HYLEBOS FISH INJURY STUDY
ROUND II

Part 2: Effects of Chemical Contaminants from the Hylebos Waterway on Growth of Juvenile Chinook Salmon

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EXECUTIVE SUMMARY

The Hylebos Waterway of Commencement Bay, an urban estuary in central Puget Sound in the state of Washington, is severely contaminated with a variety of organic and inorganic chemicals. Juvenile salmon inhabit this waterway in the late spring and early summer before initiating their ocean migration. In 1994, Round I of the Hylebos Fish Injury Study was initiated to determine contaminant exposure in juvenile salmonids which migrate through this waterway. The findings showed that juvenile chinook and chum salmon sampled from this site were exposed to a wide range of chemical contaminants, and the levels of exposure were comparable to levels which have previously been shown to cause impaired growth, immunosuppression, and increased mortality following pathogen exposure in juvenile salmon (Collier et al. 1998). Whether juvenile salmon exposed specifically to chemical contaminants characteristic of the Hylebos Waterway suffer injurious biological effects as a result of the exposure, however, was not determined in this initial study.

The objective of the present laboratory study (Part 2 of Round II of the Hylebos Fish Injury Study) was to determine which class of chemical compounds in the contaminants associated with the Hylebos Waterway can alter (impair) the growth of juvenile chinook salmon. Specifically, juvenile salmon were exposed to either 1) hexachlorobutadiene (HCBD), a signature compound of the Hylebos Waterway, 2) an extract made from Hylebos Waterway sediment using methylene chloride as solvent (HWSE-M), 3) an extract made from Hylebos Waterway sediment using pentane as solvent (HWSE-P), 4) a model mixture composed of 10 high-molecular weight polycyclic aromatic hydrocarbons (PAHs) in proportion to PAHs in a sediment sampled from the Hylebos Waterway, 5) the PCB mixture, Aroclor 1254, 6) a reference sediment extract made from Nisqually River estuary sediment (NQSE), or 7) acetone/Emulphor, the solvent vehicle as control. Juvenile chinook salmon were exposed to the chemicals by intraperitoneal injection to ensure consistent delivery of a specific dosage. Growth was assessed by measuring changes in length and weight of the fish after a 60 day exposure period.

Significant ($p \leq 0.05$) differences in growth between juveniles exposed to contaminants and juveniles administered the solvent vehicle or the reference sediment extract were observed. Exposure to the two sediment extracts from the Hylebos Waterway (HWSE-M and HWSE-P), HCBD, as well as to the PCB mixture, Aroclor 1254, were associated with decreased growth of juvenile chinook salmon. These findings support the hypothesis that chemical contaminant exposure of juvenile salmon in the Hylebos Waterway influences their physiology such that their survival potential may be reduced. Because recruitment of salmon appears to be strongly influenced by factors acting on the first year of ocean life (Pearcy 1992), these results suggest that the risk for increased juvenile mortality and subsequent decreased adult recruitment is

potentially greater for juveniles exposed to contaminants during their residence in the Hylebos Waterway. However, the level of increase risk cannot be determined from the current information.

INTRODUCTION

Estuaries are critical habitats for juveniles of several Pacific salmon species during their transition to life in the ocean (Levings and Bouillon 1997). Estuarine habitats provide refuge from predators, rich food supply to support rapid growth, and are where juveniles make the final transition from freshwater to marine conditions (Thorpe 1994). Urban estuaries, however, receive inputs of toxic anthropogenic substances from a variety of sources, and many of these chemicals can accumulate in sediments and thus can be retained in the estuary. There is growing concern that while juvenile salmon are undergoing numerous physiological adaptations during their residence in estuarine environments, any additional stress such as exposure to toxic chemicals, may be injurious to their growth and survival.

The Hylebos Waterway of Commencement Bay, in central Puget Sound, in the state of Washington, is severely contaminated by a variety of organic and inorganic chemicals; and juvenile chinook salmon inhabit this waterway in the late spring and early summer. In 1994, Round I of the Hylebos Fish Injury Study was initiated to determine whether efforts to reduce chemical contaminant input into the waterways of Commencement Bay (contaminant source control) had resulted in improvements in habitat quality, as determined by: 1) decreases in prevalence of liver disease in flatfish, 2) normal reproductive function in flatfish; and 3) minimal contaminant exposure in juvenile salmonids migrating through this waterway. The findings of this study (Round I) showed that there have been no appreciable changes in disease prevalence or apparent contaminant exposure of flatfish from this site since the 1970s. Moreover, female flatfish from the Hylebos Waterway were showing evidence of precocious sexual maturation in young animals and inhibited gonadal development in older fish. More importantly for the report provided below, the results also showed that two species of juvenile salmon sampled from this site were exposed to a wide range of chemical contaminants and in particular showed increased exposure to chlorinated compounds (HCBD, HCB) that are elevated in Hylebos Waterway sediments compared to other sites in Puget Sound. HCBD is considered a marker chemical for the Hylebos Waterway. The levels of exposure to CHs and PAHs are comparable to levels which have previously been shown to cause impaired growth, immunosuppression, and increased mortality following pathogen exposure in juvenile salmon from the contaminated Duwamish Waterway, Puget Sound (Arkoosh et al. 1998, Casillas et al. 1995b). Thess studies provide the scientific rationale for determining if juvenile salmon, exposed to chemical contaminants characteristic of the Hylebos Waterway, exhibit injury from such exposure. However, the level of increased risk cannot be determined from the current information.

In our previous studies (Casillas et al. 1995a, b) designed to assess the effects of contaminants on the growth, juvenile fall chinook salmon were collected from an urban estuary

(Duwamish Waterway) and from non urban estuaries (Skokomish River and Nisqually River) as well as their respective releasing hatcheries. Collected fish were held in the laboratory for up to 90 days and differences in growth were evaluated with respect to previous exposure to urban contaminants. We found that juvenile chinook salmon from the contaminated estuary did not grow as well as fish from the corresponding hatchery on the Green River. In contrast, juvenile fall chinook salmon from the non urban estuaries showed no difference in growth compared to fish from the corresponding hatchery on the Skokomish River. Furthermore, when we measured the concentration of plasma hormones that are involved in regulation of growth in fish, such as thyroxine (T4), triiodothyronine (T_3), and insulin-like growth factor (IGF), we found that fish from the urban estuary had lower IGF levels than fish from the corresponding hatchery as well as from the non urban estuary and hatchery. These findings indicated that juvenile chinook salmon exhibiting contaminant-associated modulation of endocrine factors had impaired overall growth (Casillas et al., 1995a, b). The ecotoxicological implication is that mortality is potentially increased for juvenile salmon using contaminated estuary sites.

Accordingly, having previously shown that juvenile fall chinook and chum salmon are exposed to contaminants during residence in the Hylebos Waterway (Collier et al. 1998), our aim in this study (Part 3 of Round II of the Hylebos Fish Injury Study) was to determine if contaminants associated with the Hylebos Waterway can impair the growth of juvenile chinook salmon. Specifically in June 1997, juvenile chinook salmon were exposed to one of the following compounds or suite of compounds: 1) hexachlorobutadiene (HCBD), a signature compound of the Hylebos Waterway, 2) a methylene chloride extract of sediment from Hylebos Waterway (HWSE-M), 3) a pentane extract of sediment from Hylebos Waterway (HWSE-P), 4) a model mixture composed of 10 high-molecular weight polycyclic aromatic hydrocarbons (PAHs) in proportion to these PAHs in a sediment sample from the Hylebos Waterway, 5) the PCB mixture (Aroclor 1254), 6) a reference sediment extract made from Nisqually River estuary sediment (NQSE), or 7) acetone/Emulphor, the solvent vehicle control. The chemical contaminants were administered at sublethal concentrations. These were determined from the results of the 96 hr LD_{50} experiment for PAHs and PCBs with juvenile chinook salmon (Arkoosh et al. 1994) or based on our experience with sediment extracts. HCBD was administered at 20% of the 96-hr LD_{50} data for fish other than salmonids (Jorgensen et al. 1991). Growth was subsequently measured after a 60 day period. The hypothesis being tested was that growth of juvenile chinook salmon is reduced by contaminant exposure and impairment of growth is dependent on the chemicals or chemical mixtures used.

METHODS

Experiments in the growth study are described in detail in the SAP (Appendix 1). The chemicals identified in the NQSE, HWSE-M, HWSE-P and their concentration are listed in Table 1, 2, 3, respectively. The composition of the PAH model mixture is listed in Table 4.

Exposure Assessment

Information on the level of exposure for each treatment generated in subsequent experiments of the growth study is described in the SAP (Appendix 1).

Statistical methods - growth studies

Differences in mortality among the treatment groups were evaluated using contingency analysis (Zar 1978). Statistical significance of the growth studies were assessed using one factor ANOVA to assess treatment effects and a two factor nested ANOVA to evaluate the influence of tanks on the outcome. Significant differences compared to the control groups of fish (fish receiving acetone/Emulphor or the reference Nisqually River estuary sediment extract) were evaluated using Dunnett's Multiple Comparison Test (Zar 1978) at $\alpha = 0.05$. A one-tailed test was employed, because the hypothesis being tested was whether contaminant treatment reduced growth. ANCOVA was used to evaluate differences in growth over time amongst the treatments at $\alpha = 0.05$.

RESULTS

Sediment Extract Chemistry

The objective of administering HCBD, Aroclor 1254, HWSE-M, HWSE-P, and a model mixture of PAHs was to expose juvenile salmon to compounds representative of chemical contaminants in the Hylebos Waterway. HCBD was administered at a dose of 21 mg/kg of body weight. NQSE was prepared from sediment collected near the mouth of the Nisqually River. Chemical amounts and type in this extract are representative of sediment from a site that is not urbanized (Table 1). Juvenile chinook salmon treated with NQSE served as an alternate control group, providing a more direct opportunity to evaluate the influence of contaminant extracts, via injections, on fish growth. HWSE-M and HWSE-P were prepared from sediment collected near the mouth of the Hylebos Waterway using two different methods. Sediments from the Hylebos Waterway were taken at Stations HY-07, -08, and -09. All sediment sites were designated and analyzed during the sediment injury studies conducted during Phase 1 of the Hylebos Damage Assessment investigations. The objective for preparing these sediment extracts was to obtain test solutions which include chemical compounds that are present in sediment from the Hylebos Waterway using two different methods to isolate non polar toxic CHs and AHs (polar compounds were removed from the extracts by eluting through a silica column). The composition of analytes in the two Hylebos Waterway extracts is listed in Table 2 and 3. The

major difference in these methods was that pentane or methylene chloride were used as solvents in the extraction process. In the pentane extract of the Hylebos Waterway sediment (HWSE-P), the proportion of high-molecular weight AHs (HMWAHs) was reduced relative to the CHs; therefore, the ratio of CHs to HMWAHs was increased (from a ratio of 1.7 in the HWSE-M to a ratio of 2.8 in the HWSE-P). As described in the SAP, it was anticipated that a fraction containing reduced levels of both HMWAHs and low-molecular weight AHs (LMWAHs) could be prepared; however, from the perspective of the experimental design, it was not practical to eliminate or greatly reduce the proportion of both HMWAHs and LMWAHs without losing the CHs as well. Nevertheless, using pentane rather than methylene chloride in the extraction process reduced HMWAHs nearly 47% while maintaining the chlorinated butadienes at nearly 88% of original levels (Tables 2 and 3). Hence, these two extraction approaches did provide an opportunity to evaluate whether there is an interaction between HMWAHs and CHs in affecting the growth of juvenile salmon. The chemicals and concentrations present in the HWSE-M represent the full range of chemicals (AHs and CHs) that may be available to fish. The final concentration of chemicals in each of the extracts (NQSE, HWSE-M) was 200 g sediment/ml of acetone, and the final concentration of chemicals in the HWSE-P was 400 g sediment/ml of acetone. A model mixture of polycyclic aromatic hydrocarbons (PAHs) containing 10 high molecular weight PAHs was also prepared to reflect the same ratios of these analytes previously found in sediment during Phase I of the Hylebos Damage Assessment studies at Station HY-24. The PCB mixture, Aroclor 1254, was administered to evaluate the effect of PCBs using a mixture which is representative of PCB congeners found in the Hylebos Waterway sediment. Concentrations of the PCBs, and the model mixture of PAHs given to juveniles were equal to 20% of the 96hr LD_{50} (Arkoosh et al. 1991). All test solutions, including controls were each administered at a volume equivalent to 1.5 µl solution/g fish. Dosages administered to experimental fish were described in detail in the methods section of the Hylebos Interpretive Report /Round II- Tissue concentrations and biochemical responses.

Temperature Profile, Feeding Rates, and Mortalities

Ambient water temperatures during the course of the study ranged from 11.6°C at the start of the study (June 26, 1997) to 12.8 °C at the end of the 60 day growth period (Figure 1). The mean (± SD) of water temperature was 12.2± 0.4 ^{0}C. The small variation in temperature was in part due to drawing seawater from a depth of approximately 50 feet, thereby minimizing the influence of more rapid changes in sea surface temperatures.

Feeding rates ranged from 3.6% to 3.7% body weight/day for each treatment and ranged from 3.5% to 3.7% body weight/day for each of the tanks of juvenile chinook salmon (Table 5). Replicate groups of tanks were maintained for each injected control or treatment group of

juvenile chinook salmon, primarily to minimize the risk of loss of all experimental fish for each treatment from an unforeseen catastrophic event.

Recorded mortalities of experimental fish ranged from 5.5% to 18% (11 to 36 animals) after a 60 day growth period from a total of 200 fish for each of the treatment groups (Figure 2). The highest number of mortalities were observed in juveniles receiving either the model PAH mixture or the PCB formulation (Aroclor 1254) with only the group receiving the model PAH mixture exhibiting a significantly higher mortality than the average mortality (9.4%) among all groups. In contrast, the lowest mortalities were observed in juveniles receiving the reference Nisqually sediment extract or the Hylebos Waterway pentane-extract. Most of the mortalities were observed during the first 2 to 3 weeks of the growth study (Table 6). Mortalities were low and stable throughout the remainder of the growth period. At the end of the 60 day period, the number of observed mortalities and the number of fish remaining in all treatments did not always add up to 200. The number of fish at the end of the experiment was typically less (ranged from 1 to 10 fewer animals per tank) than what was expected based on recorded mortalities. This difference was attributed to losses of fish that escaped from the tanks through the exiting water standpipe, or by jumping out of the covered tanks. For example, 3 dead fish were found on the floor one day during the experiment; and we were not able to determine which tank (treatment) they came from, therefore they were not included in our mortality record. Although nets were carefully placed on top of tanks to minimize escapes, it is difficult to make the tanks escape-proof. Moreover, in one instance, we had more fish (2 "extra" fish) in a tank than was expected. These excess fish may be due to an error in the mortality record.

Growth Studies

The average starting size of juvenile chinook salmon in the seven different treatment groups ranged from 93 mm to 94 mm in fork length (Table 7) and 7.9 g to 8.2 g in weight (Table 8). An approximate 43% increase in length and a 290% increase in weight was observed after a 60 day growth period for all fish. At the end of the study period, fork lengths ranged from 133 mm to 135 mm and weights from 29.6 g to 32.1 g for the various treatment groups of fish. Juvenile chinook salmon exposed to the PCBs (Aroclor 1254), HCBD, the HWSE-M, or the HWSE-P were significantly smaller (ANOVA $p \leq 0.05$, Dunnett's One-Tailed Test) in length, or weight than fish exposed to the solvent vehicle. Fish exposed to either the PCBs (Aroclor 1254), HCBD, or the Hylebos Waterway sediment extracts were generally 2 mm shorter in fork length or 2.5 g lighter in mass than fish exposed to the solvent vehicle, or the reference sediment extract (NQSE). It is important to note that the size of juveniles exposed to the two Hylebos Waterway sediment extracts at the end of the of the exposure period may be, in part, due to the significantly smaller lengths and weights of these groups at the start of the experiment. These fish were

smaller by approximately 1 mm and 0.3 g at the start of the experiment than fish exposed to the solvent vehicle, or the reference sediment extract (NQSE). However, this difference at the start of the experiment was not considered to affect the differences at the end of the 60-day exposure period. Moreover, because juveniles exposed to either PCBs or HCBD at the start of the experiment were not significantly different in size than fish exposed to the solvent vehicle, or the reference sediment extract (NQSE), the differences after the 60 day exposure period are likely the result of these particular treatments.

The experimental design in this study was to include a replicate tank for each treatment to insure that a treatment was not lost if there was an accidental loss of water to a tank. If, however, we evaluate the differences in length or weight of fish for each of the treatments, and included tanks as a nested variable within the experimental design, the variation with respect to tanks was significant (ANOVA, $p \leq 0.05$), and treatment differences in length or weight were no longer significantly different ($p > 0.05$). The tank effect is attributable to the greater variation between tanks in mean length and weight of juveniles exposed to either PCBs, HCBD, or the model PAH mixture (Figures 3 and 4). In one of the two tanks of fish exposed to either PCBs, HCBD, or the model PAH mixture, mean length or weight falls well below the 95% confidence interval for expected length or weight after a 60 day growth period. This confidence interval was generated from juvenile salmon exposed to the solvent vehicle - acetone:Emulphor, or exposed to the reference sediment extract (NQSE). In the remaining tanks of fish exposed to these three treatments, mean length or weight is above or within the 95% confidence interval for control mean length or weight after a 60 day growth period, thereby contributing to the greater variation in size between tanks. The variation in size between replicate tanks of fish receiving Aroclor 1254 or HCBD was not sufficient to negate the significant effect on size based on treatments alone, whereas the variation between tanks of fish exposed to the model PAH mixture was great enough such that fish undergoing this specific treatment was not significantly different from fish in the control or reference groups. It is important to note that for both Hylebos sediment extracts the mean lengths and weights for the replicate tanks of both extracts fell below the 95% confidence intervals of the expected length and weight for the 60 d growth period.

To evaluate differences in length or weight of juvenile chinook salmon over time and incorporate differences with respect to initial starting size, an ANCOVA was performed using the solvent vehicle as control. The increase in length of juvenile chinook salmon exposed to the PCBs (Aroclor 1254) or HCBD was significantly less (ANCOVA, $p \leq 0.05$) than fish exposed to the solvent vehicle; the NQSE group was not significantly different ($p = 0.7$) from the solvent vehicle group. The initial starting lengths of fish exposed to the Hylebos Waterway sediment extracts (HWSE-M and HWSE-P) were significantly smaller than fish exposed to the solvent vehicle, as noted earlier, and the ANCOVA showed that the increase in length over time was not

different than observed for juveniles exposed to the solvent vehicle. However, the increase in weight of juvenile chinook salmon exposed to the PCBs (Aroclor 1254) and HCBD, as well as the HWSE-M and HWSE-P was significantly less (ANCOVA, $p \leq 0.05$) than fish exposed to the solvent vehicle. No difference in starting weights among the treatment groups were observed in this analysis.

Differences in growth of fish can also be visualized by plotting the cumulative frequency distribution of all fish exposed to the various treatments in this study. Although the size distribution in lengths and weights of juvenile chinook salmon at the start of the experiment were not large, the ranges at the end of the 60 day growth period revealed considerable expansion of this distribution. For example, the smallest to the largest fish at the start of the experiment ranged from 85 to 98 mm in fork length and 5.5 to 11.0 g in weight. At the end of the experiment, however, the size range expanded to 112 to 153 mm in fork length and 18.0 to 48.7 g in weight for all treatment groups. Displaying size distribution characteristics provides a better means to visualize differences in growth between groups over the experimental period, but statistical support for interpretation is drawn from the ANCOVA and ANOVA results. When we plot the cumulative weight frequency distribution of juvenile chinook salmon exposed to the solvent vehicle (acetone/Emulphor) and the reference sediment extract from the Nisqually River estuary (NQSE), after a 60 day growth period, the cumulative size distribution is essentially identical (Figure 5). This is consistent with the similarity in average weight of these fish as shown in Table 8. In addition, the plot of the weight distribution of juvenile chinook salmon exposed to the model PAH mixture (Figure 6) as compared to that of juvenile chinook salmon exposed to acetone/Emulphor, does not reveal any significant difference. This, again, is consistent with the overall similarity of the weight of these fish at the end of the experimental period. In contrast, the weight frequency distribution plot of juveniles exposed to HCBD (Figure 7) begins to reveal differences, which become more marked for fish exposed to the PCB mixture (Figure 8), or to the Hylebos Waterway sediment extracts (Figures 9 and 10) when compared to that of fish exposed to acetone/Emulphor. This finding agrees with the results shown in Table 8. It is noteworthy that, although the differences in absolute weight were moderate, the weight distribution pattern of fish exposed to Aroclor 1254 or the Hylebos Waterway sediment extracts revealed a clearer difference when compared to fish from the control groups.

DISCUSSION OF MAJOR FINDINGS

Juvenile chinook salmon exposed to contaminants specific to the Hylebos Waterway showed reduced growth.

The findings from this study showed that chemical contaminant exposure of juvenile salmon in the Hylebos Waterway can decrease their growth. Alterations in growth are linked to increased mortality in wild fish, including salmon (Sissenwine 1984, McGurk 1996). Clearly other ecological factors can also affect growth in fish (Brandt 1992). Although the magnitude of an increase in mortality in relation to the growth inhibition observed cannot be determined from the current findings, the results of this laboratory study are supportive of the hypothesis that chemical contaminants can decrease growth and consequently increase the risk of mortality. Juvenile chinook salmon exposed to contaminants associated with the Hylebos Waterway exhibited slower growth than did juvenile chinook salmon treated with the solvent vehicle or a sediment extract from a reference area. Although the growth suppression was statistically not large, representing a maximum 6% and 7% decrease in length or weight gain, respectively, the reduction was significant. These findings suggest that reduced growth may occur in salmon exposed to some of the chemical contaminants specific to the Hylebos Waterway. At the dosages tested, some of the treatment groups, which represent specific subsets of chemical contaminants characteristic of the Hylebos Waterway, appeared to equally suppress the growth of juvenile chinook salmon. Chlorinated hydrocarbons, characterized by the PCB mixture and HCBD, affected the growth of juvenile chinook salmon. The two sediment extracts from the Hylebos Waterway (HWSE-M and HWSE-P) containing chemical contaminants, including chlorinated hydrocarbons, and polycyclic hydrocarbons, also reduced growth, whereas growth of juvenile salmon over the 60-day period was not significantly affected by the high-molecular weight PAHs. These findings suggest that the chlorinated hydrocarbons including PCBs and the chlorinated butadienes, rather than the aromatic hydrocarbons may be the contaminants primarily contributing to the reduced growth of juvenile chinook salmon exposed to the two Hylebos Waterway sediment extracts. The conclusion of an apparent lack of ability of PAHs to impair growth of juvenile salmon must be tempered when we consider the variation in growth observed between tanks of fish receiving this contaminant type. The mean length and weight of at least one tank of fish receiving the model mixture of PAHs grew as poorly as fish exposed to PCBs or either of the Hylebos Waterway sediment extracts. However, because the duplicate tank of fish grew as well, if not slightly better than the control groups, a significant reduction in growth of juveniles exposed to the model PAH mixture was not evident. Nevertheless, overall chlorinated hydrocarbons, exemplified by the PCBs and HCBD, appeared to be consistently associated with

a small but significant reduction in the growth of juvenile salmon under the experimental conditions employed.

Juvenile chinook salmon in this study were injected with sediment extracts or model compounds in order to administer the contaminants at well-defined and controlled dosages to facilitate comparisons among treatment groups. The overall objective was to assess the impacts of chemical contaminant types found in the Hylebos Waterway on growth of juvenile chinook salmon. While juvenile chinook salmon were exposed to chemical contaminants at a dosage higher than realistically present in the environment, they were held under rather optimum conditions (they were well fed and did not have any competition or predators during the experiment). Thus, the impacts on growth of experimental fish may have been minimized when interpreted in the context of the ecosystem and integrated with the activities that fish must undertake to survive in the natural environment.

The reduced growth of salmon exposed to contaminants associated with the Hylebos Waterway observed in this study are consistent with previous findings (Casillas et al. 1995a, b) showing impaired growth and altered immune function in juvenile chinook salmon from the Duwamish Waterway in Elliott Bay, also located in Puget Sound, WA. We found that juvenile salmon from the Duwamish Waterway estuary showing increased exposure to CHs and AHs did not grow as well as juvenile salmon that are out migrating through the reference estuaries of the Nisqually and Skokomish Rivers. In addition, the reduced growth of juveniles exposed to chemical contaminants in the Duwamish Waterway, after a 60 to 90 day period, was of approximately the same magnitude as that observed in this study. It is important to note that the level of exposure to chemical contaminants in fish sampled from the Duwamish estuary was lower than exposure in the laboratory study reported here. The finding of reduced growth in both studies suggests that the threshold for chemical contaminants affecting growth of juvenile salmon is within the range of environmental exposure.

Chemical contaminants also affect other physiological functions in juvenile salmon. Arkoosh et al. (1991, 1998) showed a suppressed secondary immune response in white blood cells of anterior kidney to specific model antigens and a greater percent of cumulative mortality to *V. anguillarum* after natural exposure to chemical contaminants, including PAHs and PCBs in the waterway. In addition, juvenile salmon exposed to a model PAH, dimethylbenz[a]anthracene (DMBA) or a PCB mixture (Aroclor 1254) in the laboratory exhibited a similar suppressed secondary immune response in white blood cells of anterior kidney to model antigens (Arkoosh et al. 1994). Both PAHs and PCBs are known to induce immunosuppression in other species (Thomas and Hinsdill 1978; Ward et al. 1985). Thus, our findings are consistent with the observations that chemical contaminants, including chlorinated hydrocarbons found in the Hylebos Waterway, could affect a range of physiological functions, including

immunocompetence and growth, in juvenile salmon. A follow up study, Round III is being designed to determine the sediment levels of contaminants at which effects (e.g. impaired growth, disease resistance) would be observed.

The ecological significance of reduced growth from exposure to chemical contaminants specific to the Hylebos Waterway is likely to be manifest during the initial phase of early ocean life and consequently could affect their survival potential in the estuary and early ocean environment.

The effect of the reduced growth observed after exposure to contaminants specific to the Hylebos Waterway, although not large, may significantly reduce the ability of juvenile salmon to effectively compete and exist in their natural environment for several reasons. First, fish growth is highly pliable and integrates various habitat conditions, thus growth has been used frequently as an indicator of ecosystem health (Brandt 1992). Moreover, the ability of a fish to achieve its optimum growth rate as a juvenile is not only a relative measure of the fish's health and conditions, it is also a measure of survival and reproductive output potential (Brandt 1992). Thus, growth is linked to a variety of important life functions supporting propagation of the species. The links in growth, survival, and reproduction among fish foster the hypothesis that slower growing or smaller animals, as they proceed through successive life stages, inherently acquire a greater chance of mortality and have lower fecundity at reproduction (Banse and Mosher 1980). In this context, factors such as chemical contaminants, which are shown to reduce growth rate in the pre-adult stages, can be linked to reduced reproductive and survival potential.

Secondly, the reduced growth of juvenile salmon is occurring as they enter the marine environment where mortality is a significant factor in controlling the numbers that are recruited to the fishery and back to their natal streams. Approximately one-half of the loss of pre-adult (egg through juvenile stage) salmon has been shown to occur in the marine environment (Bradford 1995). Predators, inter- and intra-specific competition, food availability, smolt quality and health, and environmental factors (chemical and physical) are all potentially and likely important factors that influence survival of salmon in the estuarine and near shore marine environment. Thus, factors that affect the ability of salmon to function normally in their natural environment in the short-term are likely to influence longer term survival to their reproductive stage. The reduced growth of juvenile salmon shown in the current study is therefore an indication of a decrease in survival potential; especially in an environment where extensive mortality has been documented (Bradford 1995).

Third, the observed reduced growth of juvenile chinook salmon induced by chemical contaminant exposure is occurring during a phase where growth has been shown to be critical to success of the population. Growth of salmon during the first year of ocean life appears to be critical to recruitment success (Pearcy 1992, Unwin 1997, Unwin and Glova 1997, Heath et al. 1997). This understanding is based on the positive relationship between the number of precocious male salmon (fish that mature earlier than-normal) and the commensurate success of the adult population of salmon for each year class (Pearcy 1992, Gudjonsson et al. 1995). Precocious maturation, further, appears to be linked to growth rates. Higher growth rates are associated with a higher proportion of precociously maturing salmon in the population (Friedland and Haas 1996). The issue, however, may not be if an animal is growing or is of a larger size, but rather what is the proportion of fish with the appropriate growth rate. Dickhoff et al. (1995) and Beckman et al. (1997) have shown that out migrating juvenile salmon smolts with the highest growth rates at the time of release, and not necessarily the largest smolts, survived better than juveniles exhibiting slower growth rates. Holtby et al. (1990) supported this contention for juvenile coho salmon, showing that in typical years, fish with higher growth rates survived better than juveniles with slower growth rates. It is in this context that reduced growth caused by factors, such as chemical contaminants, as shown in our study, may have long term ecological consequence for the survival of juvenile salmon inhabiting urban estuaries for part of their life cycle.

Finally, the salmon in the present study were found to have altered growth that extended well past the initial chemical contaminant exposure period, that is, reduced growth was evident two months after the initial exposure. This finding is also supported by a previous field study showing that juvenile salmon collected from the Duwamish estuary and held for two months in uncontaminated seawater exhibited suppressed growth. Our previous studies suggest that

although juvenile chinook salmon are only briefly exposed to contaminants in an urban estuary as they migrate to sea, growth altering events persist for at least 2 months, and may extend into their early ocean life. The findings in Round I of these studies showed that during a relatively brief residence in the Hylebos Waterway, juvenile chinook and chum salmon were exposed to significant levels of chemical contaminants which resulted in induction of early biological alterations indicated by elevated hepatic CYP1A and DNA damage. In the present study we demonstrated that contaminants characteristic of sediments from the Hylebos Waterway reduced the growth of juvenile chinook salmon. Because recruitment of fish to the adult stage is considered to be dependent on factors acting during the first year of life (Sissenwine 1984, Pearcy 1992), the potential for contaminants to influence size dependent mortality and mortality rates within the population is a possibility. There is uncertainty in relating the observed reduction in growth as a result of exposure to chemical contaminants described in this study to potential impacts on mortality and population structure. However, the findings from studies cited above suggest that the observed reduction in growth has the potential to increase mortality of juvenile salmon through other ecological interactions (e.g. predation, inability to acquire appropriate prey, disease) which they encounter in their natural habitat.

Quantitating the level of increased risk however, cannot be assessed from the findings of the present study. When we examined the adult return data for fall chinook salmon to urban associated river systems, such as the Puyallup (Hylebos Waterway) or the Green River (Duwamish Waterway), using the Coded Wire Tag database (PFMSC 1993), we found, however, that the average returns during the period of 1971-1991 to these urban systems were less than the average for Puget Sound river systems (Washington State hatcheries) or for major river systems for the entire state of Washington (Table 11). Although we cannot define the causal factors which affect the lower than average adult returns for fish migrating through urban estuaries, as there are many factors which contribute to the outcome, such as predation, food availability, ocean conditions, etc., the finding that chemical contaminants reduce growth of juvenile salmon, and that reduced growth is associated with lower survival potential in chinook salmon (Unwin 1997) is not inconsistent with the lower than average return rates from these urban systems.