarbon concentration (high dose). The 10-d exposure regime was chosen after consultation with the oil and gas industry regarding the duration of oil spill events from exploration and production facilities.

The hydrocarbon concentrations were selected to be in the sublethal range for juvenile pink salmon based on previous toxicological work by Moles et al. (1987) and ourselves, and also to be within the range of anticipated concentrations encountered after spill events: Short and Rounds (in press), indicated that dissolved aromatic hydrocarbons from the *Exxon Valdez*

were present in the upper water column for 1-2 weeks after the spill while particulate oil was most prevalent off oiled beaches for 1-2 months.

Logistical constraints necessitated replicate experiments conducted over three consecutive years. About 30,000 fry were coded-wire tagged for each treatment group in each year. A total of 268,680 pink salmon fry were tagged, adipose-clipped for subsequent separation from other adult pink salmon captured in the commercial fishery or their natal stream, exposed to crude oil (or remained untreated as controls), and released for long term survival assessment. Each year, the acute toxicity of the water soluble fraction of the crude oil was determined using a flow-through bioassay technique. The influence of hydrocarbon exposure on short term growth was determined using measurements of length and weight before and after treatment.

Secondary study objectives provided information on the sublethal effects of hydrocarbon exposure on pink salmon fry, and included effects on gill, liver, and kidney tissue (Brand et al. 1996) and the influence of hydrocarbon pre-exposure on subsequent hydrocarbon avoidance responses (Department of Fisheries and Oceans: unpublished data).

Detailed methodology and tabular summaries of results for water quality, fish husbandry, coded-wire tagging, hydrocarbon concentrations and acute lethal bioassays, have been reported separately (Fink et al. 1995), and only brief mention will be provided here.

Selection of Test Organism

The choice of pink salmon as the test species for the study was based on a number of biological, economic and logistical considerations. In the early part of their life pink salmon are biased towards the surface of the water and frequently occupy shallow (often less than 20 cm depth) nearshore habitats. This seemingly obligatory behaviour would facilitate exposure to spilled oil on the water surface with the potential for detrimental effects. This species is ecologically important in a range of nearshore Pacific marine communities (Heard 1991; Groot and Margolis 1991) and, consequently, any serious effects on individuals could lead to effects at the ecosystem level. This, in turn could have economic consequences for pink salmon are important to the Pacific coastal fisheries of Washington, Alaska, and British Columbia.

This species fulfils many of the recommended criteria outlined by Cairns (1988) for predicting ecological consequences based on laboratory experiments with a single species. From a toxicological perspective, pink salmon are as sensitive to crude oil as many other marine species, and the newly-emerged saltwater-acclimated fry are the particularly sensitive life cycle stage (Moles et al. 1979, 1987). While their small size at emergence and rapid migration to sea was conducive to handling and exposing high numbers of individuals to oil (i.e. low loading densities in the holding and exposure facilities were possible), the mandatory invasive tagging (half-length coded-wire) of such small fish was a potential

problem which we overcame through the use of technology previously only employed in Alaska.

Other species of salmon were considered for the study, but practical considerations favoured the Quinsam River pink salmon stock which is one of the most important in British Columbia supporting an annual harvest of 80,000-500,000 adults from a release of 1-3.5 million fry (1983-89; Department of Fisheries and Oceans, unpublished data). This stock of salmon is noted for its health, and is supplied as eyed eggs to other hatcheries along the south coast of British Columbia (Jim Van Tine, Department of Fisheries and Oceans, pers. comm.). A major factor in the selection of the species was their comparatively short life span which permitted three experimental releases and the return of adults within 4 years. Other Pacific salmon species would have required an additional 2-4 years or more for the return of adults.

Study Area

This investigation was conducted near the town of Campbell River, Vancouver Island, towards the north end of the Strait of Georgia, British Columbia, Canada (Figure 2). Juvenile pink salmon fry were obtained, at emergence, from the Department of Fisheries and Oceans' Quinsam River salmon hatchery. A field laboratory and saltwater rearing facility for the research was constructed each year on the seaward side of Tyee Spit near the mouth of the Campbell River (Campbell River field location courtesy of Westmin Resources Ltd., Myra Falls Division). This location permitted transport of fry from the freshwater hatchery to saltwater rearing troughs within 15-20 minutes. Strong tidal action in the adjacent Discovery Passage provided cool and well mixed sea water which was pumped to the field laboratory.

MATERIALS AND METHODS

Experimental Seawater System

The sea water experimental system outlined in Figure 3, consisted of six main components, 1) a pumping system, a sea water air equilibration tower (2) and distribution system (3), three fish holding troughs (4), a dissolved hydrocarbon preparation system (5) and a flow-through bioassay apparatus (6).

Sea water was pumped from a submerged, screened intake located approximately 2.5 m above the sea floor. Two 1/4 HP Franklin single stage stainless steel well pumps in series delivered 225 L $\cdot min^{-1}$ sea water, at high tide, to a 2 m x 15 cm PVC air equilibration column filled with plastic rings. In 1992 and 1993, a 1/2 HP pool pump replaced one of the two well pumps. Air equilibrated sea water (9.8 - 10.4 mg$\cdot L^{-1}$ dissolved oxygen at 28 - 30 ‰ salinity) was fed into a covered 200 L polyethylene header tank and from there

distributed by gravity to the fish holding troughs, dissolved hydrocarbon preparation system, and the flow-through bioassay apparatus.

Each of three $2m^3$ capacity "Capilano" holding troughs received 25 $L \cdot min^{-1}$ sea water. To prevent fish from escaping, the outflow from each trough was screened with fine nylon mesh (1.5mm) held in plywood frames. Black nylon mesh covers were placed over each trough to reduce direct sunlight and provide cover for the fish. Sea water depth (60 cm) in the troughs was regulated by externally adjustable standpipes and the freeboard was 10 cm (Figure 3). According to calculations provided by Sprague (1969), the 90% replacement time for these troughs was 4 h. The holding troughs, bioassay apparatus and hydrocarbon preparation system were housed in a temporary wooden structure glazed with 6mm U.V. grade polyethylene film.

Coded-wire Tagging of Pink Fry

Fry were anaesthetized with 2-phenoxyethanol, their adipose fins clipped, and then tagged with half-length binary-coded wire tags using a Northwest Marine Technology Ltd. Mark IV tagging machine. A monocular hand lens was used to monitor tag location. A 24 - 48 h evaluation of tag loss was made by passing 150 fish through a North West Marine Technology quality control device. Similarly, overall tag retention at release (27-29 d) was determined for each treatment group. The 1990 and 1991 tagging programs were conducted by LGL Environmental Research Associates Ltd. Sidney B.C., and the 1992 tagging program was conducted by Axys Environmental Sciences Ltd, Sidney B.C.

This experimental program represented the first large scale tagging program of pink salmon fry in Canada, and was made possible through the provision of technical expertise (S. G. Taylor) from the National Marine Fisheries Service, Auke Bay Laboratory in Juneau, Alaska, and logistical support from the Quinsam River salmon hatchery near Campbell River, B.C.

Transport and Acclimation of Pink Salmon Fry to Sea Water

At the end of each day of tagging, pink salmon fry (pre-assigned by code number to one of three treatment groups) were transported from the hatchery to the experimental facility in 60 L polyethylene containers filled with fresh water: fry loading density ranged from 10 - 20 $g \cdot L^{-1}$. Two battery powered pumps continuously introduced air into the water in the containers during the 15-20 min transport period. Upon arrival at the field laboratory, sea water (28 - 30 ‰) from the experimental trough into which the fish were to be placed was siphoned at 6 $L \cdot min^{-1}$ into each container to progressively replace the fresh water. Full exchange of fresh water with sea water was accomplished within 15-30 min after which the fry were poured directly into the appropriate holding trough.

Fish Husbandry

Our objectives were to maintain the overall health of the fry, and to reduce factors that might interfere with experimental results. Accordingly, fry were fed 3-4% of their body weight per day, an amount sufficient to produce moderate growth, while preserving acceptable dissolved oxygen levels within the holding troughs. On each day of tagging, new batches of fish were introduced to the troughs after the final evening feeding and were not fed until the following morning. Fry delivered from the hatchery during the first 5 d of tagging were fed frozen Biodiet Starter Mash #1 (Bioproducts Inc, Warrenton, Oregon). Biodiet Starter Mash #2 was used for the remainder of the experimental period. Sea water delivered to the holding troughs was screened through multiple layers of 1 cm welded nylon fish net, but otherwise unfiltered. Therefore a small amount of natural food was always available in each of the holding troughs. The sides and bottoms of each trough were siphoned after the last feeding each day to remove uneaten food and feces. Any fry mortalities were recorded and the carcasses removed from each trough twice daily.

Fish loading densities in the holding troughs varied from 0.25 $kg \cdot L^{-1} \cdot min^{-1}$ at the end of the tagging period to 0.42 $kg \cdot L^{-1} \cdot min^{-1}$ at the end of the exposure period. This change in loading density reflected the growth of the fish during the experimental period.

Water Quality Monitoring

Sea water temperature, dissolved oxygen and salinity were monitored twice daily throughout the holding and hydrocarbon exposure periods. Dissolved oxygen was measured to the nearest 0.1 $mg \cdot L^{-1}$ with a portable YSI Model 57 (Yellow Springs Instruments Ltd.), calibrated against air saturated sea water, while temperature and salinity were measured to the nearest 0.1 ^{o}C and ‰ respectively with a custom-made recording oceanographic probe (Axys Group, Sydney B.C.).

Production of Crude Oil Water Soluble Fraction (WSF)

The glass extraction column used to provide the water soluble fraction (WSF) of crude oil in this investigation was modified from a similar device described by Moles et al. (1985) and used for several years in toxicological studies conducted at the National Marine Fisheries Service Laboratory in Auke Bay, Alaska. The critical element of this extraction column was a pipette plate situated above a constantly replenished floating oil layer, which dispersed a continual "rain" of small sea water droplets onto the oil (Figure 4). The droplets, having a higher specific gravity, sank through the oil layer dissolving low molecular weight aromatic hydrocarbons in the process. Subsequently, these hydrocarbon-carrying droplets exited the oil/water interface and coalesced to form a particulate-free water soluble fraction which flowed by gravity through a glass column and tubing to a hydrocarbon header tank.

Fresh North Slope crude oil (donated by Atlantic Richfield Co., Ferndale WA) was pumped to the WSF extraction column oil layer at the rate of 4-6 ml•min^{-1}. The crude oil from which water soluble components had been extracted, was automatically siphoned from the bottom of this layer to a waste storage container for subsequent disposal (Figure 4). The flow of sea water through the extraction column was 2.2 L •min^{-1}. Although several different mechanical systems were tested initially, gravity siphons were chosen to distribute WSF from the hydrocarbon header tank to the holding troughs because of their simplicity and reliability.

Each year, the glass column, associated tubing and hydrocarbon header tank were cleaned on exposure Day 5 to remove the coating of hydrocarbon tolerant bacteria. The process required 1-3 h, during which time hydrocarbon input was interrupted. In 1991 and 1992, a slowly rotating (60 r.p.m.) stirrer was positioned in the column below the oil/water interface in order to add a small amount of energy to the system which helped stabilize the interface and prevented entrainment of whole oil into the WSF stream.

Dissolved Hydrocarbon Determinations

Water samples for dissolved hydrocarbon analyses were collected in new 40-mL glass vials with screw caps and Teflon seals. Vials were inverted at mid-depth in each of the troughs and brought to the surface. A single crystal of mercuric chloride was added as a preservative to prevent biological activity. The lid was then screwed in place so that all air bubbles were excluded. Each vial was labelled for identification and sealed with black vinyl tape. Samples were stored in small Thermos® brand coolers at 4 $\pm$ 2 ^{o}C. Samples were shipped by air to AGAT Laboratories (Calgary, Alberta). Analyses were initiated less than 48 h after sample collection, using a modified EPA 602 method for purgable aromatic hydrocarbons (GC-Mass Spectrometry: EPA, 1984).

Water samples were collected daily from the two hydrocarbon exposure troughs, control trough and the hydrocarbon header tank. Additional samples were periodically collected to characterize the nature and stability of WSF concentrations in the exposure troughs and to verify the low hydrocarbon concentration in the effluent discharge.

Procedures employed during the 1990 experiment did not adequately characterize the WSF produced in the extraction system. An assessment of hydrocarbon concentrations to which fry were exposed in this year was deduced from post-experimental analyses. The oil extraction apparatus was reassembled at the West Vancouver Laboratory (Department of Fisheries and Oceans) to determine the production of dissolved hydrocarbons in relation to sea water flow rate. From these data we made a retrospective estimate of hydrocarbon concentrations used in the 1990 experiment.

Acute Toxicity of WSF to Pink Salmon Fry

Acute toxicity of the WSF was determined according to methods described by Litchfield and Wilcoxon (1949) and Sprague (1969). To assess the acute toxicity of the crude oil WSF, 96-h flow-through bioassays were conducted prior to the main hydrocarbon exposure experiment. Four-litre glass jars were used as aquaria and quick-connect control valves were assembled to provide proportional sea water and dissolved hydrocarbon flow. Flow rates were checked three times daily. Each treatment jar received 0.5 L $\cdot min^{-1}$ of air-saturated sea water and a specific concentration of WSF. Eighty fish were randomly selected from the three holding troughs and equally distributed among eight 4 L glass jars. Fish loading density at 10 fish per container was 0.55 $g \cdot L^{-1}$. The 90% replacement time for water in the bioassay aquaria was 0.4 h (Sprague 1969). Dissolved hydrocarbons were metered to the jars as proportional dilutions. Fry were not fed during the bioassay, although in 1992 food was presented to the bioassay survivors, to determine whether feeding activity would recommence following near lethal exposures.

Ten Day Exposure of Pink Salmon Fry to Crude Oil WSF

Pink salmon fry received one of 2 hydrocarbon doses commencing at noon on the first day of exposure; a high dose (178-349 $\mu g \cdot L^{-1}$), a low dose (25-54 $\mu g \cdot L^{-1}$). Minute levels of dissolved hydrocarbons reported in several sea water control tank samples (0-6 $\mu g \cdot L^{-1}$) may represent sampling or analytical error (practical quantitation limit of 0.1$\mu g \cdot L^{-1}$), or perhaps low background hydrocarbon levels from nearby marine industrial and recreational centers. Hydrocarbon exposures were maintained for just over 10 d.

Short-term Growth

We anticipated that the brief experimental exposure periods (10 d) would not result in detectable differences in growth between the treatment groups, but data obtained in 1990 prior to release of the fry suggested otherwise. Five random samples of over 100 fry were removed by net from each trough. Surplus water was absorbed through the net's mesh with tissue paper, and the samples were placed in pre-weighed polyethylene bags. The bags were weighed to the nearest 0.1 g, and the mean weight of individuals in each bag calculated. Mean standard length of fry from each sample was determined by measuring 10 randomly chosen fish from each bag. These preliminary measurements suggested the potential for short-term growth differences between the treatment groups, and a more rigorous protocol was adopted for the subsequent two years. Immediately prior to the release of the fry in 1991 and 1992, several random samples of fish taken from each trough were combined in one of three 60L plastic containers until at least 300 fish from each trough were obtained. These fry were maintained in running sea water for 8-12 h, after which time they were anaesthetized with tricane-methane-sulfonate (MS 222). Anaesthetized fry were placed on, and covered with, wet paper towels. Standard length (nose to hypural plate) was measured ($\pm$1mm) and the fry were immediately transferred to a tared electronic pan balance, and weighed to the nearest 0.01 g.

During 1992, a further 300 fry per treatment group were removed from the holding troughs just prior to oil exposure to provide a pre-treatment sample. Pre-treatment length and weight in 1990 and 1991 were obtained during the tagging program thus encompassing the tagging (13-14 d) and holding periods (3 d).

Fry Release

The same method was used to release the pink salmon fry to the marine environment in each of the three consecutive years of the experiment. The fry were released from the troughs to the marine environment of Discovery Passage to complete their life cycle. An evening release (after 2100h) was chosen to avoid excessive avian predation on the abundance of prey concentrated near the discharge site. Although predation is a natural part of fry mortality we concluded that releasing large numbers of fry under high visibility conditions could have stimulated a higher than normal rate of predation from the many flocks of mergansers, cormorants and gulls present near the Campbell River estuary. Prior to the release, samples of fry from each treatment trough were retained for length and weight determination, histological examination, and disease assessment.

A gravity siphon system facilitated the movement of the fish with the minimum of handling stress. One end of a 30 m length of flexible, smooth-walled, 5-cm ID PVC hose was connected to the outlet standpipe of each holding trough and the other end (with solid PVC pipe extensions) was positioned 2-3 m beyond the tide line in 40-60 cm deep water. The fish retention screen at the downstream end of the holding trough was removed and the inflow of sea water to the trough stopped. The latter step was necessary to reduce a flow-orientation effect on the fry. The standpipe with drain hose attached was then lowered and, accordingly, the pink salmon fry were siphoned from the trough, through the PVC hose to the nearshore marine environment. Nylon screen pole seines were used to guide the fry towards the outlet in the floor of the trough and the few fry which remained in the very shallow residual water were flushed into the drain with sea water.

About 45 min was required to drain all three troughs. The three treatment groups were released sequentially but in random order and transit time within the release hose was 10-12 sec. The release of fry in 1990 and 1991 was conducted under extreme conditions of wind and rain, while calm conditions prevailed in 1992.

Adult Returns

From August through October in 1991, 1992, and 1993, employees of the Quinsam River Hatchery sampled the returning adult pink salmon selecting those without adipose fins. Adults returning to the Quinsam River were collected during dead pitch surveys, counting fence checks, and from broodstock holding at the hatchery. Data for these adipose-clipped fish included a head identification number, sex, length (post-orbital to hypural) and for some fish, weight ($\pm$ 0.1 kg). Heads were removed, frozen and labelled prior to transport to the laboratory for tag detection and recovery. Together with these samples were fish with

"stubby" adipose fins. While this condition could have occurred naturally it could also represent partly regenerated adipose fin tissue.

Adipose fin-clipped pink salmon were also recovered from the commercial fishery. Length (post-orbital to hypural), location, and date of capture were recorded for these fish, and the heads were labelled and sent to the laboratory for tag recovery and identification. Analyses of the binary codes on the tags was completed under the direction of Margaret Birch (Department of Fisheries and Oceans, Vancouver; 1991 and 1992 adult returns) and J.O. Thomas and Associates, Vancouver B.C., for the 1993 returns.

Adipose-clipped adult fish captured in the commercial fishery or returning to spawn (escapement) were assumed to be part of this investigation since, with the exception of fish from the Quinsam River marking program (adipose and ventral clip), no other group of similarly marked pink salmon (ie. adipose-clipped only) were reported from the study area (Brian Anderson, Department of Fisheries and Oceans, pers. comm.). However, a high percentage of adipose-clipped adults in the 1992 and 1993 commercial catch were without tags, thus strongly suggesting the presence of an additional group of adipose-clipped pink salmon.

Since a proportion of returning adults were expected to have lost their coded-wire tags, the heads were first passed through a quality control device to determine the presence and location of the tag. Heads containing tags were then sectionally dissected and the individual parts checked until the tag was recovered. The binary code on the tag was read under a microscope and the number recorded. A few tags were lost in the dissection and analysis process, or were otherwise unreadable.

Long-term Growth

The lengths ($\pm$ 1mm) and weights ($\pm$ 0.1kg) of adult pink salmon that returned to the Quinsam River were obtained from the Quinsam River Hatchery records, while only lengths ($\pm$ 1mm) were obtained from adults captured in the commercial fishery. In order to minimize differences related to sexual maturation and morphological changes to the head, length was determined from post occipital boundary to the hypural plate.

RESULTS

Tagging Program

The numbers of tagged fry released following each field program are summarized in Table 1. Of the 268,680 pink fry released during the investigation, 230,355 (86%) were estimated to have had coded-wire tags. This deduction is based on assessments of tag retention by the fry made during the tagging program and just prior to their release. Tag

retention at release in 1992 (79.1%) was lower than either previous year (91.3% and 86.8% for 1991 and 1992 respectively), and the variation reflects the difficulties of tagging the small (~23mm) pink fry, as well as year to year differences in individual tagging machine performance, the precision of fit of the tagging head molds, operator skill, and frequency of tag placement monitoring (quality control).

When the data for all years are combined, the estimated numbers of tagged pink fry in each treatment group did not vary more than 2.4% with the high dose group having the highest number of fry released (77,463), and low dose group having the least number of fry released (75,635).

Water Quality

The quality of sea water used in this study remained high throughout the three year investigation. Average afternoon values for temperature, salinity and dissolved oxygen in the holding troughs, and maximum and minimum values for temperature and oxygen are presented in Table 2. These represent the lowest water quality conditions present during the experiments (ie. the highest daytime water temperatures and the lowest dissolved oxygen concentrations after the afternoon feeding). Mean temperatures ranged from a high of 9.7 ^{o}C in 1990 to a low of 8.6^{o}C in 1992. The temperature of sea water (Figure 5) varied in response to changes in the weather. Average salinity ranged from 28.4 (1992) to 29.7 ‰ (1990; Figure 6). Within year variation in salinity was less than 1.5 ‰ over each experimental period and did not vary significantly between individual exposure troughs (Table 2). Average dissolved oxygen concentrations were somewhat higher in 1992 (8.3-8.5 mg•L^{-1}) and lowest in 1990 (7.2-7.4 mg•L^{-1}). The difference reflects the generally warmer weather, and the installation of the sea water air equilibration tower prior to the hydrocarbon exposure period in 1990. In 1991 and 1992, the air equilibration tower was used from the beginning of the tagging period. Despite the admission of air-equilibrated sea water, the dissolved oxygen concentrations in the exposure troughs decreased during the holding period (Figure 7) in response to the growth of the fish and hence an increase in loading density and oxygen requirements.

Dissolved Hydrocarbon Concentrations

Dissolved hydrocarbon concentrations in the holding troughs varied between years in response to changes in incoming sea water temperature and air temperatures (the temperatures of the holding troughs and the WSF production column fluctuated slightly in response to diurnal and weather-related changes in air temperatures), consequent differences in biological activity (i.e. bacterial and algal growth) and extraction efficiency in the WSF production column, as well as minor differences in composition of the whole crude oil (Figure 8). Our original experimental design (Duval et al. 1990) required dissolved hydrocarbon exposure concentrations of 500 μg•L^{-1} for the high dose, and 50 μg•L^{-1} for the low dose treatment groups. Hydrocarbon losses within the toxicant preparation and delivery systems, as well as evaporative losses from the exposure troughs reduced actual hydrocarbon

concentrations. The concentrations of the crude oil WSF determined from samples taken from the troughs during the three year investigation are shown in Figure 9. Mean (± S.D.) high dose concentrations ranged from 178.3±78.4 $\mu g \cdot L^{-1}$ (1992) to 348.6±58.4 $\mu g \cdot L^{-1}$ (1991). Low dose concentrations ranged from 25.3±6.8 $\mu g \cdot L^{-1}$ (1992) to 54.2±17.5 $\mu g \cdot L^{-1}$ (1991).

We expected to be able to calibrate dissolved hydrocarbon flow rate to the troughs in order to compensate for variation in WSF extraction column output. The analyses of hydrocarbon concentrations in samples in 1991 and 1992, for example, were initiated within 40 - 50 h of collection, and would have permitted coarse corrections to be made in exposure concentrations. However, the high sea water flow rate required to maintain acceptable dissolved oxygen concentrations in the exposure troughs (25 $L \cdot min^{-1}$) meant that the WSF extraction column was operating at full capacity in order to produce the nominal dilution concentrations for the high and low dose exposures. A higher extraction column output was attempted but resulted in unstable operating conditions (ie. whole oil particulates were observed in the toxicant header when the extraction column seawater flow rate exceeded 2.2 $L \cdot min^{-1}$).

The WSF consisted of benzene (43%), toluene (38%), *o, m* and *p* xylenes (15%), ethylbenzene (3%), and naphthalene (1%) (Figure 8). The composition of North Slope WSF differed only slightly from that reported for Cook Inlet WSF (Korn et al. 1979), and Prudhoe Bay WSF (Moles et al. 1985). The pink salmon fry were exposed to 248-250 h commencing near 12:00 noon (Day 1) and suspended between 19:00 and 22:00 h (Day 10).

Acute Toxicity

Toxicity of the water soluble fraction to the pink salmon fry was comparable between years. Flow-through bioassays conducted just prior to the hydrocarbon exposure period produced 96h LC50 values for 1990, 1991 and 1992 of 2.2, 2.8, and 1.0 $mg \cdot L^{-1}$, respectively. The tests were conducted each year using sea water at ambient temperature; in 1990, 1991 and 1992, sea water temperature during the test was 8.9, 10.0, and 7.7 °C, respectively (In tests conducted between 4 and 12 °C, Korn et al. (1979) found that lower temperature generally increased the toxicity of toluene and Cook Inlet WSF to pink salmon fry, while decreasing the toxicity of naphthalene).

Short Term Growth

Each treatment group cohort was fed a total of 5-6 kg Biodiet starter mash during the 28-30 d experimental period. The ration was 2.5-3.0% of body weight per day and the fry were not satiated (during the latter stages of the experimental holding period in each of the test years, several instances of cannibalism were observed). Intensity of the feeding response appeared unaffected by hydrocarbon exposure, and was vigorous in all treatment groups, especially when the full compliment of fish was present in each trough. Food conversion rates ranged between 1.0-2.2 units of feed to 1 unit of growth (some natural food was

present in the coarsely screened sea water), and were within expected ranges (Piper et al. 1986; Sedgwick, 1988). Low dose fry had the lowest feed conversion rates of all treatment groups in both 1990 and 1991 (1.7 and 2.2, respectively), but not in 1992, suggesting that the differences may not necessarily be attributable to hydrocarbon exposure.

The influence of hydrocarbon exposure on the early growth of pink salmon fry was determined by comparing length and weight data recorded prior to, and at the conclusion of, hydrocarbon exposure. Fry from each treatment group grew during the hydrocarbon exposure period. Standard length (all treatment groups combined) increased 0.16 - 0.24 $mm \cdot d^{-1}$ and mean weight between 3-6 $mg \cdot d^{-1}$.

Mean lengths for all treatment groups after exposure to dissolved hydrocarbons ranged from 33 - 35 mm. Mean weight ranged from 0.29 - 0.43 g, and mean condition factors were between 0.81 and 1.00. There were significant differences in short term growth between treatment groups, but these differences were not consistent between years or between treatment groups. For example, in 1990 and 1991, low dose fry were found to be significantly shorter and lighter than either the high dose or control fry, and in 1991, the high dose fry were significantly shorter than the control but not different in weight ($p<0.05$). By comparison, in 1992, the post-exposure lengths and weights for all treatment groups were not significantly different from each other (in 1991, pre-exposure length and weight data were derived from the tagging program, whereas in 1992, they were more precisely determined from measurements taken immediately prior to hydrocarbon exposure). Since the 1992 estimates for fry length and weight are thought to be more accurate than those from 1990 and 1991, we conclude that 10 d dissolved hydrocarbon exposures did not influence short term growth in pink salmon fry. When adult data are considered, low dose fish were slightly heavier than either control or high dose (see below), whereas as fry they were lighter in two of three years.

Short Term Survival

Where the number of test organisms is high, and the test organisms themselves are relatively fragile, some mortality of test subjects would be expected. In this investigation, large numbers of pink salmon fry were maintained in close proximity to each other, competing for space and food, and subjected to periodic disturbance (feeding and trough maintenance) as well as exposure to dissolved hydrocarbons. A small number of deaths occurred in the troughs each day irrespective of treatment.

In general, the size of individual dead fry was considerably less than that of the main population. For example, in 1991 the average size ($\pm$ S.D.) of dead fry was 0.15 $\pm$ 0.05 g (n=364), 0.13 $\pm$ 0.04 g (n=489), and 0.16 $\pm$ 0.06 g (n=319) for the control, low dose and high dose groups respectively. Fry at emergence (i.e. before the initiation of feeding behavior) in 1991 were 0.22 $\pm$ 0.03 g (n=907) for all groups. Those fish which died in the early and middle periods of the experiment were either slow growing individuals or those

which probably failed to initiate feeding. Towards the end of the hydrocarbon exposure period larger individuals died.

Cumulative mortality (expressed as a percent of the total number of fry in each holding trough) is shown in Figure 10. The mortality versus time curves for each experimental year had features in common. They were characterized by three distinct periods, an initial mortality phase during the time when fish (recently stressed from tagging, transport, and salt water acclimation) were added to the trough each day, a middle phase where no new fish were added and mortality was slight, and a later phase when the mortality rate accelerated.

Mortalities in the initial phase represented 47%, 59% and 35% of the total number of mortalities removed from the troughs in 1990, 1991 and 1992 respectively, and most individuals are thought to have succumbed within the first 24 h of holding (Table 3). The middle phase comprised 4%, 8%, and 4%, while the later phase was 49%, 33% and 61% of the total fry mortality in 1990, 1991 and 1992 respectively. Toward the end of the exposure period in each of the three years, the rate of fry mortality increased. In 1990, this increase occurred in both high and low dose treatment groups, but in 1991 and 1992, control group fry showed a similar increase, though of lesser magnitude. This late phase in the mortality curve is coincident with higher fish loading densities (a consequence of growth), and a general decline in dissolved oxygen concentrations (Figure 7).

There was a general trend towards higher mortality in the pink salmon fry exposed to hydrocarbons (Table 4a). Percent mortality at release, however, was not significantly affected by hydrocarbon exposure ($F = 4.469 \leq F_{.95} = 6.944$; Table 4b) when data were corrected for mortality occurring before the exposure period began. The difference in cumulative mortality was significant with respect to study year ($F = 9.262 \geq F_{.95} = 6.944$) with 1991 fry mortality about half of that in 1990 or 1992. We initially considered normalizing the 1991 data to correct for this lower overall mortality before testing for a relationship between hydrocarbon exposure and fry mortality. However, the genetic isolation of odd and even year pink salmon, together with the significantly smaller size of emerging fry in 1991, mitigated against any corrective factor being applied to the mortality results (eliminating the 1991 data from the analyses, did not alter the result).

While short term mortality was not affected by hydrocarbon treatment, it was, nevertheless, highly correlated with the combined number of adults recovered from the fisheries and the Quinsam River (Figure 11: $r_{Pear.} = 0.917$). Variance in cumulative percent fry mortality at the time of release accounted for 87% of the variance in the number of returning adults, suggesting that fry mortality at the time of release was a predictor for and inversely related to the number of fish that would survive to adulthood from each trough. This relationship implies that some of the factors contributing to the long term survival of pink salmon fry may be determined very early in their life cycle.

Overall, mortality of fry during the holding period was low. The mean cumulative percent mortality during the 27-30 d experimental period (all treatment groups combined) for 1990, 1991, and 1992 was 2.2%, 1.2%, and 2.4%, respectively. Daily mortality averaged over the last 5 d of the hydrocarbon exposure period ranged between 0.03 and 0.19% of the populations (Table 3). Parker (1968; cited in Pearcy, 1992) estimated pink salmon average daily loss rates of 2-4% for the first 40 d of ocean life after leaving the Bella Coola River, British Columbia, and subsequent daily loss rates of 0.4-0.8% for their remaining 410 d in marine waters. The mortality rate of pink salmon fry held in captivity in this investigation is, therefore, approximately one order of magnitude lower than that of a similar population under natural conditions.

Fry Release

The release operation proceeded efficiently in 1990 and 1991. In 1992 a rapidly falling tide, minimal wave activity, and the probable attraction to artificial light in the experimental compound resulted in the shoreline stranding of an estimated 1000 fry out of 88,331 released (1.1%). A total of 69 dead fry were collected from the cobble beach the following morning, 8 h after the release. Of these, 59.4%, were controls, 15.9% were high dose, and 8.7% were low dose fish; tags were not recovered from the remaining 16%. Control group fry had been released first, followed by the high and low dose groups. Compound lights had been extinguished part way through the high dose release, and before the low dose release was started.

Effects of Hydrocarbon Exposure on Numbers of Returning Adults

Recovery of adults from the fisheries

Adipose-clipped adult pink salmon recovered from the commercial fishery were predominantly from Johnstone Strait (Department of Fisheries and Oceans Statistical Areas 12 and 13), near their natal Quinsam River. Only 6% were caught in other areas (Figure 12). Eleven adults were captured in Area 26 in 1992 (northwest coast of Vancouver Island), and 1 fish was captured in Area 20 in 1991 (southwest coast of Vancouver Island).

The number of adult pink salmon recovered in the commercial fishery varied between years with 46, 243, and 517 obtained in 1991, 1992, and 1993, respectively. The number of adult fish with readable coded-wire tags was 44 (95.8%), 109 (44.9%), and 80 (15.5%) for the same years (Table 5). In relation to the total estimated number of tagged fry released each year (total released x % tag retention) this represents a percent recovery of 0.06 (1991), 0.13 (1992), and 0.11 (1993).

When data from all years are combined, 81 control fish, 79 low dose fish, and 73 high dose fish were obtained from the commercial fishery (Figure 13). The implied within-year variation in survivorship between treatment groups was considerable. For example, in

1991, twice as many high dose adults (20) returned as did control adults (9), whereas in 1993 the opposite occurred with 32 control fish returning and only 16 high dose fish.

Recovery of adults from the Quinsam River (escapement)

The numbers of male and female adults recovered from each treatment group are shown in Table 5 and Figure 14. Of the 658 adult fish obtained from the Quinsam River that were from our experiment, 220 were from the high dose treatment group, 216 from low dose fish and 222 from the control group.

Expected versus actual returns of adult pink salmon

Tagging required up to 14 d to complete in each of the three years of the study, giving rise to test populations which comprised individuals of different age and background (i.e. the oldest were held for over two weeks, while others were held for as little as 3 d prior to exposure to hydrocarbons). Within each treatment group, fry that were tagged early in the tagging program had different codes from those tagged later (Table 6). These differences in code numbers allowed for an assessment to be made as to whether fry tagged early in the program survived to adult in higher numbers than those tagged later. In order to verify that each tagged fish had an equal chance of being recovered as an adult (notwithstanding experimental treatment), a comparison was made between the expected number of recoveries of fish in each tag code category and the actual proportion recovered.

The expected number of adults was determined by taking the number of adult pink salmon with legible tag codes in each treatment group, and proportioning them according to the original distribution of tag codes at release . For example, in 1990, 64.9% of the high dose fry were tagged with the number 20102. Of the 83 high dose adult fish recovered in 1992, we would expect that 64.9% of the adults (about 54) would have the tag code 20102. However, 69 of the adults obtained from this treatment group (about 83%) were found to have the 20102 code. Thus a greater number of high dose fry tagged early in 1990 were recovered as adults than would have been predicted based upon the proportion of these fish in the group that was released. Similarly, low dose and control fry tagged early in 1990 were also recovered as adults in higher proportions (15% and 8% respectively) when compared with the later-tagged fry. However, the difference was only significant for the low dose fry (χ^2 Goodness of Fit: $P = 0.95$: Table 6).

The overall pattern of higher survival to adult of fry tagged early in the 1990 tagging program was not repeated in 1991 and 1992, suggesting that some factor unique to the 1990 program was responsible for the differences in that year.

Overall Recovery of Adults

When adult fish from the commercial fishery and those returning to the Quinsam River to spawn are combined the following results were obtained. In 1991, adult pink

salmon from the high dose treatment group outnumbered adults from both the low dose and control groups, while in 1993 adults from the low dose group slightly outnumbered both control and high dose groups. In 1992 returns from each treatment group were similar. The total number of adult fish that we obtained with readable tags (harvest and escapement) was 891 of which 34.0% were from the control group, 32.9% were from the high dose group, and 33.1% were from the low dose group (Figure 15).

The release of 230,355 tagged pink salmon fry over 3 years resulted in a recovery of 303, 295, and 293 adults with legible tags from the control, low and high hydrocarbon exposure groups respectively. The corresponding recoveries were 0.39, 0.39 and 0.38%.

A difference in tag retention between the fish taken from the commercial catch and those from the natal Quinsam River strongly implied that some adipose-clipped fish were present in the salmon population that did not originate from our study (Table 7a). Using the tag retention information derived from adult pink salmon that entered the Quinsam River a correction factor was applied to provide a more accurate estimate of the recovery of adults from this study (Table 7b). Based upon this information 0.4, 0.7 and 0.6% of the fry with tags were recovered in 1991,1992, and 1993 respectively. This represented an overall recovery of 0.6% (1324 individuals) from 230,355 tagged fry. This is not a survival estimate for the experimental population, however, for we do not have data on the total number of pink salmon captured in the fishery but not recorded (i.e. missed fish) nor the sampling efficiency on the Quinsam River.

Adult Sex, Length and Weight

Sex, length and weight of adult pink salmon taken from the Quinsam River are shown in Table 5. Control males outnumbered their female counterparts 122 to 100 when all data are combined. Low dose males returned in comparable numbers to low dose females (106 to 110), while high dose males were less numerous than high dose females (105 to 115). The greatest departure from these relatively close sex ratios was in the 1993 high dose fish where females outnumbered males by 2.6:1 (36 to 14).

On average, control males weighed less (1.18 $\pm$ 0.31 kg: $\bar{x}$ $\pm$S.D. n=122) than either low dose (1.26 $\pm$ 0.32: n=104) or high dose males (1.23 $\pm$ 0.30: n=105); however the differences were not significant ($F=1.404 \leq F_{.95}(2,4)= 3.036$). Low dose females in 1992 were significantly heavier (1.11 $\pm$ 0.17kg:n =55) than high dose females (1.02 $\pm$ 0.10kg: n=43) ($F=3.124 \geq F_{.95}(2,4)= 3.120$). They were also slightly heavier than high dose females in 1991 and 1993, but the differences were not significant. During the same period low dose females tended to be equal to, or heavier than, the control females but none of the differences were significant. While differences in adult weight as a function of treatment group were minor, differences between experimental years were considerable. Male and female adults were significantly shorter and lighter in 1992, than in either 1991 or 1993 (Table 8), probably reflecting the genetic isolation of odd and even year stocks (Groot and Margolis 1991).

Two trends in the adult length and weight data are apparent; control males appear to be lighter on average than low dose males, while low dose females are, on average, heavier than high dose females and heavier, or equal to control females. Neither trend is significant at the 5% level. No significant differences in mean length of commercially caught fish as a function of treatment were observed ($p \leq 0.05$: post occipital to hypural plate; range 468-486 mm).

DISCUSSION

We speculated that significant differences in the number of returning adult pink salmon from treated and untreated groups of newly emerged fry would suggest that sublethal hydrocarbon exposures had resulted in differential survival under natural conditions. Conversely, however, if equal numbers of adult fish returned from all treatment groups the inference would be that the sublethal exposures employed did not affect long term survival.

We found that the exposure of pink salmon fry to 25-54 $\mu g \cdot L^{-1}$ or 178-349 $\mu g \cdot L^{-1}$ of the WSF of North Slope crude oil for 10 d did not result in a measurable difference in survival to adulthood relative to control fish. While between year variation in the number of recovered adults was considerable, when data from all years are combined, adult fish from the control treatment group were only 1.1% more numerous than those from the high dose group, while adults from the low dose group were intermediate between these two groups. Further, fish from hydrocarbon treatment and control groups returned in a state of advanced reproductive maturity. In 1993, a shortage of returning pink salmon adult fish necessitated including the experimental fish in the Quinsam River Hatchery egg takes.

This overall study result was obtained despite the presence, immediately after exposure, of histological effects in liver, kidney, and gill tissues in a significant proportion of the hydrocarbon-exposed test groups (Brand et al. 1996). In addition, avoidance trials conducted during the second and third year of this investigation suggested the possibility that pink fry pre-exposed to the high hydrocarbon dose had reduced ability to detect and avoid dissolved hydrocarbons (Department of Fisheries and Oceans, unpublished data) implying a detriment to some physiological and behavioral functions. Babcock (1985) found loss of cilia and increased mucus production in olfactory rosettes of pink salmon fry exposed for 12-29 days to 0.51 $mg \cdot L^{-1}$ benzene (a primary constituent of the WSF of North Slope crude oil used in our investigation) and speculated that these effects could give rise to decreased chemosensory reception, with possible consequences to homing, feeding behavior and avoidance of predators.

In the absence of any adult return data, it would have been reasonable to propose that the sublethal effects observed in hydrocarbon-exposed fry in this investigation (i.e. gill, liver, and kidney damage and potential olfactory loss) would signify a probable loss of health and longevity of exposed individuals. That this did not translate to a differential survival to adulthood requires careful examination of the experiment and the conditions under which it

was conducted. It is obvious that the experiment did not simulate all the conditions that may be encountered at the time of an oil spill. For example, the absence of oil droplets, or floating oil, oiled prey and predators were potentially significant factors not accounted for. In addition, it is conceivable that those fish which exhibited sublethal responses to the exposure conditions we employed were the weaker or more sensitive individuals of the population. It is therefore possible that these would have succumbed to discriminate and indiscriminant selection pressures in the wild before the more robust individuals in the population.

Rather than estimate the potential effects on fry maintained in captivity, we chose to evaluate the effect of the hydrocarbon treatment under "natural" conditions. We sacrificed a measure of certainty in the result by releasing the fry to complete their lifecycle outside our sphere of knowledge or control, and in doing so gained a large measure of relevance in that the determination of survival frequency of treated versus untreated fish was completely objective, and ecologically meaningful. The necessity of replicating the experiment three times in succession however, (a direct consequence of logistical constraints), introduced the potential for between-year variability in experimental conditions, and also in post-experimental oceanic growth conditions which may have masked potential differences in survival among treatment groups. Vallion (1981; cited in Pearcey, 1992) found extreme differences in ocean survival of pink salmon from fry to adult stage at Sashin Creek, AK, where, in a 16 year period, survival ranged from a low of 0.2% to a high of 23% with a coefficient of variation of 159%. Where natural variation is high, long term effects resulting from brief toxic exposures are difficult to elucidate. Barnthouse et al. (1989) point out that "for typical fisheries data sets, where sample size is limited and variability is high, changes of 50% or more may be undetectable using conventional statistical criteria". Thus, even if there was differential survival in our experiment, it may have been masked by such variability.

Survival of pink salmon from fry to adult is generally low, and the average survival of pink fry released from the Quinsam River Hatchery, for example, ranges from 1-10 % for unfed fry and from 2-19 % for diet-supplemented fry (1979-1989: DFO unpublished data). Thus, pink salmon face major challenges to survival throughout their two year life cycle. Parker (1968) estimated that instantaneous mortality of Bella Coola River pink salmon was higher than 0.7 for the first few weeks of the marine phase of their lifecycle and remained as high as 0.45 for the first 5 months of coastal feeding. The effect on survival of a sublethal toxic challenge administered within a protected, food-enhanced environment for a duration amounting to 1.4% of a typical life cycle may easily be overshadowed by the influence of later, more rigorous natural and anthropogenic challenges. Over the course of the three year investigation, at least 230,000 tagged fry were released from the experimental holding troughs, and only a very small proportion of those were ever recovered (891 or 0.4% with legible tag codes). The fate of 99% of the fry used in this experiment remains unknown. What can be stated with certainty is that the vast majority of test subjects were neither recaptured in the commercial fishery nor returned as mature adults to the Quinsam River (nor were any strays reported from other nearby river systems). Thus, there is a possibility that

post-experimental selection for, or against oil-exposed fish could have taken place but remained undetected because of the more significant normal mortality events (predation, fishing, disease).

With their documented ability to metabolize hydrocarbons (Rice et al. 1977) pink salmon fry exposed in this investigation may have recovered following treatment. Following the 96 h acute lethal bioassay in 1991, 4 of 5 fry surviving 3.1 $mg \cdot L^{-1}$ dissolved hydrocarbons and 10 of 10 fry surviving 1.5 $mg \cdot L^{-1}$ resumed feeding activity after oil exposure ended (fry were not fed during the bioassay) and subsequently survived through an additional 6 d, at which time the experiment was terminated. This indicates a potential for recovery following near-fatal hydrocarbon exposure. In the 1991 high dose trough, dissolved hydrocarbon concentrations (349 $\mu g \cdot L^{-1}$) were one order of magnitude lower than the acute bioassay concentration that produced 50% mortality after 96h (from which an apparent recovery was made by fry surviving the exposure). Rice et al. (1977) found that tissue hydrocarbon concentrations in pink salmon exposed to Cook Inlet WSF increased rapidly at first, but then equally rapidly returned to baseline levels despite continued exposure to the oil.

The partitioning of hydrocarbons between water and fish tissue is regulated by simple concentration equilibria: aromatic hydrocarbons are lipophilic in nature, concentrating in yolk, liver, brain or fatty tissues at higher levels than in muscle, heart or gills (Malins and Hodgins (1981). In fish where lipid-rich, low metabolic rate tissues are present, toxic effects on the organism may be reduced (Moles et al. 1987). Uptake and persistence of tissue hydrocarbons is, in part, regulated by the type of molecules present. Coho salmon smolts and starry flounder (Roubal et al. 1978) accumulated alkyl-substituted aromatic hydrocarbons in preference to un-substituted structures, while salt-water acclimated Dolley Varden juveniles (Thomas and Rice, 1981) retained a higher proportion of ^{14}C-labelled naphthalene after 24 h than ^{14}C-labelled toluene. The former authors also observed that tissue accumulation increased in relation to the number of benzene rings in the molecules.

In this investigation, monoaromatics comprised over 95% of the WSF of North Slope crude oil, with naphthalene as the only diaromatic hydrocarbon recorded. Rapid excretion of tissue hydrocarbons when their concentration in the water returns to background levels, coupled with metabolism of residual hydrocarbons could afford oil-exposed pink fry the means to avoid any long term influence following non-lethal hydrocarbon exposure. Rice and Thomas (1989) found that pre-exposure of pink fry to naphthalene (and to a lesser extent toluene) activated a mixed function oxidase (MFO) system which conveyed an enhanced survival benefit during subsequent acute hydrocarbon exposure. They also suggested, however, that the metabolism of hydrocarbons in pink salmon was not without cost citing higher oxygen consumption in oil exposed fry, and the potential that the higher energy utilization necessary for hydrocarbon metabolism could affect other physiological and behavioral functions, including swimming performance, plasma cortisol, and ultimately growth. In our study, with the possible exception of low dose female adults, which were slightly larger than control females, and significantly larger than high dose females, effects

on growth to the adult stage following exposure of fry to dissolved hydrocarbons were relatively minor, especially in relation to the more significant between-year size variation (Table 5). We did not measure respiration in this investigation, however, there appeared to be only minor differences in the oxygen saturation of the sea water in the holding troughs during the hydrocarbon exposure period (the combination of fish respiration and any chemical oxygen demand from the dissolved hydrocarbons resulted in a 20-30% drop in oxygen saturation in the troughs compared to the oxygen saturation in the sea water header tank; Figure 7).

In the absence of further hydrocarbon exposure, dilution of tissue borne hydrocarbons resulting from an increase in body mass through growth could diminish potential physiological consequences of early life cycle stage exposure to oil. Provided that hydrocarbon exposure did not produce higher predation or disease related mortality in hydrocarbon exposed fry during the initial ocean phase, the passage of time would gradually reduce any difference between treatment groups.

The fry release method employed in this investigation, which was chosen to control the immediate predation of test groups by large flocks of seabirds prevalent near the Campbell River study site in spring, may have provided hydrocarbon exposed fry with an additional 8h of recovery before having to cope with a normal selection pressure, thus eliminating a potential mortality source whose effects may have resulted in differential mortality among treatment groups.

It seems likely that any selective disadvantage experienced by hydrocarbon-treated versus untreated fry may have been relatively short-lived, perhaps not extending much beyond the duration of the experimental holding period itself. In the protected, food-enriched, predator-free, holding troughs, selection pressure would be minimized, and potential differences in the individual "fitness" of fry from each test groups could have been masked. Following release, hydrocarbon-exposed fry from this investigation demonstrated the ability to grow, avoid predators, mature, and home on their natal stream in numbers equivalent to untreated control fry. In a conceptually similar experiment, Ostrander et al. (1989) exposed coho salmon eggs to a single dose of benzo[a]pyrene (B[a]P) given either early or late in egg development, and subsequently determined the number and size of returning adult fish. No significant differences could be detected in either the number or reproductive state of returning adults irrespective of treatment group, despite earlier studies by these authors which indicated significant metabolic effects and tissue retention of B[a]P following brief exposure. Isolation of experimental fish from natural predation and other competitive stressors during and after exposure was cited as a possible reason for the subsequent lack of discernable effects at the adult stage.

A number of unanswered questions remain. Within a population, a range of physiological responses to hydrocarbon exposure is possible. Many of the cytological effects observed in pink fry following dissolved hydrocarbon exposure, were limited to a fraction of the individuals examined (Brand et al. 1996), suggesting that considerable variation in

hydrocarbon tolerance among individuals may have been present in the test populations. It is not immediately clear from our results whether hydrocarbon-exposed fry returning 2 years later as adults represent individuals that recovered from tissue damage, individuals that never developed those pathologies, or perhaps some combination of tolerance and tissue recovery.

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REFERENCES

Babcock, M.M. 1985. Morphology of olfactory epithelium of pink salmon, Oncorhynchus gorbuscha, and changes following exposure to benzene: a scanning electron microscopy study, p. 259-267. *In* J.S. Gray, and M.E. Christiansen [ed.] Marine biology of polar regions and effects of stress on marine organisms. John Wiley and Sons Ltd. New York.

Barnthouse, L.W., G.W. Suter II, and A.E. Rosen. 1989. Inferring population-level significance from individual level-effects: an extrapolation from fisheries science to ecotoxicology, p.289-300. *In* G.W. Suter II, and M.A.Lewis [ed.] Aquatic toxicology and environmental fate: Eleventh Volume ASTM Special Technical Publication 1007.

Brand, D.G., R. Fink, W. Bengeyfield, I.K. Birtwell, and C.D. McAllister. 1996. Histological analysis of salt water-acclimated pink salmon fry, (Oncorhynchus gorbuscha), after 10 day exposure to sublethal concentrations of the water soluble fraction of North Slope crude oil. Submitted to Toxicol. Path.

Cairns, J., Jr. 1988. What constitutes field validation of predictions based on laboratory evidence?, p. 361-368. *In* W.J. Adams, G.A. Chapman, and W.G. Landis [ed.] Aquatic toxicology and hazard assessment: Tenth Volume, ASTM Special Technical Publication 971.

Chevron Canada Resources Ltd. 1982. Initial environmental evaluation for renewed petroleum exploration in Hecate Strait and Queen Charlotte Sound. Volumes 1 and 2.

Duval, W.S., S. Hopkinson, W.R. Olmsted, and R. Kashino. 1989. The *Nestucca* oil spill: preliminary evaluation of impacts on the west coast of Vancouver Island. ESL Environmental Sciences Ltd., Vancouver, B.C. Prepared for Environment Canada and B.C. Ministry of Environment. 62 p.

Duval, W., R. Fink, R. Olmsted, B. Humphrey, and K. English. 1990. A research program to determine the effects of sublethal crude oil exposures on long-term survival, escapement and harvest of pink salmon (Oncorhynchus gorbuscha). ESL Environmental Sciences Ltd.Vancouver, B.C., and LGL Ltd.Sidney, B.C. Prepared for Department of Fisheries and Oceans, Nanaimo and West Vancouver, B.C. 28 p.

E.P.A. 1984. Guidelines establishing test procedures for the analysis of pollutants under the Clean Water Act. October 26, 1984. U.S. Environmental Protection Agency.

FEARO. 1986. Offshore hydrocarbon exploration: Report and recommendations of the West Coast Offshore Exploration Environmental Assessment Panel, April, 1986. West Coast Offshore Exploration Environmental Assessment Panel, Vancouver, B.C. Prepared for the Government of Canada, Ministry of Environment, and Ministry of Energy Mines and Resources, and for the Province of British Columbia, Ministry of Environment, and Ministry of Energy Mines and Petroleum Resources. 123 p.

Fink, R., R. Alexander, W. Bengeyfield, I.K. Birtwell, and C.D. McAllister. 1995. The influence of sublethal crude oil exposure on long term survival, escapement, and harvest of pink salmon (Oncorhynchus gorbuscha). Can. Data Rep. Fish. Aquat. Sci. 949: 78 p.

Galt, J.A., W.J. Lehr, and D.L. Payton. 1991. Fate and transport of the *Exxon Valdez* oil spill. Environ. Sci. Technol. 25(2): 202-209.

Groot, C., and L. Margolis [ed.]. 1991. Pacific Salmon Life Histories. Department of Fisheries and Oceans, Biological Sciences Branch, Pacific Biological Station, Nanaimo, British Columbia, Canada. UBC Press.Vancouver, B.C. 564 p.

Heard, W.R. 1991. Life history of pink salmon (Oncorhynchus gorbuscha), p. 120 -230. *In* C. Groot, and L. Margolis [ed.], Pacific Salmon Life Histories, UBC press, Vancouver. 564 p.

Kelso, D.D., and M. Kendziorek. 1991. Alaska's response to the *Exxon Valdez* oil spill. Environ. Sci. Technol. 25(1): 16-23.

Korn, S.D., A. Moles, and S.D. Rice. 1979. Effects of temperature on the median tolerance limit of pink salmon and shrimp exposed to toluene, naphthalene, and Cook Inlet crude oil. Bull. Environ. Contam. Toxicol. 21: 521-525.

Litchfield, J.T., and F. Wilcoxon. 1949. A simplified method for evaluating dose-effect experiments. J. Pharmacol. Exp. Ther. 96: 99-113.

Maki, A.W. 1991. The *Exxon Valdez* oil spill: initial environmental impact assessment. Environ. Sci. Technol. 25(1): 24-29.

Malins, D.C., and H.O. Hodgins. 1981. Petroleum and marine fishes; a review of uptake, disposition and effects. Environ. Sci. Technol. 15: 1272-1280.

Moles, A., M.M. Babcock, and S.D. Rice. 1987. Effects of oil exposure on pink salmon, Oncorhynchus gorbuscha, alevins in a simulated intertidal environment. Mar. Environ. Res. 21: 49-58.

Moles, A., S.D. Rice, and S. Andrews. 1985. Continuous-flow devices for exposing marine organisms to the water-soluble fraction of crude oil and it's components. Can. Tech. Rep. Fish. Aquat. Sci. 1368: 53-61.

Moles, A., S.D. Rice, and S.Korn, 1979. Sensitivity of Alaskan freshwater and anadromous fishes to Prudhoe Bay crude oil and benzene. Trans. Amer. Fish. Soc. 108: 408-414.

Ostrander, G.K., M.L.Landolt, and R.M. Kocan. 1989. Whole life history studies of coho salmon (Oncorhynchus kisutch) following embryonic exposure to benzo(*a*)pyrene. Aquatic Toxicology 15: 109-126.

Parker, R.R. 1968. Marine mortality schedules of pink salmon of the Bella Coola River, central British Columbia. J. Fish. Res. Bd. Can. 25: 757-794.

Pearcy, W.G. 1992. Ocean ecology of North Pacific salmonids. University of Washington Press. 179 p.

Piper, R.G., I.B. McElwain, L.E.Orme, J.P. McCraren, L.G. Fowler, and J.R. Leonard. 1986. Fish Hatchery Management. United States Department of the Interior Fish and Wildlife Service, Washington, D.C. 517 p.

Rice, S.D., and R.E.Thomas. 1989. Effect of pre-treatment exposures of toluene or naphthalene on the tolerance of pink salmon (Oncorhychus gorbuscha) and kelp shrimp (Eualis suckleyi). Comp. Biochem. Physiol. 94(1): 289-293.

Rice, S.D., R.E. Thomas, and J.W.Short. 1977. Effect of petroleum on breathing and coughing rates, and hydrocarbon uptake-depuration in pink salmon fry, p. 259-277. *In* F.J. Vernberg, A. Calabrese, F.P. Thurberg, and W.B. Vernberg [ed.], Physiological Responses of Marine Biota to Pollutants. Academic Press Inc. New York.

Rice, S.D. 1973. Toxicity and avoidance tests with Prudhoe Bay crude oil and pink salmon fry. p.667 - 670. *In* Proceedings of 1973 Joint Conference on Prevention and Control of Oil Spills. American Petroleum Institute, Washington, D.C.

Roubal, W.T., S.I. Stranahan, and D.C. Malins. 1978. The accumulation of low molecular weight aromatic hydrocarbons of crude oil by coho salmon (Oncorhynchus kisutch) and starry flounder (Platichthys stellatus). Arch. Environm. Contam. Toxicol. 7(2): 237-244.

Sedgwick, S.D. 1988. Salmon Farming Handbook. Dorsett Press, Dorchester, Great Britain. 207p.

Short, J.W., and P.M.Rounds. 1996. Chemical sampling and analysis of petroleum hydrocarbons in the near-surface seawater of Prince William Sound, Alaska, after the *Exxon Valdez* oil spill. *In* S.D. Rice, R.B. Spies, D.A. Wolfe, and B.A. Wright [ed.], *Exxon Valdez* Oil Spill Symposium Proceedings. American Fisheries Society Symposium No. 18: 0000-0000 (In Press).

Schnieder, D. 1993. Oil spill has long term effects. Water Environ. Technol. 5(9): 30-32.

Sprague, J.B. 1969. Measurement of pollutant toxicity to fish: I. Bioassay methods for acute toxicity. Water Res. 3: 793-821.

States/British Columbia Oil Spill Task Force, Final Report. 1990. Province of British Columbia, State of Washington, State of Oregon, State of Alaska, State of California. 146 p.

Thomas, R.E., and S.D. Rice. 1981. Excretion of aromatic hydrocarbons and their metabolites by freshwater and seawater Dolly Varden char, p. 425-448. *In* F.J. Vernberg, A. Calabrese, F.P. Thurberg, and W.B. Vernberg [ed.], Biological Monitoring of Marine Pollutants. Academic Press, New York.

Table 1. Number of pink salmon fry coded-wire tagged and released (1990-1992).

Year	Numbers of Fry	Treatment Group		
		Control	Low Dose	High Dose
1990	Total Number of Fry	31,985	31,207	32,323
	Number of Fry Removed *	2,800	3,139	2,808
	Total Number of Fry Released	29,185	28,068	29,515
	Tag Retention (%)	91	91	92
	No. Tagged Fry Released →	**26,617**	**25,430**	**27,213**
1991	Total Number of Fry	31,502	33,028	31,784
	Number of Fry Removed *	834	1,119	780
	Total Number of Fry Released	30,668	31,909	31,004
	Tag Retention (%)	85	86	89
	No. Tagged Fry Released →	**26,190**	**27,378**	**27,656**
1992	Total Number of Fry	31,067	31,255	31,210
	Number of Fry Removed *	1,609	1,725	1,867
	Total Number of Fry Released	29,458	29,530	29,343
	Tag Retention (%)	83	77	77
	No. Tagged Fry Released →	**24,450**	**22,827**	**22,594**
	All Years Combined	**77,257**	**75,635**	**77,463**

* Removed due to mortality or sampled for additional experiments

Table 2. Experimental sea water quality (1990-1992).

	1990			1991			1992		
	Control	Low	High	Control	Low	High	Control	Low	High
Temperature (° C)									
Mean*	9.8	9.7	9.7	9.0	9.0	9.0	8.6	8.7	8.6
s.d.	0.5	0.5	0.5	0.6	0.5	0.5	0.4	0.4	0.4
n	28	27	28	28	28	28	29	29	29
maximum	11.2	11.1	11.0	10.5	10.1	10.1	9.6	9.7	9.7
minimum	9.0	8.7	8.8	8.2	8.3	8.2	8.1	8.0	8.0
Dissolved Oxygen (mg/L)									
Mean*	7.2	7.4	7.3	7.9	8.2	8.0	8.3	8.4	8.5
s.d.	0.4	0.5	0.5	0.9	0.7	0.8	0.8	0.7	0.7
n	29	28	28	22	22	22	26	26	26
maximum	8.3	8.3	8.3	10.0	9.8	9.9	9.5	9.7	9.6
minimum	6.6	6.6	6.4	6.5	7.3	6.9	7.3	7.3	7.2
Salinity (ppt)									
Mean	29.7	29.7	29.6	29.3	29.2	29.3	28.4	28.4	28.5
s.d.	0.2	0.4	0.5	0.2	0.2	0.2	0.3	0.3	0.3
n	29	28	28	22	22	22	26	26	26

* = the mean of all afternoon readings when dissolved oxygen was lowest and temperature was typically highest.

Table 3. Fraction (%) of pink salmon fry dying in each of three experimental phases relative to the total number that died, and daily mortality rates just prior to release.

Year	Period	Percent of total fry mortality			
		Control	Low dose	High dose	Combined
1990	During tagging	70	38	32	**46.7**
	After tagging	5	3	5	**4.3**
	After oil exposure	25	59	63	**49.0**
	Daily mortality rate (%)*	0.06	0.24	0.17	**0.16**
1991	During tagging	67	40	70	**59.0**
	After tagging	8	7	10	**8.3**
	After oil exposure	25	53	20	**32.7**
	Daily mortality rate (%)*	0.04	0.11	0.07	**0.07**
1992	During tagging	40	29	35	**34.7**
	After tagging	5	4	3	**4.0**
	After oil exposure	55	67	62	**61.3**
	Daily mortality rate (%)*	0.13	0.19	0.18	**0.17**

* Calculated for the final five days before fry release (period of maximum mortality)

Table 4a. Cumulative fry mortality at release versus hydrocarbon exposure.

Treatment group	Year	Corrected mortality (%) *	Average		Standard deviation
Control	1990	0.52			
	1991	0.33			
	1992	1.11	**0.65**	±	**0.41**
Low Dose	1990	1.68			
	1991	0.82			
	1992	1.55	**1.35**	±	**0.46**
High Dose	1990	1.15			
	1991	0.26			
	1992	1.72	**1.04**	±	**0.74**

* cumulative percent mortality corrected for deaths occuring before hydrocarbon exposure.

Table 4b. Mortality versus treatment and year (2-factor Anova without replication).

Summary	Count	Sum	Average	Variance	
Control	3	1.96	0.653	0.165	
Low Dose	3	4.05	1.350	0.215	
High Dose	3	3.13	1.043	0.541	
1990	3	3.35	1.117	0.337	
1991	3	1.41	0.470	0.093	
1992	3	4.38	1.460	0.099	
Source of Variation	*SS*	*df*	*MS*	*F*	F_{95}
Treatment	0.731	2	0.366	**4.469**	**6.944**
Year	1.516	2	0.758	**9.262**	**6.944**
Error	0.327	4	0.082		
Total	2.575	8			

Table 5. Number of recovered male and female pink salmon with legible tags.

Year	Treatment Group	Commercial Catch	Quinsam River Escapement			Combined Recovery
			Males	Females	All Fish	
1991	Control	9	31	31	62	71
	Low Dose	15	22	22	44	59
	High Dose	20	27	36	63	83
	Total	44	80	89	169	213
1992	Control	40	54	46	100	140
	Low Dose	32	55	55	110	142
	High Dose	37	64	43	107	144
	Total	109	173	144	317	426
1993	Control	32	37	23	60	92
	Low Dose	32	29	33	62	94
	High Dose	16	14	36	50	66
	Total	80	80	92	172	252

Combined year totals

Treatment Group	Commercial Catch	Quinsam River Escapement			Combined
		Males	Females	All Fish	
Control	81	122	100	222	303
Low Dose	79	106	110	216	295
High Dose	73	105	115	220	293
All groups	233	333	325	658	891

Table 6. Actual versus expected adult recovery by year and treatment group.

Year	Treatment group	Tag code	Tag Days	% Distrib. of codes	Number of tagged fry released	Adult returns by tag code: Actual recovery	Expected recovery	% difference	Goodness of Fit: Critical value	Chi Squared	df
1990	Control	20101	1-9	59.8	15917	48	42	7.81	7.81	4.71	3
		20401	10-12	22.0	5856	15	16	-0.87			
		20402	12	12.8	3407	8	9	-1.53			
		20202	12	5.4	1437	0	4	-5.40			
				100.0	**26617**	**71**					
	Low Dose	20404	1-8	56.6	14393	42	33	14.59	**3.84**	**4.53**	1
		20405	8-12	43.4	11036	17	26	-14.59	Significant (P=0.95)		
				100.0	**25430**	**59**					
	High Dose	20102	1-9	64.9	17661	69	54	18.07	**5.99**	**12.00**	2
		20501	10-11	17.8	4844	8	15	-8.43	Significant (P=0.95)		
		20502	10-11	17.3	4708	6	14	-9.64			
				100.0	**27213**	**83**					
1991	Low Dose	211110	1-7	25.9	7091	30	37	-4.77	7.81	2.11	3
		211111	8-10	33.7	9226	51	48	2.22			
		211112	11-14	33.9	9281	53	48	3.42			
		211113	14	6.5	1780	8	9	-0.87			
				100.0	**27378**	**142**					
	Control	21123	1-7	32.6	8538	39	46	-4.74	7.81	4.92	3
		21124	8-10	45.0	11786	60	63	-2.14			
		21125	14	5.0	1310	7	7	0.00			
		21126	11-14	17.4	4557	34	24	6.89			
				100.0	**26190**	**140**					
	High Dose	211114	1-7	31.2	8629	37	45	-5.51	7.81	7.17	3
		211115	8-10	41.8	11560	63	60	1.95			
		21121	11-14	21.0	5808	40	30	6.78			
		21122	14	6.0	1659	4	9	-3.22			
				100.0	**27656**	**144**					
1992	Control	21134	1-5	41.5	10147	39	38	0.89	5.99	0.07	2
		21135	5-9	40.5	9902	36	37	-1.37			
		21136	9-12	18.0	4401	17	17	0.48			
				100.0	**24450**	**92**					
	Low Dose	21137	1-5	34.2	7807	38	32	6.23	5.99	1.91	2
		211214	5-12	26.0	5926	20	24	-4.68			
		211215	8-11	39.8	9085	36	37	-1.50			
				100.0	**22827**	**94**					
	High Dose	21131	1-5	34.3	7750	21	23	-2.48	5.99	0.28	2
		21132	5-8	30.6	6914	22	20	2.73			
		21133	9-12	35.1	7931	23	23	-0.25			
				100.0	**22594**	**66**					

Table 7a. Proportion of recovered adult pink salmon with legible tags.

Year	Description	Commercial Catch	Quinsam River Escapement	Total
1991	total adults captured	46	255	301
	total with legible tags	44	169	213
	adult tag retention (%)	NA *	**66.3**	
1992	total fish captured	243	444	687
	total with legible tags	109	317	426
	adult tag retention (%)	**44.9** **	**71.4**	
1993	total fish captured	517	277	794
	total with legible tags	80	172	252
	adult tag retention (%)	**15.5** **	**62.1**	
			total fish captured	**1782**
			total with legible tags	**891**

* - Only adults with legible tags reported for 1991 commercial returns: two tags lost or illegible.

** - Lower tag retention in commercial catches for 1992, 1993 in relation to the escapement tag retention, implies the presence of an additional group of pink salmon in the study area that were marked but not tagged.

Table 7b. Recovery of adult salmon in relation to the number of fry released.

Year	Description	Result
1990-91	Estimated number of tagged fry released	79,260
	Actual no. of tagged adults recovered	213
	Adult tag retention (%)*	66.3
	No. of surviving adults (corrected)**	321
	Estimated % recovery	0.405
1991-92	Estimated number of tagged fry released	81,224
	Actual no. of tagged adults recovered	426
	Adult tag retention (%)	71.4
	No. of surviving adults (corrected)**	597
	Estimated % recovery	0.735
1992-93	Estimated number of tagged fry released	69,871
	Actual no. of tagged adults recovered	252
	Adult tag retention (%)	62.1
	No. of surviving adults (corrected)**	406
	Estimated % recovery	0.581
All Years	Total estimated no. of recovered adults	**1324**
	Estimated % recovery	**0.575**

* - tag retention for escapement adults used for both escapement and commercial catch

** - (No. commercial adults with tags + No. escapement adults with tags) / % tag retention of escapement adults

Table 8. Mean lengths and weights of adult pink salmon from the Quinsam River.

Sex	Year	Treatment Group	Mean Length (mm)	Standard Deviation	Mean Weight (kg)	Standard Deviation	n	ANOVA 1-Factor $p \leq 0.05$
Males	1991	Control	385.90	26.11	1.28	0.23	31	ns*
		Low Dose	393.73	25.99	1.47	0.32	22	ns
		High Dose	391.30	24.52	1.44	0.25	27	ns
	1992	Control	366.93	23.99	1.08	0.24	54	ns
		Low Dose	372.45	23.57	1.13	0.25	55	ns
		High Dose	371.45	22.27	1.09	0.24	64	ns
	1993	Control	387.57	27.66	1.26	0.39	37	ns
		Low Dose	398.24	23.99	1.36	0.32	27	ns
		High Dose	393.21	26.45	1.36	0.30	14	ns
	By Year	1991	**389.88**	25.44	**1.38**	0.27	80	
		1992	**370.36**	23.22	**1.10**	0.24	173	**92< 91or 93**
		1993	**392.43**	26.29	**1.31**	0.35	78	
	By Treatment	Control	378.01	27.34	1.18	0.31	122	ns
		Low Dose	383.92	26.83	1.26	0.32	104	ns
		High Dose	379.46	25.29	1.23	0.30	105	ns
Females	1991	Control	396.37	17.80	1.35	0.15	31	ns
		Low Dose	399.95	12.94	1.36	0.14	22	ns
		High Dose	398.03	18.96	1.34	0.16	36	ns
	1992	Control	372.07	16.45	1.07	0.12	46	
		Low Dose	374.22	17.63	**1.11**	0.17	55	**Low Dose**
		High Dose	371.65	13.38	**1.02**	0.10	43	**>High Dose**
	1993	Control	393.68	18.45	1.30	0.18	23	ns
		Low Dose	394.85	18.33	1.30	0.30	33	ns
		High Dose	391.14	19.00	1.23	0.19	36	ns
	By Year	1991	**397.94**	17.08	**1.35**	0.15	89	
		1992	**372.76**	16.02	**1.07**	0.14	144	**92< 91 and 93**
		1993	**393.10**	18.49	**1.27**	0.24	92	
	By Treatment	Control	384.36	20.74	1.21	0.19	100	ns
		Low Dose	385.55	20.44	1.23	0.25	110	ns
		High Dose	385.90	20.45	1.17	0.20	115	ns

* ns = not significant

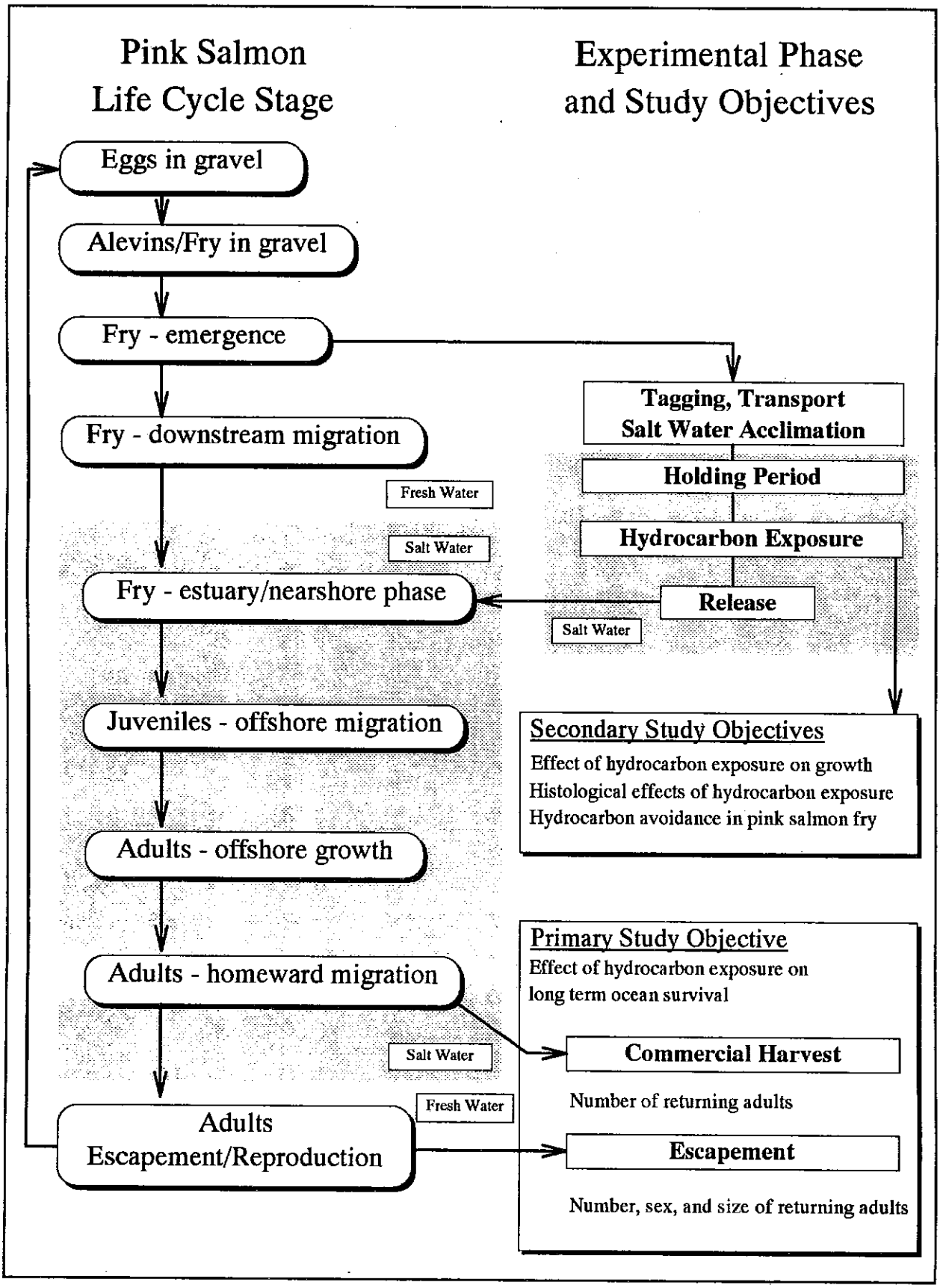


Figure 1. Relationship between pink salmon life cycle and study objectives.

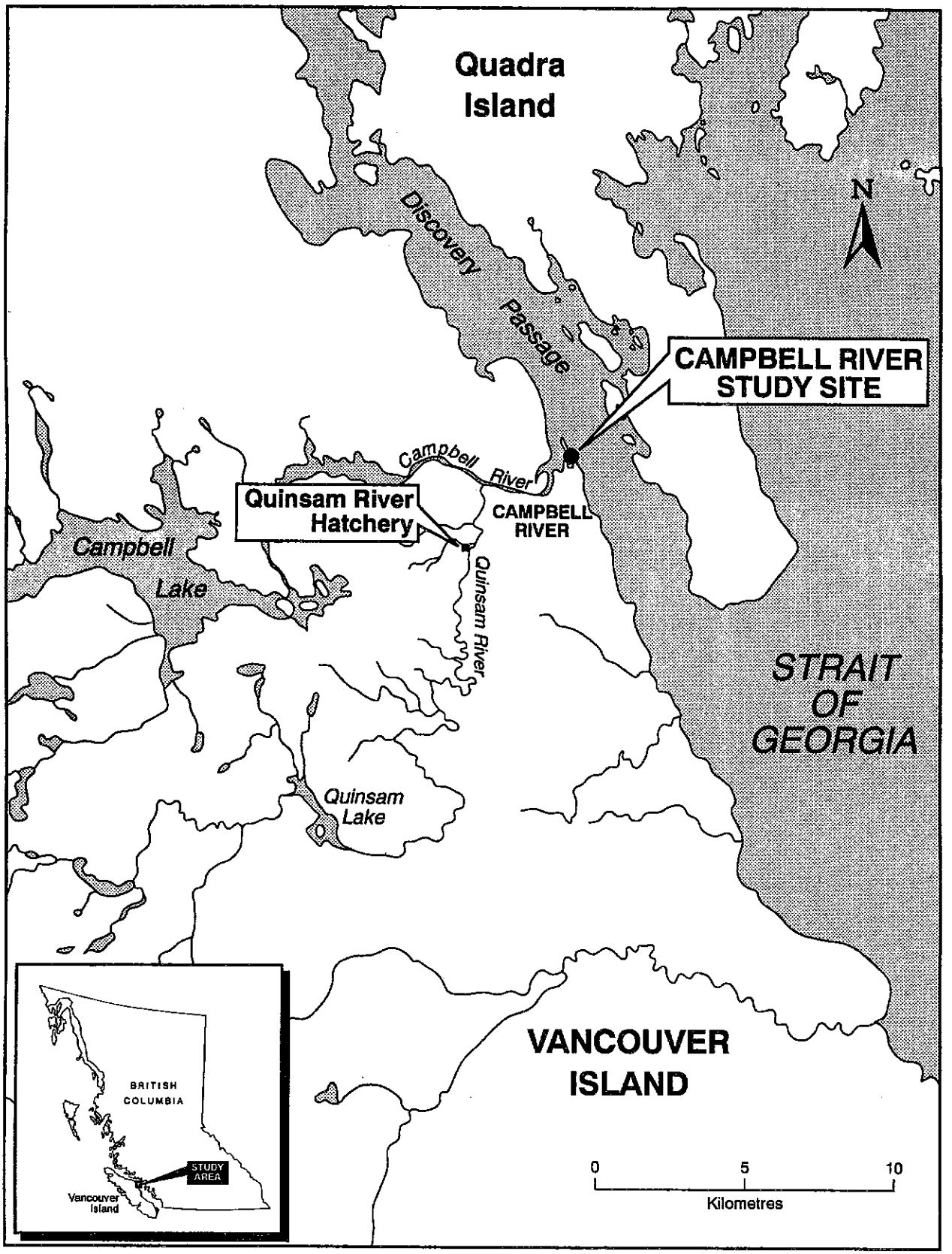


Figure 2. Map of study area near Campbell River B.C.

[Figure 3: Legend]

1. Primary seawater pump.
2. Secondary seawater pump.
3. Air equilibration tower.
4. Seawater header tank.
5. PVC flow control valve.
6. Fish holding trough.
7. Trough outflow (to waste).
8. Polyetheylene wool filter.
9. Seawater supply to WSF apparatus.
10. WSF apparatus.
11. Crude oil supply.
12. Oil metering pump.
13. WSF header tank.
14. WSF flow control valves.

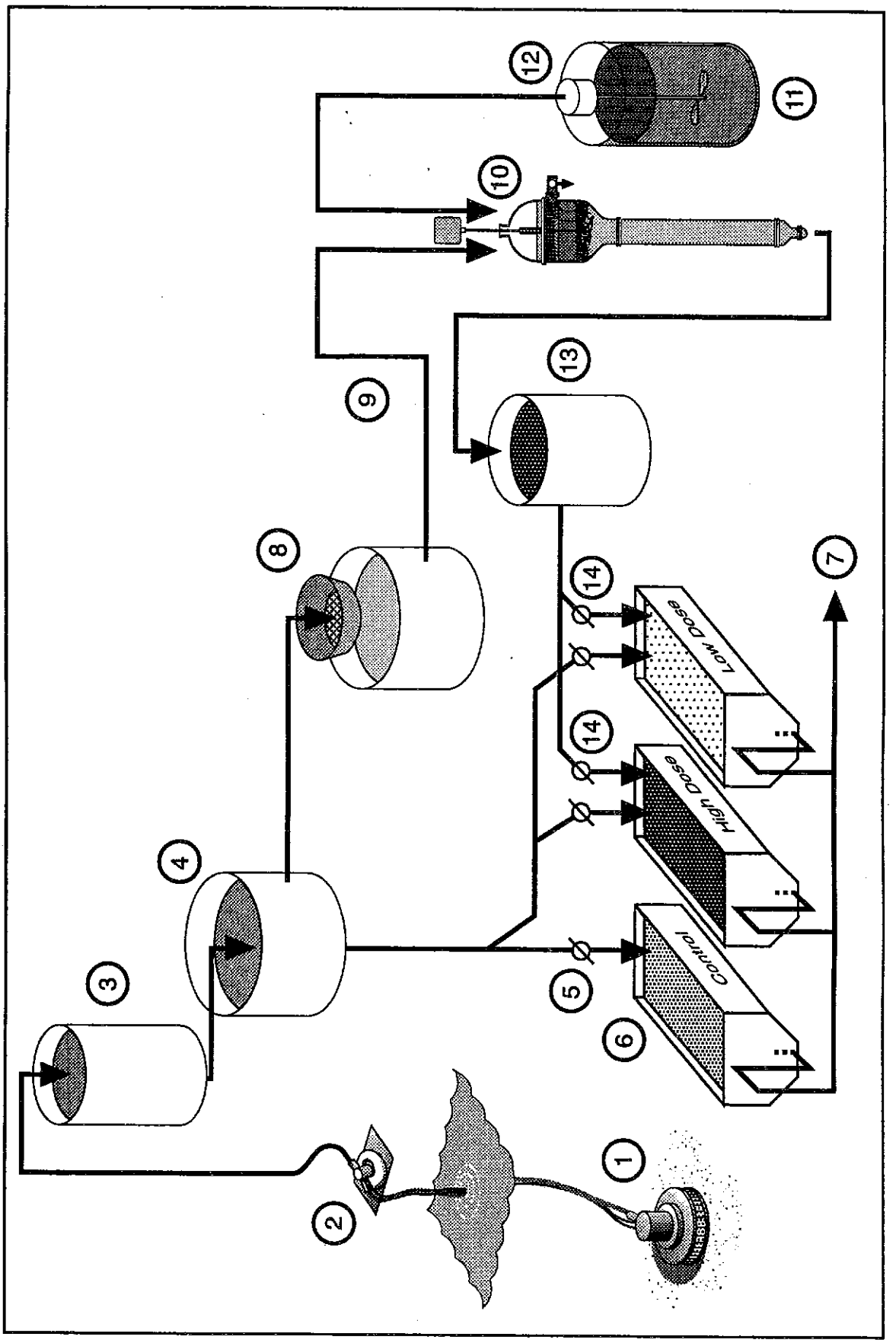


Figure 3. Schematic diagram of experimental apparatus.

[Figure 4: Legend]

1. Polyetheylene wool filter.
2. Filtered sea water
3. teflon-glass flow control valve.
4. 60RPM stirrer.
5. Upper seawater chamber.
6. Pipette plate.
7. Floating crude oil layer.
8. Crude oil-sea water interface (bubbles)
9. Crude oil water soluble fraction (WSF)
10. Cleanout drain.
11. WSF output flow
12. WSF reservoir.
13. To exposure troughs or bioassay apparatus.

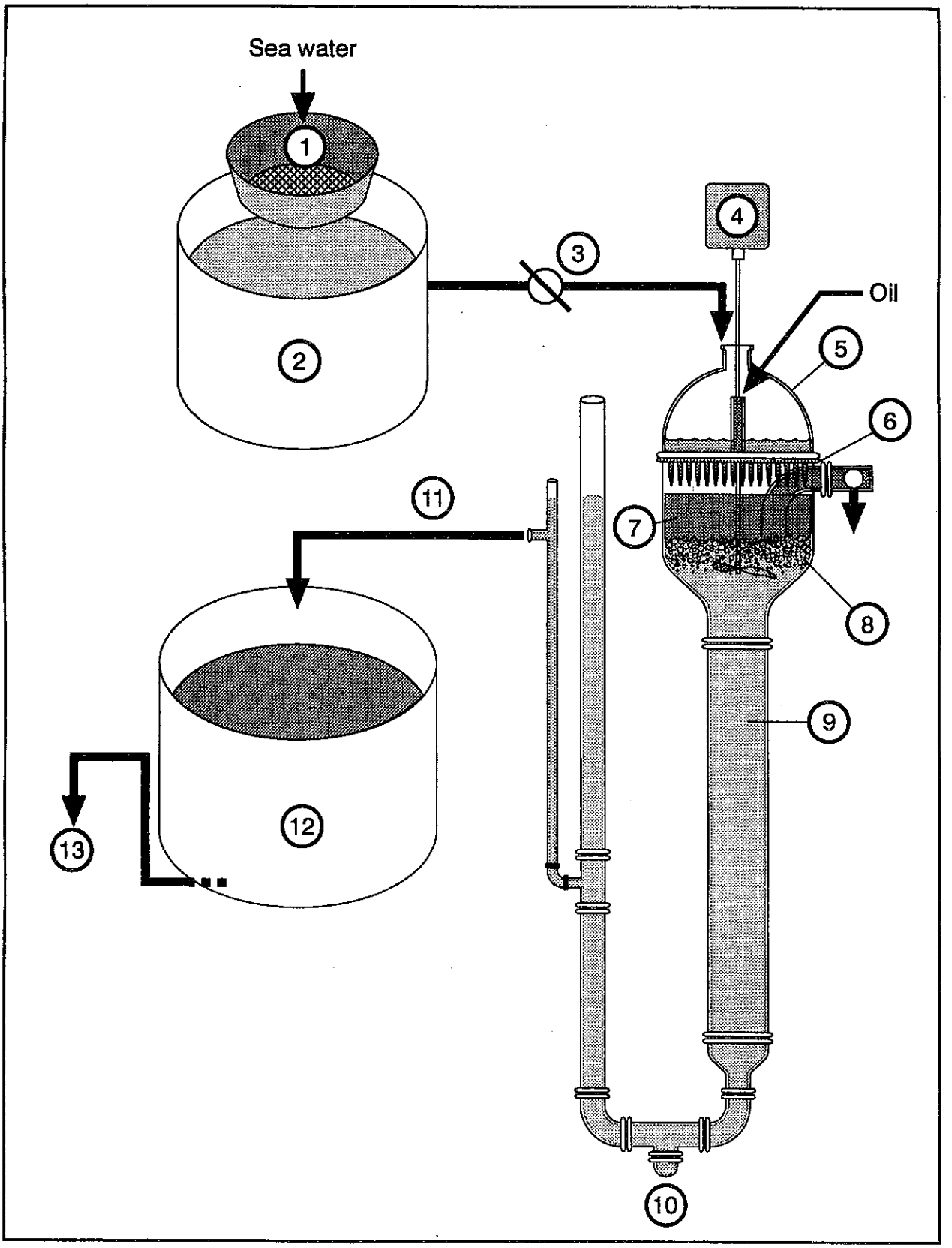


Figure 4. Crude oil WSF extraction apparatus.

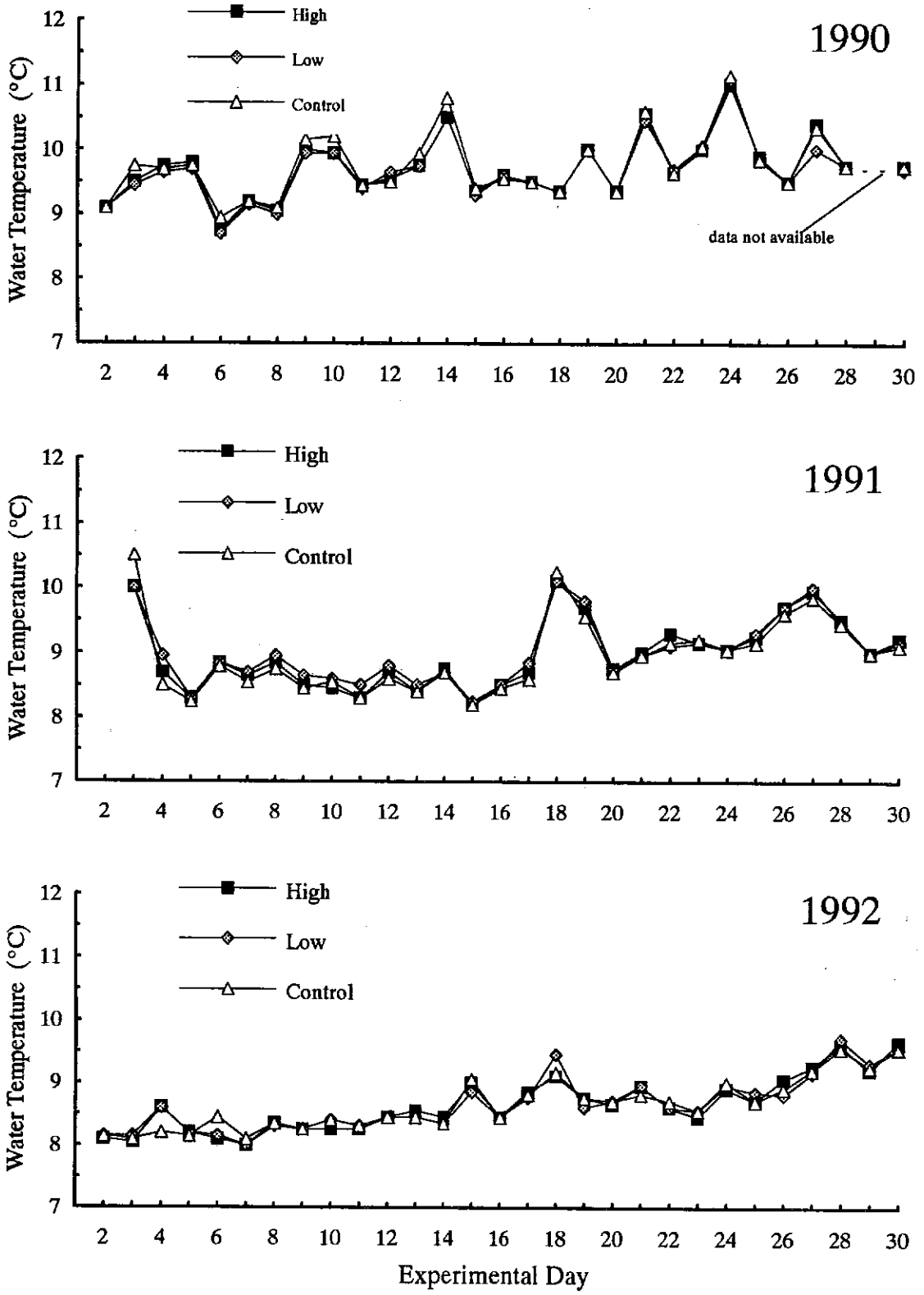


Figure 5. Seawater temperature in the holding troughs.

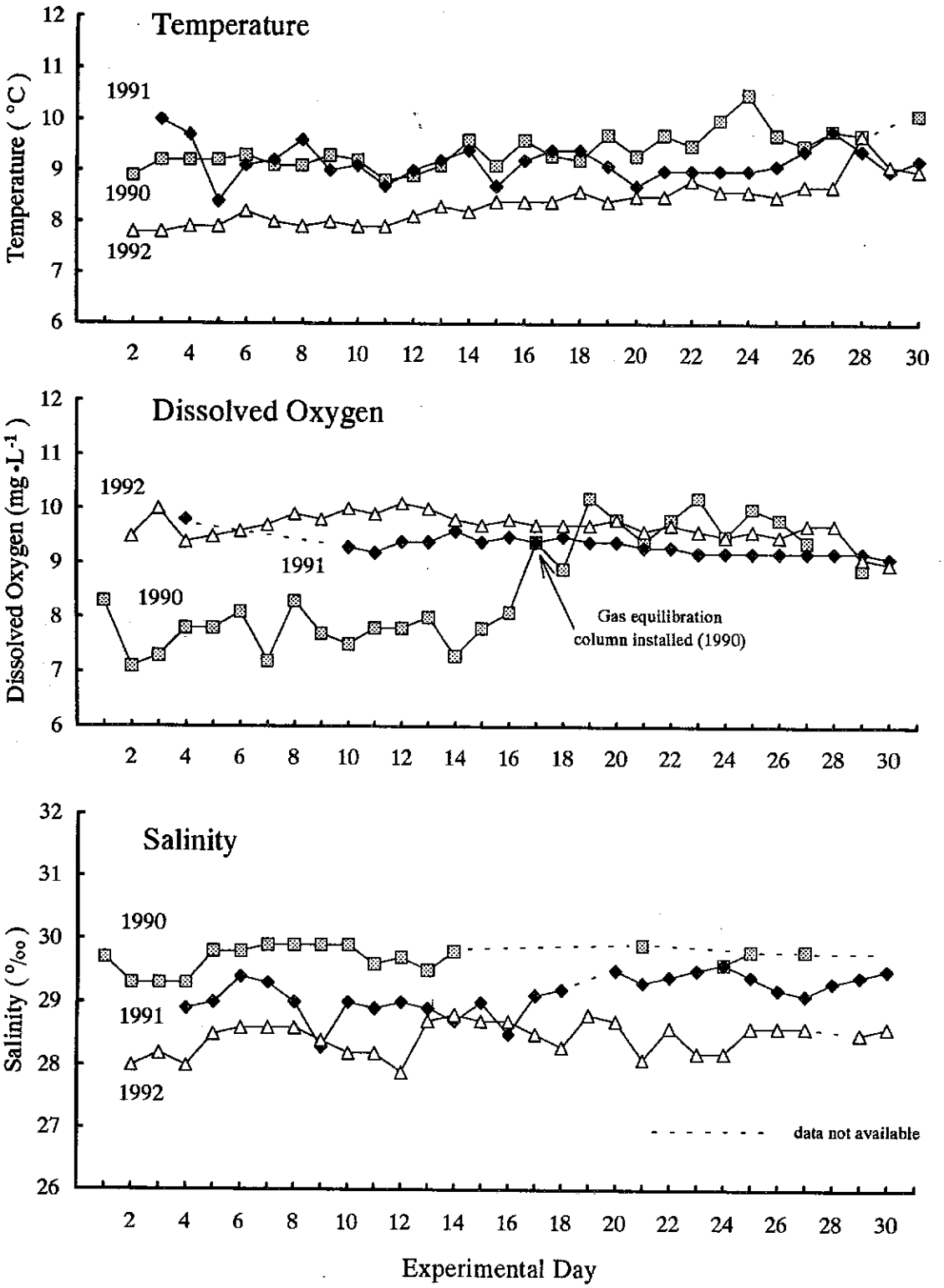


Figure 6. Seawater header temperature, dissolved oxygen and salinity.

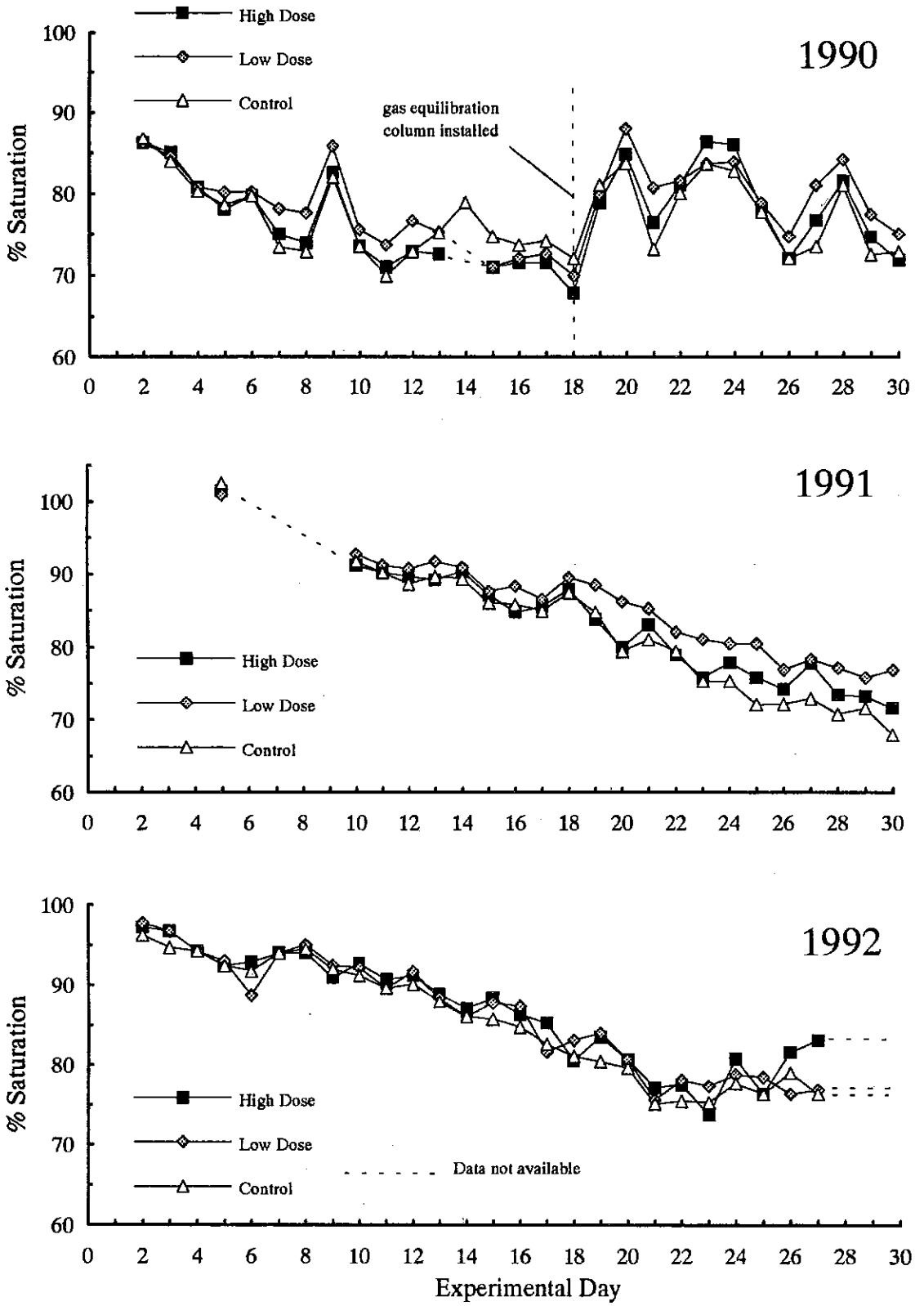


Figure 7. Dissolved oxygen saturation in the holding troughs for each treatment (1990-1992).

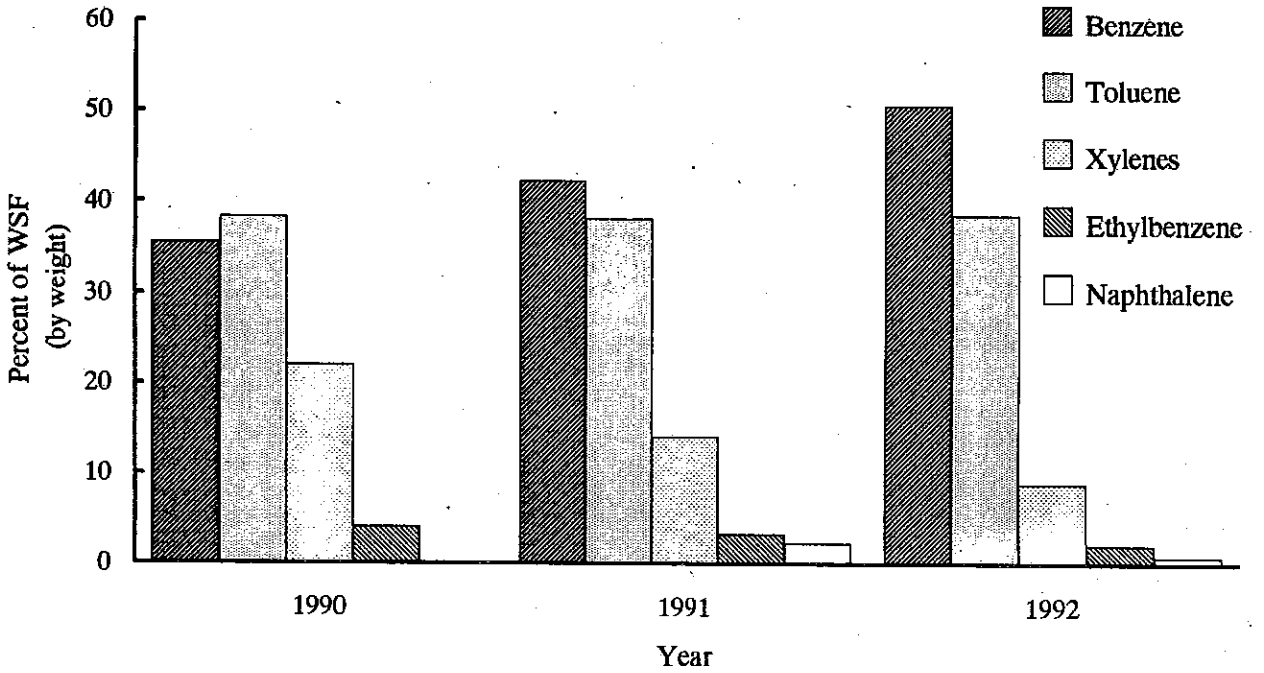


Figure 8. Composition of WSF (%) in pink salmon exposure troughs.

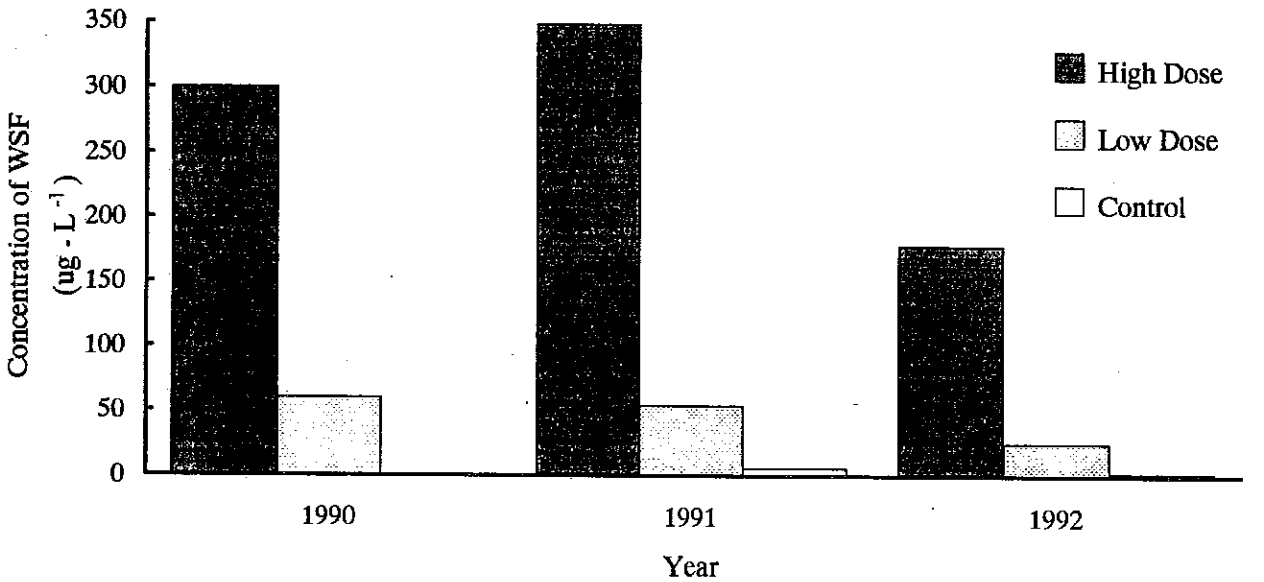


Figure 9. Concentration of WSF in pink salmon exposure troughs.

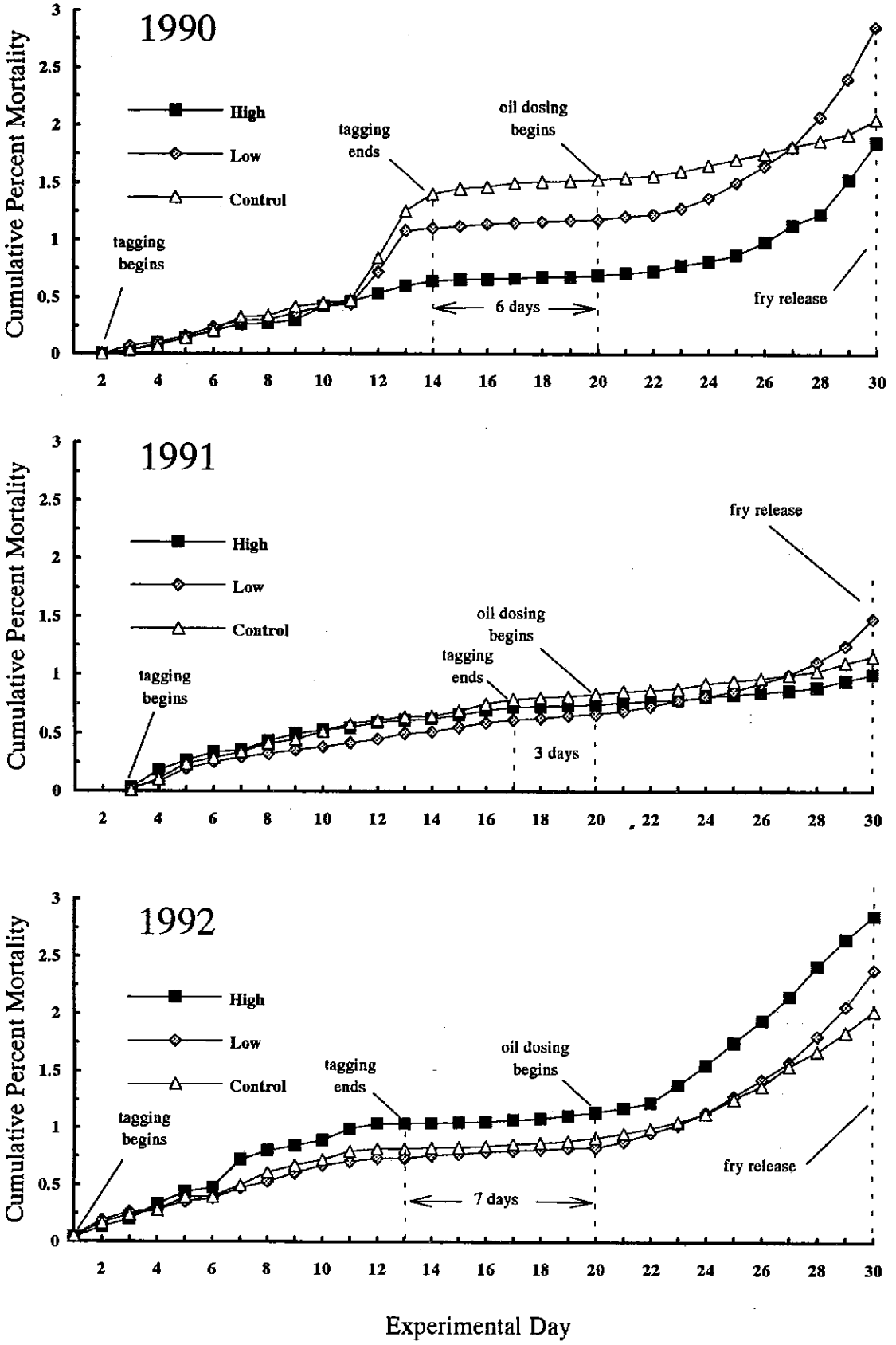


Figure 10. Cumulative fry mortality by treatment group and year.

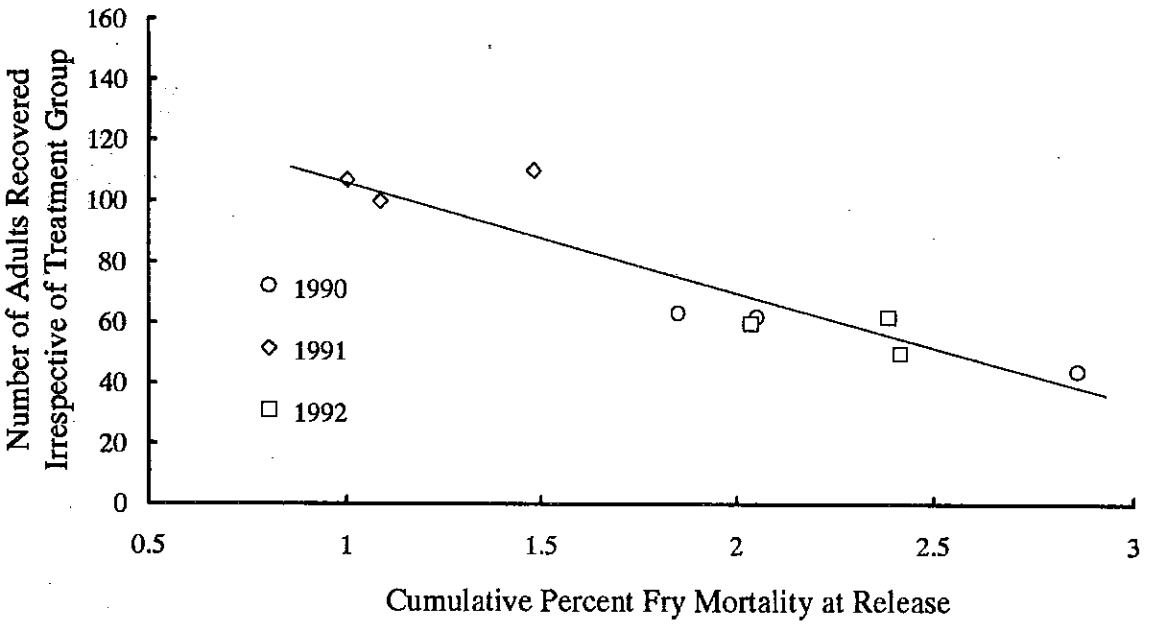
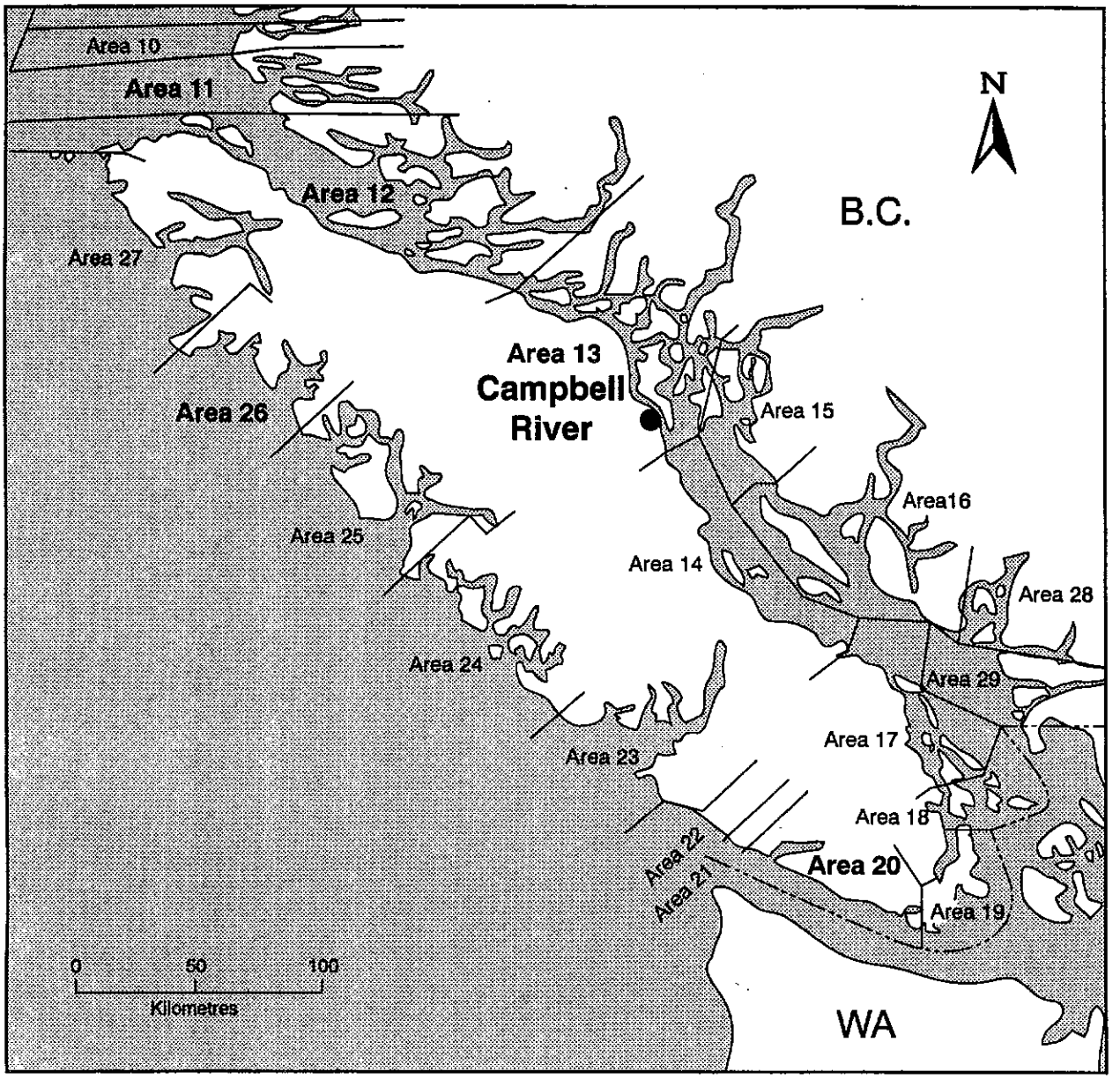


Figure 11. Correlation between fry mortality and the number of adults recovered.



Capture year	No. of tagged adult pink salmon recovered in fishery					
	Area 11 *	Area 12	Area 12 or 13	Area 13	Area 20	Area 26
1991	-	25	3	14	1	-
1992	1	14	19	64	-	11
1993	-	2	-	77	-	-

* - Department of Fisheries and Oceans Statistical Area

Figure 12. Location of adult pink salmon recovered from the commercial fishery.

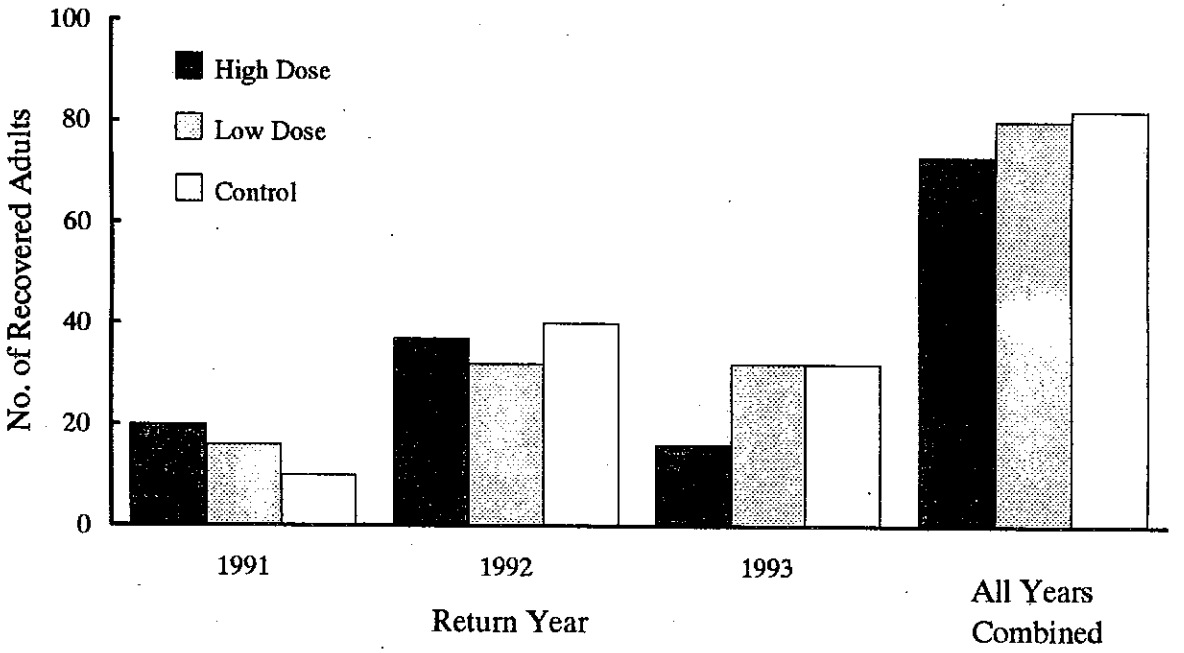


Figure 13. Number of tagged adult pink salmon recovered from the commercial fishery.

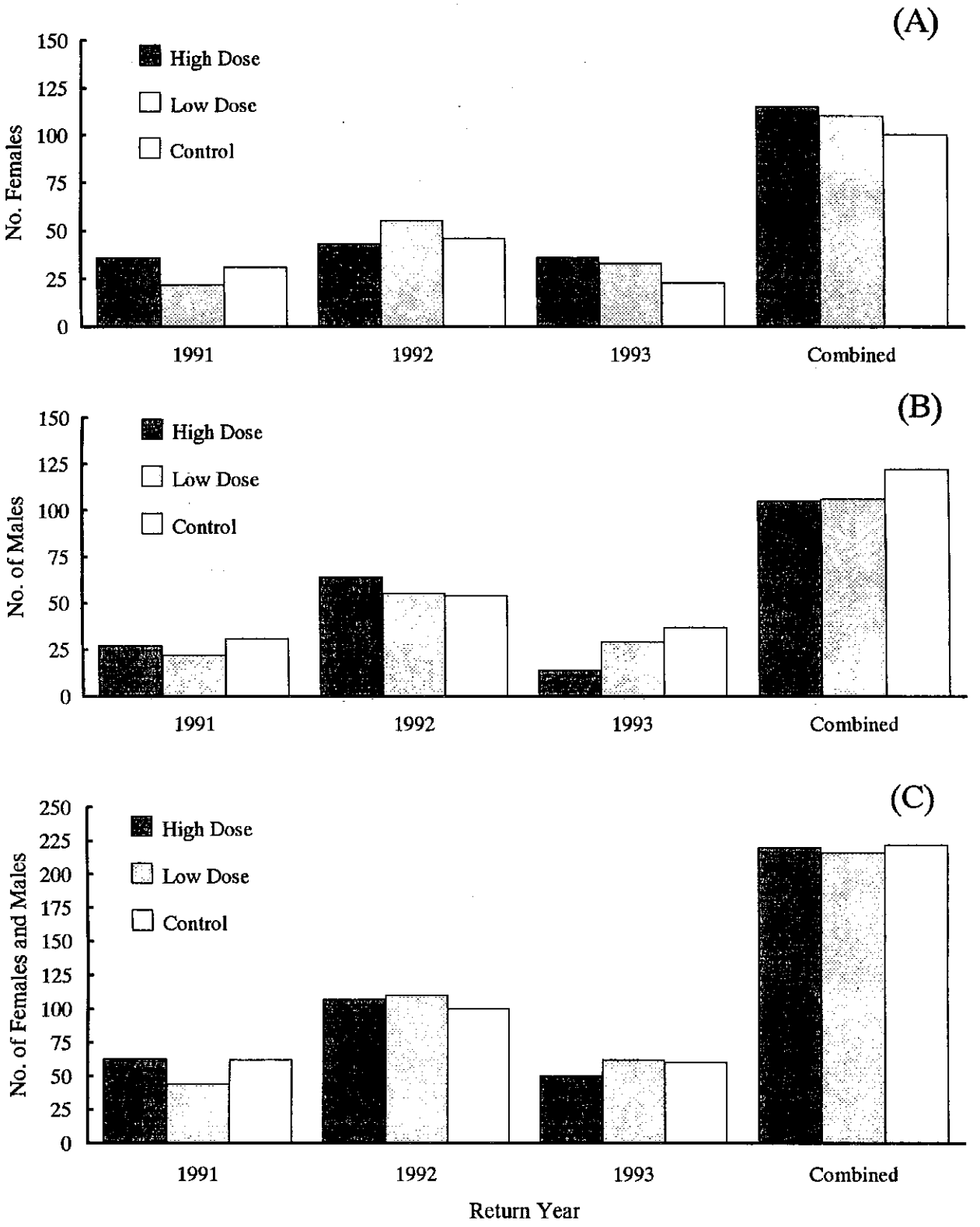


Figure 14. Number of tagged adult pink salmon recovered from the Quinsam River. (A) - Females: (B) - Males: (C) - Females and Males

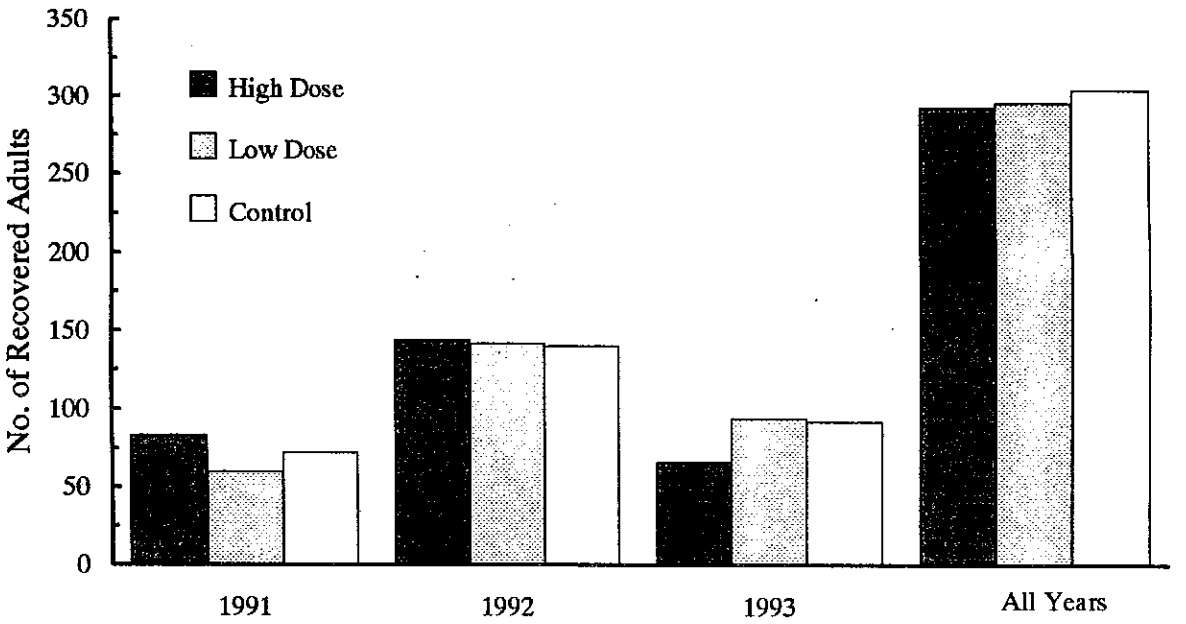


Figure 15. Total number of tagged adult pink salmon caught in the commercial fishery or in their natal stream.