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THE EFFECTS OF OIL-CONTAMINATED PREY ON THE FEEDING, GROWTH, AND RELATED ENERGETICS ON PINK SALMON, ONCORHYNCHUS GORBUSCHA WALBAUM, FRY

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THE EFFECTS OF OIL-CONTAMINATED PREY

ON THE FEEDING, GROWTH, AND RELATED ENERGETICS

OF PINK SALMON FRY, ONCORHYNCHUS GORBUSCHA WALBAUM, FRY

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ABSTRACT

Pink salmon, Oncorhynchus gorbuscha Walbaum, fry were exposed to oil-contaminated prey (OCP) in a series of experiments to determine the effects of oil exposure via the diet on the ability of pink fry to survive. Brine shrimp, Artemia salina, nauplii were contaminated with petroleum hydrocarbons by exposure to the water-soluble fraction (WSF) of Cook Inlet crude oil and fed to the fish. Feeding rates were measured for 10 days using OCP and for 5 days using uncontaminated prey (post-exposure period). In a separate experiment, fry growth was measured over a 50 day period. In another experiment, fry oxygen consumption, food absorption and utilization, and ammonia excretion was measured to determine the effects of OCP on fry metabolic activity.

Fry feeding rates were reduced by exposure to OCP, and remained suppressed during the post-exposure period. Chronic exposure to OCP for 50 days reduced fry growth. OCP were not lethal to the fry. There was no change in fry oxygen consumption or ammonia excretion from exposure to OCP, but the fish exposed to OCP absorbed less food than controls and continued to absorb less food for 7 days after exposure. Results indicate that exposure to OCP can reduce fry growth primarily by reducing food intake, but additional nutrition is lost from the non-absorption of ingested food. Reductions in growth could decrease fry survival, and thereby reduce the number of returning adult pink salmon.

TABLE OF CONTENTS

	Page
List of Figures	v
List of Tables	vi
Acknowledgements	vii
Chapter 1: Introduction	1
Chapter 2: Effects of Oil-contaminated prey on the Feeding and Growth Rates of Pink Salmon Fry	8
Chapter 3: Effects of Oil-contaminated prey on the Oxygen Consumption, Food Absorption, and Ammonia Excretion of Pink Salmon Fry	32
Chapter 4: Summary of Experimental Results	70
Chapter 5: Implications of Results	73
Literature Cited	78
Appendices	87
Appendix A	87
Appendix B	89
Appendix C	90
Appendix D	92
Appendix E	94
Appendix F	96
Appendix G	98

LIST OF FIGURES

Page

Figure 1. Average daily feeding rate (no. prey eaten/day/fish) of control fry, and fry fed oil-contaminated prey (± 1 SE)..... 15

Figure 2. Average length of control fry, fry fed oil-contaminated prey, and fry exposed to 0.7 ppm WSF over a 50 day period (± 95% confidence interval)..... 19

Figure 3. Average wet weight of control fry, fry fed oil-contaminated prey, and fry exposed to 0.7 ppm WSF over a 50 day period (± 95% confidence interval)..... 20

Figure 4. Average dry weight of control fry, fry fed oil-contaminated prey, and fry exposed to 0.7 ppm WSF over a 50 day period (± 95% confidence interval)..... 21

Figure 5. Growth rates (% increase in wet weight/day/fish) and corresponding feeding rates (no. prey eaten x 1,000/day/fish) of fry for each hydrocarbon concentration of oil-contaminated prey..... 24

Figure 6. Average daily feeding rate of control fry, fry fed oil-contaminated prey, fry exposed to 0.7 ppm WSF, and fry fed reduced rations (± 1 SE)..... 44

Figure 7. Average daily absorption efficiency of control fry, fry fed oil-contaminated prey, fry exposed to 0.7 ppm WSF, and fry fed reduced rations (± 1 SE)..... 48

Figure 8. Average daily oxygen consumption rate of control fry, fry fed oil-contaminated prey, fry exposed to 0.7 ppm WSF, and fry fed reduced rations (± 1 SE)..... 52

Figure 9. Average daily ammonia excretion rate for control fry, fry fed oil-contaminated prey, fry exposed to 0.7 ppm WSF, and fry fed reduced rations (± 1 SE)..... 56

Figure 10. Average daily O:N ratio of control fry, fry fed oil-contaminated prey, fry exposed to 0.7 ppm WSF, and fry fed reduced rations (± 1 SE)..... 60

LIST OF TABLES

		Page
Table 1.	Average aromatic hydrocarbon concentration of WSF-exposed brine shrimp prey.....	11
Table 2.	Two-way ANOVA tables comparing feeding rates of control fry and fry fed OCP.....	16
Table 3.	Two-way ANOVA tables comparing feeding rates among fry fed OCP.....	18
Table 4.	Average length, wet weight, dry weight, and percent dry weight of control fry, fry fed oil-contaminated prey, and fry exposed to 0.7 ppm WSF for 50 days.....	22
Table 5.	Concentrations of aromatic hydrocarbons (ppm) in fry exposed to 0.7 ppm WSF and fry fed 6.5 ppm OCP.....	26
Table 6.	Average aromatic hydrocarbon concentration of WSF-exposed brine shrimp prey.....	39
Table 7.	Two-way ANOVA tables comparing fry feeding rates.....	45
Table 8.	Two-way ANOVA tables comparing fry absorption efficiencies.....	49
Table 9.	Two-way ANOVA tables comparing fry oxygen consumption rates.....	53
Table 10.	Two-way ANOVA tables comparing fry ammonia excretion rates.....	57
Table 11.	Two-way ANOVA tables comparing fry O:N ratios.....	61

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CHAPTER 1

INTRODUCTION

Fishes are an integral part of the marine ecosystem, and provide an important source of food for the world's human population. Previous studies have found that crude and refined petroleum components could destroy or injure fishes in the marine environment (Moore and Dwyer 1974; Malins 1977; Wolfe 1977). Inputs of petroleum to the marine enviroment can come from oil tanker spills, urban runoff, offshore oil well blowouts, and accidental discharges when oil is transferred between land and sea (National Academy of Sciences 1975; Food and Agricultural Organization 1977). With the exception of tanker spills that could occur in the open ocean, virtually all sources of petroleum to the marine environment exist along continental shelves, which are biologically the most productive regions in the ocean. Continental shelves are also the spawning and nursery grounds for many fish species, and support most of the world's commercial fisheries. It is reasonable to assume that offshore oil exploration and development will continue as terrestrial petroleum deposits become depleted. Because of the close proximity of petroleum development to the coastal marine environment, considerable effort has been made to understand the effects of oil on fishes.

Oil in seawater can be acutely toxic to fishes depending on the concentration of aromatic hydrocarbons contained in different crude and refined petroleum components, and the fish species being tested. For example, Neff et al. (1976) found that the acute toxicity of oil to various invertebrate and fish species increased with greater concentrations of aromatic hydrocarbons. Refined petroleum products, such as fuel oils, contain greater concentrations of aromatic hydrocarbons and are more toxic than crude oil which contains a greater proportion of paraffins. However, when Neff et al. exposed two different fish species, silverside minnow, Menidia beryllina, and gulf killifish, Fundulus similis, to the same water-soluble fraction (WSF) of Louisiana crude oil, the 96 hr LC-50 concentration of WSF for the silverside was 5.5 ppm, but was 16.8 ppm for the killifish. Both fish species exist in the same coastal environment, but the silverside was more sensitive than the killifish.

Sublethal concentrations of petroleum hydrocarbons can also be harmful to fishes. Although sublethal effects of oil are not acutely toxic, they do indicate that chronic exposure to oil has the potential for reducing the survival of fishes. Most studies involving sublethal effects of petroleum on fishes have exposed fishes to petroleum components in water. Numerous studies have documented histological changes to different fish tissues resulting from exposure to oil. For example, tissues of the liver (Sabo and Stegeman 1977), spleen (Hodgins et al. 1977), gill (Engelhardt et al. 1980), vertebrae (Linden et al. 1980), eye lens (Hawkes 1977), brain (Dimichelle and Taylor 1978), and

olfactory organ (Babcock in press) of various fish species have been shown to become necrotic, functionally impaired, or deformed when exposed to oil. Other studies have demonstrated that exposure to oil changes the metabolic activity of fishes (Brocksen and Bailey 1973; Struhsaker et al. 1974; Thomas and Rice 1975; Eldridge et al. 1977; Thomas and Rice 1979), and can induce the production of enzymes used by fishes to metabolize oil (Payne and Penrose 1975; Gruger et al. 1977; Spies et al. 1982). Still other studies demonstrated that exposure to oil can impair the feeding behavior of fishes (Wang and Nicol 1977; Folmar et al. 1981), and elicit an avoidance response to oil (Rice 1973; Maynard and Weber 1981; Weber et al. 1981). Overall, oil in water can have numerous and widespread sublethal effects on fishes, and prolonged exposure to oil could reduce the ability of fishes to survive and propagate.

Information concerning the effects of oil on fishes from dietary exposure is less extensive than information on the effects of oil in water. The uptake, metabolism, and excretion of petroleum components acquired from the diet has been documented for various fish species (Dixit and Anderson 1977; Roubal et al. 1977; Whittle et al. 1977; Collier et al. 1978; Nava and Engelhardt 1980; Thomas and Rice 1981; Reichert and Varanasi 1982) because exposure to oil from the diet was recognized as an important pathway of contamination for fishes. Fishes exposed to oil from the diet have become depleted of stored energy and experience poor growth and histological damage to gut tissues in contact with oil (Hawkes 1977; Hawkes et al. 1980). Although oil in

the diet of juvenile fishes is considered a major source of contamination for fishes that feed upon zooplankton species (Whittle and Mackie 1976; Teal 1977), the effects oil in the diet on the survival of juvenile fishes have not been studied.

Laboratory experiments have demonstrated that zooplankton species can accumulate petroleum hydrocarbons from seawater (Corner 1975; Lee 1975), and petroleum hydrocarbons have been found in zooplankton organisms following several major oil spills (Conover 1971; Grose and Mattson 1977; Mackie et al. 1978). Corner et al. (1976) reported that the concentration of naphthalene (a hydrocarbon found in oil) in the marine copepod, Calanus helgolandicus, exposed to naphthalene in seawater was an order of magnitude greater than the concentration of naphthalene in seawater, and that more naphthalene was acquired from feeding on contaminated food organisms than from naphthalene in seawater. They also found that one-third of the naphthalene acquired from the diet was still present ten days after exposure, but most of the naphthalene accumulated from seawater was not detected within three days after exposure. Corner et al. concluded that oil-contaminated zooplankton prey species are a likely source of exposure to oil by planktivorous fishes. Since many larval fish species depend on zooplanktonic organisms for food, juvenile fishes could be exposed to petroleum hydrocarbons by ingesting oil-contaminated prey organisms.

Sublethal effects of oil in water on juvenile fishes have received considerable attention because early life stages of fishes are more sensitive to oil than adult forms (Rosenthal and Alderdice 1976). May

(1974) described the developmental changes of fishes from egg, to embryo, to feeding larvae, as the most critical period for the survival of fishes to adulthood. Many fish species produce larvae in the coastal marine environment. Since most petroleum-related industrial activities occur along continental shelves, larval fishes are likely to be exposed to petroleum, and less likely to survive than adults. Although the effect of exposure to oil-contaminated prey on the survival of juvenile fishes has not been studied, it is possible that exposure to oil-contaminated prey species could reduce the survival of juvenile fishes because of the increased sensitivity of juvenile fishes to oil, and the ability of zooplankton prey species to accumulate and retain petroleum components.

The effects of exposure to oil-contaminated prey on the growth of juvenile fishes are not known. However, the effects of oil in water on the growth of juvenile and larval fishes have been studied, and provide an indication of the potential effects oil-contaminated prey could have on the growth of juvenile fishes. Sublethal concentrations of petroleum hydrocarbons in water, sometimes as low as 20% of the LC-50, have been shown to reduce the growth of several juvenile and larval fish species (Rice et al. 1975; Linden 1978; Sharp et al. 1979; Moles et al. 1981; Woodward et al. 1981; Moles and Rice 1983; Rowe et al. 1983). Although there are numerous adverse effects resulting from exposure to petroleum, reduced food intake and increased energy requirements are two factors often cited as causing reduced growth of juvenile and larval fishes (Rice in press). Reductions in food intake

can by itself reduce the growth of fishes (LeBrasseur 1969; Brett and Groves 1979), but an additional energy burden resulting from exposure to petroleum hydrocarbons could cause further reductions in growth.

The reduced growth of juvenile fishes could decrease their survival during early sea life. For example, Parker (1971) observed that juvenile coho salmon, Oncorhynchus kisutch, preferred to feed on pink salmon fry, O. gorbuscha, instead of chum salmon fry, O. keta, because the pink salmon fry were smaller than the chum salmon and presumably easier to catch. When coho salmon smolts were released from hatcheries in different size groups, smaller smolts yielded fewer returning adults than larger smolts (Bilton et al. 1982). Reductions in the growth of juvenile fishes from exposure to oil could produce higher mortalities during early sea life which could result in the production of fewer spawning adults.

The primary objective of this study was to determine the effects of exposure to oil-contaminated prey organisms on the survival of pink salmon fry, Oncorhynchus gorbuscha. Pink salmon fry were chosen as the test fish species for several reasons: 1) there is information from previous studies on the effects of oil in seawater on the growth and physiology of pink salmon fry (Rice 1973; Rice et al. 1975; Thomas and Rice 1975; Rice et al. 1977; Moles et al. 1979; Thomas and Rice 1979; Moles and Rice 1983), from which information could be obtained and compared to the effects of oil-contaminated prey, 2) the fry spend their early sea life in the coastal marine environment where most petroleum-related activities occur, 3) the fry prey upon

zooplankton which can become contaminated with oil from any of the previously mentioned potential sources, 4) pink fry are found in a coastal marine environment receiving oil tanker traffic (Prince William Sound, Alaska) and chronic low-level hydrocarbon inputs (Port Valdez, Alaska) that could contaminate the coastal marine food web, and 5) adult pink salmon are an important commercial fishery (Rogers 1972).

The growth rate of pink salmon fry exposed to oil-contaminated prey was chosen as an indicator of the survival potential for these fish because survival to adulthood is to a large extent dependent on growth during early development, and because previous studies found that exposure to oil in seawater reduces fry growth (Rice et al. 1975; Moles and Rice 1983). Fry feeding rate was studied because results from previous experiments found that exposure to oil in water decreases fry feeding rates and contributes to reductions in fry growth (Moles and Rice 1983). The metabolic activity of pink salmon fry was also studied because previous studies have found that exposure to oil in seawater increases the energy requirements of pink salmon fry, and is also attributed to reducing fry growth (Thomas and Rice 1975; Thomas and Rice 1979). Oxygen consumption, absorption and utilization of food, and ammonia excretion in the fry were studied to determine if exposure to oil-contaminated prey affects the energy uptake and requirements of pink salmon fry.

CHAPTER 2

EFFECTS OF OIL-CONTAMINATED PREY ON THE FEEDING AND GROWTH RATES OF PINK SALMON FRY

The effects of oil in seawater on reducing the feeding and growth rate of pink salmon fry, Oncorhynchus gorbuscha, have been documented (Rice et al. 1975; Moles and Rice 1983). However the effects of oil-contaminated prey (OCP) on feeding and growth rates of pink salmon fry are not known. Oil-contaminated zooplankton prey species can result from accidental oil spills (Conover 1971; Grose and Mattson 1977; Mackie et al. 1978), and could become a source of petroleum contamination for pink salmon fry. Although there is no information concerning the effects of OCP on feeding and growth rates of pink salmon fry, previous experiments with other salmonids exposed to oil in seawater indicate that oil can interfere with feeding behavior (Folmar et al. 1981, Folmar and Hodgins 1982). Since exposure to oil in seawater can reduce prey consumption, exposure to OCP could also interfere with normal feeding.

Food energy required for growth of pink salmon fry could become depleted during a critical stage in their life history by prolonged exposure to OCP, which may result in reduced survival. For example, when Moles and Rice (1983) exposed pink salmon fry to sublethal concentrations of the water-soluble fraction (WSF) of Cook Inlet crude

oil for forty days, the fish ate very little food during the first several days of the experiment, and eventually utilized only ten percent of their food ration. The growth rate of these fish was one-third the growth rate of control fry. The feeding behavior of juvenile coho salmon, Oncorhynchus kisutch, was impaired by exposure to sublethal concentrations of toluene and naphthalene, and decreased food intake was attributed to reducing the growth rate of these fish (Moles et al. 1981). It is not known if exposure to OCP will effect the growth of pink salmon fry, but previous studies with juvenile salmonids exposed to petroleum components in seawater indicate that fry growth can be reduced through reductions in food intake. Parker (1971) has shown that reductions in the size of juvenile salmon can reduce their survival during early sea life. Therefore, reductions in feeding and growth rate, caused by exposure to OCP, could reduce the survival of pink salmon fry.

The objective in this chapter was to determine the effects of OCP on the feeding and growth rate of pink salmon fry, and to measure the amount of petroleum hydrocarbons acquired from the diet. The results of this experiment were used to determine the potential effect of OCP on fry survival.

Materials and Methods

Fish collection and maintenance.

Pink salmon fry in seawater (average length = 31 mm ± 0.3 mm, wet weight = 190 mg ± 7 mg) were obtained from the Douglas Pink And Chum, Inc. hatchery at Sheep Creek, near Thane, Alaska, and held in 800 L fiberglass aquaria at the Auke Bay Laboratory for 20 days prior to beginning the experiments. The fry were fed Oregon Moist Pellets (Bioproducts, Inc.) for one week then placed on a diet of frozen brine shrimp. When the fry reached 220 mg (± 8 mg) they were transferred to 4 L pvc (polyvinyl chloride) incubators. The sample size was 15 fry/incubator for feeding rate determinations and 30 fry/incubator for growth rate determinations. Seawater flow rate through each incubator was 250 ml/min, water temperature was 8.0 °C (± 1.0 °C), and salinity was 28 o/oo (± 2 o/oo).

Feeding rate.

Live brine shrimp, Artemia salina, nauplii were used as the prey organism to simulate plankton-like organisms that make up an important part of the diet of pink salmon fry (Cooney et al. 1981). Oil-contaminated prey (OCP) were prepared by exposing freshly hatched brine shrimp nauplii to the WSF of Cook Inlet crude oil (Moles et al. in press). The nauplii were placed for 24 hrs in either 10%, 50%, or 100% WSF to produce three dose levels of OCP (see Table 1). Brine shrimp were siphoned from the WSF dosing tank, partitioned with a

Table 1. Average aromatic hydrocarbon concentrations of WSF-exposed brine shrimp prey (n = 5 prey rations). Parentheses contain the standard error of the mean.

Compound	ppm (μg/g) Aromatic Hydrocarbons		
Toluene	0.04(.01)	0.06(.02)	0.12(.02)
Ethyl Benzene	0.03(.01)	0.11(.02)	0.21(.21)
Xylenes	0.05(.01)	0.26(.05)	1.26(.21)
Trimethyl Benzenes	0.21(.03)	0.24(.02)	0.37(.07)
Naphthalene	0.08(.01)	0.62(.08)	0.96(.18)
Methyl Naphthalenes	0.14(.02)	1.15(.17)	1.87(.31)
Dimethyl Naphthalenes	0.07(.01)	0.80(.08)	1.75(.11)
Total	0.62	3.24	6.54

Bourne plankton splitter (D. Bourne, Marine Research, Inc.), and fed to triplicate fry incubators within each dose level. A fourth group of fry in triplicate incubators was fed uncontaminated brine shrimp (controls).

The number of prey introduced to the incubators was estimated by counting three replicate subsamples of food rations under a dissecting microscope. Uneaten prey were collected on 100 μm plankton netting each day from the incubator effluent and counted to obtain the number of brine shrimp eaten by difference. Seawater entered the incubators from the bottom and exited from a surface drain so that uneaten prey would not settle to the bottom of the incubator. The fish were fed OCP for 10 days then fed uncontaminated prey for 5 days. The feeding rates of fish exposed to OCP were compared to controls using a two-way analysis of variance (Sokal and Rohlf 1969). There was no significant difference in the number of prey fed daily to the fish in each incubator (average = 4,420 prey/day/fish, ± 89, n = 60).

Growth rate.

Brine shrimp prey used for determining fry growth rates were removed from the WSF dosing tanks, concentrated on plankton netting, and frozen in aluminum weighing dishes. Frozen brine shrimp pellets were punched from an aluminum dish with a cork borer and placed in incubators containing the fry. Pink fry were fed 3-4% of their wet weight/day based on the wet weight of the control fish. Food rations were adjusted upwards every two weeks. The brine shrimp ration was

determined by comparing the proximate analysis of Oregon Moist Pellet (OMP) to the proximate analysis of brine shrimp (Gallagher and Brown 1975) and increasing the amount of brine shrimp until the caloric value was the same as OMP. This method for feeding the fish provided sufficient nutrition to support fry growth. Brine shrimp nauplii were nutritionally inferior to OMP, and live nauplii could not be provided daily in the large quantities necessary to feed all the fish.

Fry were fed uncontaminated brine shrimp until their wet weight reached 250 mg. Three fry groups were then fed low, medium, and high dose OCP, respectively, and a fourth group of fry was fed uncontaminated prey (controls). A fifth group of fish was placed in WSF at 50% of the 96 hr LC-50 for pink salmon fry, which is approximately 0.7 ppm WSF (Moles and Rice 1983), and fed uncontaminated prey to compare the effects of WSF on fry growth with the effects of OCP. Fry were sacrificed at 10, 23, 36, and 50 days. However, fry mortalities from exposure to WSF increased during the experiment, and a sample of the WSF exposed fish on day 36 was eliminated so that sufficient numbers of fish would be available on day 50. Triplicate fry incubators/dose were sampled on day 50 and length, wet weight, dry weight, and percent dry weight group averages were compared by Dunnett's-t (Dunnett 1964). Fry length (snout to caudal peduncle) and wet weight was measured on all fry/incubator, and ten of the fry/incubator were dried in an oven (60 °C) to determine dry weight and percent dry weight. The remaining fry from each incubator were analyzed for hydrocarbon content.

Hydrocarbon uptake.

Pink fry were frozen at 10, 23, 36, and 50 days of the growth rate experiment, and later thawed to measure the amount of hydrocarbons acquired from the OCP. Fry fed the highest dose of contaminated prey were also sampled during the first day of the experiment at 3, 7, and 12 hours in order to be certain that fish were acquiring oil from the prey organisms. Fry tissue was processed for hydrocarbons using the methods of Warner (1978), and quantified with a Hewlett-Packard model 5880A gas chromatograph using a fused silica capillary column and methyl silicone fluid as the stationary phase. The hydrocarbon concentrations of OCP (Table 1) were determined by the same methods used to analyze fry tissues. Hydrocarbon concentrations in the prey and fish were based on the prevalent mono- and bicyclic aromatic compounds.

Results

Feeding Rate.

There were no mortalities among the fish fed Oil-contaminated prey (OCP) during the feeding rate experiment. Daily fry feeding rates are presented in Figure 1. All fry fed OCP ate significantly less than controls during the 10 day exposure period (Table 2). The average daily feeding rates for fish fed control, low dose, medium dose, and high dose OCP were, respectively, 3,152 (± 422), 1,958 (± 283), 1,693

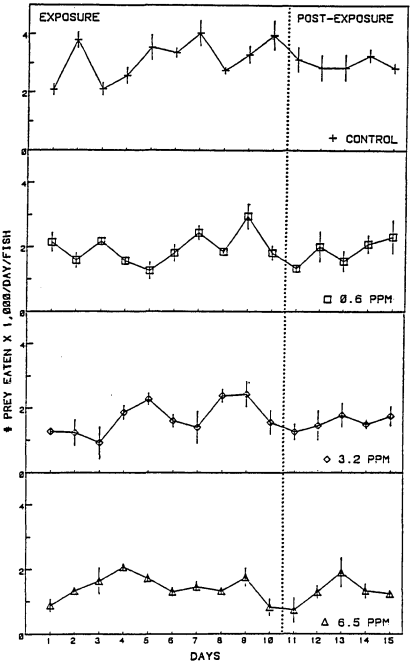

Figure 1. Average daily feeding rate (no. prey eaten/day/fish) of control fry, and fry fed oil-contaminated prey (± 1 SE).

Table 2. Two-way ANOVA tables comparing feeding rates of control fry and fry fed oil-contaminated prey during the exposure and post-exposure periods. A = time; B = treatment.

Source of Variation	S.S.	d.f.	M.S.	F-ratio
Exposure				
A	8.96×10^{6}	9	9.95×10^{5}	4.325 ***
B	5.21×10^{7}	3	1.74×10^{7}	19.892 ***
AxB	2.36×10^{7}	27	8.72×10^{5}	3.791 ***
error	1.03×10^{8}	80	2.30×10^{5}	
Post-exposure				
A	1.62×10^{6}	4	4.05×10^{5}	1.184 ns
B	2.51×10^{7}	3	8.38×10^{6}	24.511 ***
AxB	3.53×10^{6}	12	2.94×10^{5}	0.860 ns
error	1.37×10^{7}	40	3.42×10^{5}	

*** = $p < .001$; ns = not significant

(± 304), and 1,428 (± 224) prey/day/fish. Fry fed low dose, medium dose, and high dose OCP ate, respectively, 37%, 44%, and 55% less than controls. A two-way ANOVA applied to the three fry groups fed OCP indicated that the fish fed low dose OCP ate significantly more than fish fed high dose OCP (Table 3). The feeding rates of fry fed medium dose OCP were not significantly different than the feeding rates of fish fed low and high dose prey.

All of the fish fed OCP continued to eat significantly less than controls during the post-exposure period (Table 2). The average daily feeding rates during the post-exposure period for fry fed control, low dose, medium dose, and high dose OCP were, respectively, 2,992 (± 116), 1,861 (± 233), 1,551 (± 127), and 1,294 (± 242) prey/day/fish. The fish fed low dose OCP ate significantly more than the fish fed high dose OCP (Table 3).

Growth rate.

There were no mortalities among the fish fed OCP. At the beginning of the experiment, average fry length was 32 mm (± 0.2 mm), wet weight was 255 mg (± 10 mg), and dry weight was 45 mg (± 3 mg). All of the fish fed uncontaminated prey increased in size during the 50 day experiment. Fry exposed to OCP also increased in size, but the fish exposed to all three dose levels of OCP were significantly smaller than controls in length (Figure 2), wet weight (Figure 3), and dry weight (Figure 4) by the end of the experiment (Table 4). The wet weight of fish exposed to medium dose OCP did not increase during the

Table 3. Two-way ANOVA tables comparing feeding rates of fry fed oil-contaminated prey during the exposure and post-exposure periods. A = time; B = treatment.

Source of Variation	S.S.	d.f.	M.S.	F-ratio
Exposure				
A	7.28×10^{6}	9	8.09×10^{5}	3.864 ***
B	4.24×10^{6}	2	2.12×10^{6}	3.552 *
AxB	1.08×10^{7}	18	5.98×10^{5}	2.856 ***
error	1.26×10^{7}	60	2.09×10^{5}	
Post-exposure				
A	2.62×10^{6}	4	6.55×10^{5}	2.004 ns
B	2.42×10^{6}	2	1.21×10^{6}	3.706 *
AxB	2.03×10^{6}	8	2.53×10^{5}	0.775 ns
error	9.80×10^{6}	30	3.27×10^{5}	

* = $p < .05$; *** = $p < .001$; ns = not significant

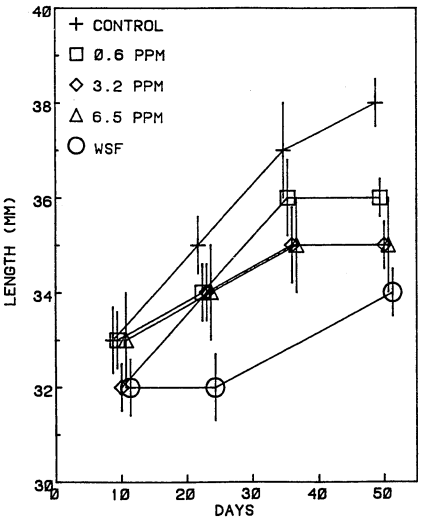

Figure 2. Average length of control fry, fry fed oil-contaminated prey, and fry exposed to 0.7 ppm WSF over a 50 day period (± 95% confidence interval).

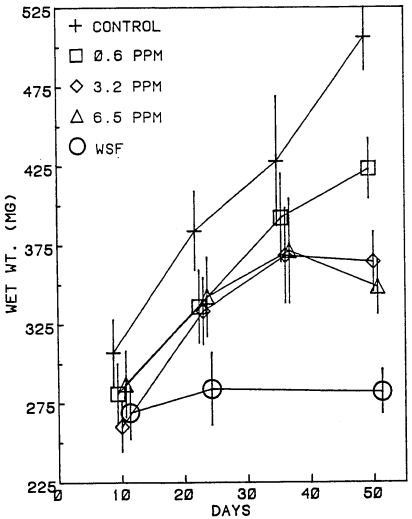

Figure 3. Average wet weight of control fry, fry fed oil-contaminated prey, and fry exposed to 0.7 ppm WSF over a 50 day period (± 95% confidence interval).

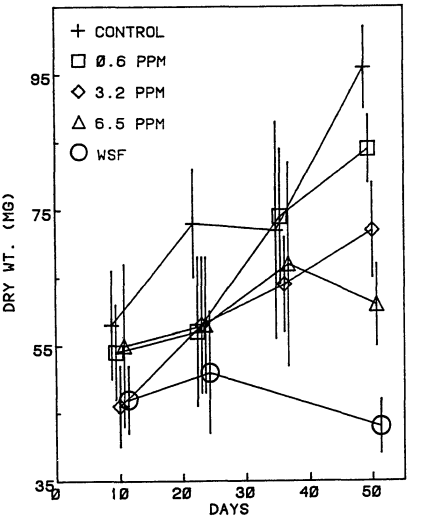

Figure 4. Average dry weight of control fry, fry fed oil-contaminated prey, and fry exposed to 0.7 ppm WSF over a 50 day period (± 95% confidence interval).

Table 4. Average length, wet weight, dry weight, and percent dry weight of control fry, fry fed oil-contaminated prey, and fry exposed to 0.7 ppm WSF in seawater for 50 days. Sample size for length and wet weight was 30 fish. Dry weight and percent dry weight sample size was 10 fish. Parentheses contain 95 percent confidence intervals. Levels of significance are based on Dunnett's-t for the three replicates within each fry group.

			ppm Oil-Contaminated Prey			
Replicate No.		Control	0.6	3.2	6.5	WSF
Length (mm)	1	38(0.8)	36(0.9)**	35(0.8)**	35(0.9)**	^{a}34(0.9)**
	2	38(0.9)	36(0.6)	35(0.8)	35(1.0)	^{b}33(0.9)
	3	38(0.6)	36(0.8)	35(0.7)	35(0.7)	^{b}34(0.7)
Wet wt (mg)	1	491(36)	423(39)**	362(39)**	352(29)**	^{a}288(32)**
	2	518(43)	420(30)	371(34)	346(33)	^{b}273(26)
	3	513(28)	422(30)	359(26)	342(28)	^{b}287(23
Dry wt (mg)	1	99(8)	87(10)*	75(18)**	66(8)**	^{c}41(7)**
	2	100(17)	80(11)	73(13)	60(14)	^{c}40(3)
	3	88(6)	85(11)	68(10)	58(12)	^{c}49(9)
% Dry wt	1	17.8(0.4)	17.4(0.2)	16.7(0.5)*	16.3(0.5)**	^{c}16.3(0.6)**
	2	17.5(0.4)	17.1(0.5)	16.8(0.3)	15.9(0.5)	^{c}15.9(0.4)
	3	17.5(0.4)	17.4(0.6)	16.6(0.4)	16.2(0.5)	^{c}16.3(1.0)

a = 10 fish, b = 13 fish, c = 5 fish
* = $p < .05$; ** = $p < .01$

last 14 days of the experiment, and fry fed high dose OCP lost weight (Figure 3). By day 50, the wet weight of fry fed low, medium, and high dose OCP was, respectively, 16%, 28%, and 36% less than controls.

Although there was no significant difference in the percent dry weight between fry fed OCP and controls 36 days into the study, the percent dry weight of fry fed medium and high dose OCP was significantly less than controls by the last day of the experiment (Table 4). This suggests that during the last 14 days of the experiment the fish fed medium and high dose OCP were being depleted of body tissue faster than it could be replaced through the incorporation of food energy.

Figure 5 presents the changes in fry growth rate over 50 days (% increase in wet weight/day/fish) and average feeding rates for fish exposed to the three hydrocarbon concentrations of OCP and controls. There was a significant correlation between feeding and growth rates (Pearson's $r = 0.980$, $p < 0.05$) with increasing hydrocarbon concentrations, even though feeding and growth rates were obtained from separate experiments.

Fry exposed to WSF had greater reductions in growth than the fish fed OCP (Table 4). The fish exposed to WSF had more food than fry fed OCP because food rations were based on the wet weight of control fish, and the number of fish exposed to WSF continuously decreased. Fry exposed to WSF increased in wet weight by only 27 mg over the 50 day exposure period, and dry weight decreased by 2 mg.

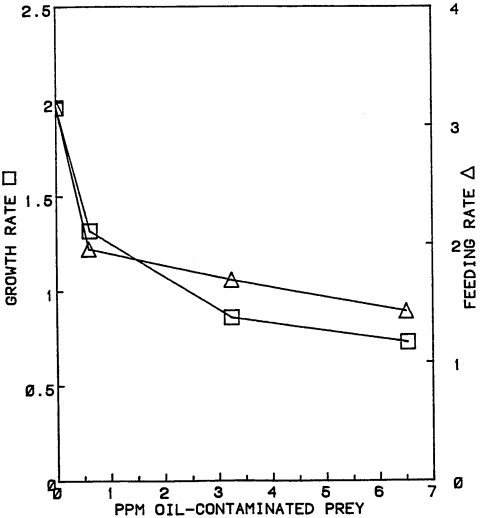

Figure 5. Growth rates (% increase in wet weight/day/fish) and corresponding feeding rates (no. prey eaten x 1,000/day/fish) of fry for each hydrocarbon concentration of oil-contaminated prey.

Hydrocarbon Uptake.

Naphthalene, methylnaphthalenes, and dimethylnaphthalenes were found in fry tissues 3 hours after the fish were fed high dose OCP (Table 5). After 12 hours, aromatic hydrocarbon concentrations were less than half the concentration detected 9 hours earlier. In contrast, fry exposed to WSF in seawater rapidly accumulated mono-and bicyclic aromatic hydrocarbons from solution during the first 24 hours of exposure.

By day 10, fry fed high dose OCP still contained naphthalene (0.01 ppm) and methyl naphthalenes (0.02 ppm), fry fed medium dose contained naphthalene (0.01 ppm), and hydrocarbon concentrations in the fish fed low dose OCP were below limits of detection. Aromatic hydrocarbons were below limits of detection in fry fed OCP at all three dose levels when the fish were analyzed again on day 23. In contrast, hydrocarbon concentrations for WSF fry at 10 days were the same as those detected after 24 hours, and did not change during the experiment.

Discussion

Pink salmon fry were able to grow when fed oil-contaminated prey (OCP) at hydrocarbon concentrations that would be lethal if present in seawater at the same levels. In contrast, the greatest reduction in fry growth resulted from exposure to WSF in seawater at a hydrocarbon concentration that was nearly an order of magnitude less than the

Table 5. Concentrations of aromatic hydrocarbons (ppm) in fry exposed to 0.7 ppm WSF in seawater for 24 hours, and for fry fed 6.5 ppm oil-contaminated prey for 12 hours.

	0.7 ppm WSF	6.5 ppm Oil-Contaminated Prey		
Compound	24 hrs	3 hrs	7 hrs	12 hrs
Toluene	0.82	nd	nd	nd
Ethyl Benzene	0.27	nd	nd	nd
Xylenes	1.23	nd	nd	nd
Trimethyl Benzenes	1.10	nd	nd	nd
Naphthalene	0.59	0.09	0.05	0.03
Methyl Naphthalenes	1.62	0.15	0.08	0.03
Dimethyl Naphthalenes	1.10	0.21	0.18	0.10

nd = not detected (below 0.01 ppm)

highest hydrocarbon concentration of OCP. Even though the hydrocarbon concentration in OCP was nearly an order of magnitude greater than the WSF concentration, the actual amount of hydrocarbons exposed to the fry from the diet was considerably less than exposure to WSF. Fry fed high dose OCP consumed approximately 4% of their wet weight each day, which amounted to less than 0.15 μg of aromatic hydrocarbons. In contrast, the fry exposed to WSF acquired much greater quantities of aromatic hydrocarbons because the volume of seawater containing hydrocarbons was much greater than the volume of prey consumed, even though the WSF concentration was nearly an order of magnitude less than the highest hydrocarbon concentration of OCP. Fishes are capable of metabolizing and excreting aromatic hydrocarbons acquired from the diet (Roubal et al. 1977; Thomas and Rice 1981), and this was demonstrated by the fry fed OCP because of the decline in concentration of aromatic hydrocarbons during the first 12 hours of exposure to OCP, and the absence of aromatic hydrocarbons for the remaining 50 days of exposure when the rates of metabolism and excretion of aromatic hydrocarbons exceeded the rate of hydrocarbon accumulation. In contrast, the fry exposed to WSF accumulated aromatic hydrocarbons to levels approximately an order of magnitude greater than the highest hydrocarbon concentration in the fry fed OCP, and retained aromatic hydrocarbons during the 50 day study. Hydrocarbons accumulated faster than they could be metabolized and excreted by the fry exposed to WSF because the fry were exposed to a relatively large amount of aromatic hydrocarbons when compared to the amount of hydrocarbons available from

OCP. Furthermore, it has been suggested that the accumulation of aromatic hydrocarbons via the gills is a more direct pathway to internal organs by way of the bloodstream than from the gut (Dixit and Anderson 1977). Fry exposed to WSF probably accumulated aromatic hydrocarbons because the bloodstream in the gills of these fish was also in close proximity to aromatic hydrocarbons in seawater, while the fry fed OCP were probably metabolizing and excreting hydrocarbons contained in the digestive tract.

Although the fry fed low dose OCP ate more than the fry fed high dose OCP, the differences in feeding rates were not as great as the differences in hydrocarbon concentrations. A ten-fold increase in hydrocarbon concentration, for example, produced only a 20% change in feeding rates. The reason for the relatively small difference in feeding rate over a wide range of hydrocarbon concentrations is not clear. Perhaps fry feeding rates were affected at a threshold OCP dose level. Further reductions in feeding rate would therefore not be in proportion to increasing OCP hydrocarbon concentrations because an impairment in fry feeding behavior was initiated at the lower OCP hydrocarbon concentration.

Pink salmon fry did not avoid OCP. In a preliminary experiment, pink salmon fry did not avoid eating OCP when given a choice between contaminated and uncontaminated prey, because petroleum hydrocarbons were later detected in fry tissues (Schwartz unpbl. data). The ingestion of petroleum-contaminated prey has also been reported with other fish allowed to choose between contaminated and uncontaminated

prey (Blackman 1974). However, fry feeding rates in the present study did not improve when the fry were later fed uncontaminated prey. This suggests that exposure to OCP had a prolonged conditioning effect on fry feeding behavior. The post-exposure feeding rates of fry previously exposed to each OCP dose level remained at or below the feeding rate of fry exposed to low dose OCP, possibly because the effect of OCP at a threshold concentration continued to mask the recognition of uncontaminated prey for the fry previously exposed to medium and high dose OCP. It is clear, however, that the effect of OCP on reducing the feeding rate of pink salmon fry is not immediately reversible when oil is removed from the diet.

It is not known if fry metabolic rate is affected by exposure to OCP, or if exposure to OCP interferes with the uptake of food energy. Although the similarity between reductions in fry feeding and growth rates at each OCP dose level suggests that reductions in growth rate were mainly the result of reductions in feeding rate, an increase in metabolic rate of fry exposed to OCP may have contributed to reducing fry growth rate. Rice et al. (1977) reported that the metabolic rate of pink salmon fry increases with exposure to WSF, and Moles and Rice (1983) concluded that both reductions in feeding rate of pink salmon fry and an additional energy burden, caused by an increase in metabolic rate, were responsible for reducing the growth rate of fry exposed to WSF. Furthermore, previous experiments with petroleum hydrocarbons in the diet of juvenile chinook salmon, Oncorhynchus tshwaytsha, indicated that gut tissues were damaged when the fish were exposed to a mixture

of aromatic hydrocarbons via the diet, remained damaged when contaminated food was removed from the diet, and suggest that ingested petroleum hydrocarbons could impair the normal uptake of food energy (Hawkes et al. 1980). Hawkes (1977) reported that chronic exposure of rainbow trout, Salmo gairdneri, to oil via the diet depleted the trout of energy reserves and reduced growth. Reductions in the percent dry weight of fry fed medium and high dose OCP in the present study indicated that after chronic exposure to OCP the fish were beginning to catabolize body tissues. Reductions in percent dry weight could have resulted from reduced food intake, but evidence from previous studies suggests that a reduction in fry growth from chronic exposure to OCP could also result from a reduction in the uptake of food energy, and an increase in fry metabolic rate.

Since the survival of juvenile salmon has been shown to decrease with size because of selective predation by large fishes (Parker 1971), OCP have the potential for reducing the survival of pink salmon fry by reducing growth rate. Although exposure to WSF had a greater effect on reducing fry growth rate than OCP in the present study, the fry can detect and avoid petroleum in seawater (Rice 1973). However, the fry in the present study did not avoid OCP, and it is possible that OCP could have a greater effect than WSF on reducing fry survival when the results of this study are applied to existing conditions in the natural environment. Prey organisms available to juvenile salmon include pelagic and benthic invertebrate species (Healy 1979; Cooney et al. 1981). Petroleum hydrocarbons introduced to the marine environment can

be absorbed from solution by pelagic prey species, as in the present study with plankton-like brine shrimp nauplii, or taken up by benthic prey species in contact with contaminated bottom sediments and become available to pink salmon fry. Even though OCP were not lethal to the fry in this study, fry feeding and growth rates were reduced, and fry survival could be inhibited as a result.

CHAPTER 3

EFFECTS OF OIL-CONTAMINATED PREY ON THE OXYGEN CONSUMPTION, FOOD ABSORPTION, AND AMMONIA EXCRETION OF PINK SALMON FRY

Sublethal concentrations of petroleum hydrocarbons can alter the metabolic activity of larval and adult fishes (Anderson et al. 1974; Struhsaker et al. 1974; Thomas and Rice 1975; Eldridge et al. 1977; Rice et al. 1977). Some changes in metabolic activity resulting from exposure to petroleum components, such as increased oxygen consumption, are indicative of additional energy requirements (Thomas and Rice 1979). Since the larval stage of fishes is a critical period for their survival (May 1974), exposure to oil could deplete larval fishes of energy used for growth, and cause reduced survival.

The results from the previous chapter found that exposure to oil-contaminated prey (OCP) can reduce the growth of pink salmon fry, Oncorhynchus gorbuscha, primarily by reducing food intake. Previous studies on the growth of other fishes also documented reduced food intake occurring with fish exposed to petroleum components (Struhsaker et al. 1974; Korn et al. 1976; Moles et al. 1981). However, it is not known if exposure to OCP affects the metabolic activity of pink salmon fry. It has also been reported that exposure to the water-soluble fraction (WSF) of Cook Inlet crude oil can increase the metabolic rate

of pink fry (Thomas and Rice 1975; Rice et al. 1977), which in addition to reductions in feeding rate is attributed to reducing fry growth (Moles and Rice 1983). An increase in the metabolic rate of pink salmon fry is atypical when food intake is reduced, since the metabolic rates of fishes decline with reductions in food intake (Brett and Groves 1979). Furthermore, the effects of exposure to oil, from food or water, on the amount of energy lost by fish through excretion are not known. Ammonia is the major non-fecal excretory product of salmonids resulting from the break down of dietary and endogenous protein (Brett and Zala 1975), and decreases with reduced food intake (Elliott 1976).

It is also not known if exposure to OCP affects the utilization of energy substrates by fishes. Salmonids derive most of their energy from dietary protein (Brett and Groves 1979), and exposure to OCP could effect the metabolic activity of pink salmon fry, as was reported for fry exposed to oil in seawater. This could alter fry utilization of different energy substrates. Oxygen:nitrogen (O:N) ratios have been used as an index of substrate utilization for marine invertebrate species (Conover and Corner 1968; Corner and Cowey 1968; Mayzaud 1973), and may provide a useful indicator for the effects of oil on substrate metabolism by fishes. An increase in O:N ratio of an animal, which is the amount of oxygen consumed divided by the amount of nitrogen excreted in atomic equivalents, indicates a greater dependence on lipid and/or carbohydrate, while a decrease in O:N ratio indicates a greater

dependence on protein. For example, Cappuzzo and Lancaster (1981) measured the O:N ratio of lobster larvae, Homarus americanus, exposed to the WSF of Louisiana crude oil and reported that O:N ratios decreased with exposure to oil. This indicated that the larvae exposed to oil were utilizing more protein than controls. Cappuzzo and Lancaster (1982) later reported that lobster larvae exposed to OCP also had lower O:N ratios than controls. They concluded that the larvae fed OCP were abnormally stressed because greater amounts of protein were being catabolized. The O:N ratios were calculated from oxygen consumption and ammonia excretion rates, since ammonia was the major excretory product of the lobster larvae. Since ammonia is also the major excretory product of salmonids, O:N ratios based on oxygen consumption and ammonia excretion rates of pink salmon fry exposed to OCP could provide an indication of changes in the type of substrate utilized by the fish.

The metabolism and excretion of ingested hydrocarbons by fish could require additional energy normally available for growth by interfering with the digestion and uptake of food energy. Hawkes (1977) reported depletion of glycogen stored in the livers of rainbow trout, Salmo gairdneri, fed Prudhoe Bay crude oil for two weeks. Prolonged feeding on oil reduced trout growth rates. She concluded that exposure to oil via the diet affected the energy storage capability of the liver, and changes in liver morphology could eventually be responsible for reducing fish survival. Hawkes et al. (1980) found that the intestinal tissues of juvenile chinook salmon,

Oncorhynchus tshwaytsha, fed a mixture of aromatic hydrocarbons for 28 days were morphologically dissimilar to control salmon tissues, remained altered for up to 21 days after exposure, and suggested that oil in the diet could sufficiently inhibit digestion and utilization of food energy to reduce the growth of juvenile chinook salmon. Previous studies on the energetics of fishes indicated that food uptake efficiency increases as ration size decreases (Solomon and Brafield 1972; Elliott 1976). Although pink salmon fry exposed to OCP eat less than fry fed uncontaminated prey, and would therefore be expected to be more efficient at utilizing ingested food, previous studies with other juvenile salmonids suggest that exposure to oil in the diet can reduce the growth and utilization of food energy by pink salmon fry.

The objective in this chapter was to determine the effects of exposure to OCP on the energy uptake and requirements of pink salmon fry, and compare the effects of dietary oil exposure on fry energetics with exposure to oil in seawater. Fry feeding rates were also monitored because changes in metabolic activity are to a large extent dependent on food intake. Oxygen consumption, food absorption and utilization, and ammonia excretion were measured to indicate whether or not exposure to OCP increases fry energy requirements and/or losses.

Materials and Methods

Fish collection and maintenance.

Pink salmon fry in seawater were collected at the Douglas Pink And Chum, Inc. hatchery at Sheep Creek, near Thane, Alaska, and reared at the Auke Bay Laboratory on a satiation diet of frozen brine shrimp. Average fry wet weight was 1.1 g (± 0.1 g, n = 10) when the experiment began. Seawater temperature was 9.0 °C (± 1.0 °C), and salinity was 28 o/oo (± 2 o/oo).

Flow-through chamber.

One week prior to the start of the experiment the fry were transferred to flow-through chambers and were fed live uncontaminated brine shrimp nauplii. Flow-through chambers were constructed using one-liter aspirator jars. Seawater entered from a reservoir opened to the atmosphere to maintain oxygen concentrations at saturation, and through the aspirator port of the jar. Seawater exited from the chamber through a tube connected to the neck of the jar. The height of the reservoir and outflow tube above the jar was adjusted to maintain a constant seawater flowrate of 40 ml/min through each chamber. The chamber was suspended upside-down in a water bath to maintain the chamber at ambient seawater temperature, and allow fecal material to settle at the outflow tube in the neck of the chamber, where it was carried away and collected with the chamber effluent.

Each chamber was masked from direct laboratory lighting because the fish became excited when shadows obscured light to the chamber, resulting in spuriously high oxygen consumption rates. In addition to masking each chamber, a series of timing switches produced a regular eight hour dark period with one hour of dim light to simulate dawn and dusk immediately before and after complete darkness. The fish appeared acclimated to the laboratory environment within two days after entering the chamber.

Experimental design.

Three chambers, each containing five fish, were exposed to one of the following test conditions: uncontaminated prey in uncontaminated seawater (controls), oil-contaminated prey (OCP) in uncontaminated seawater, uncontaminated prey in 50% of the 96 hr LC-50 of the water-soluble fraction (WSF) Cook Inlet crude oil (Moles and Rice 1983), and reduced rations of uncontaminated prey in uncontaminated seawater. The fish fed reduced rations were used to compare fry energetics resulting from reduced food intake without exposure to oil with the energetics of fish exposed to oil. Exposure to the above conditions continued for seven days, followed by seven days of post-exposure. During the post-exposure period all fry groups received the same amount of uncontaminated prey and were supplied with uncontaminated seawater. At the end of the experiment, the fish biomass in each chamber was obtained by drying the fish for 24 hours at 60 °C. Dry weight biomass in each chamber varied between 0.8 - 1.1 g

(average = 0.97g ± .02g, n = 18). Feeding, oxygen consumption, and ammonia excretion rates were expressed as a proportion of dry weight.

Feeding rate.

The fish were fed prey organisms once every day by adding live brine shrimp, Artemia salina, nauplii to the inflowing seawater of each chamber from a 20 ml syringe. Uneaten prey were collected on 100 μm plankton netting from the chamber effluent. The number of prey in replicate food rations and uneaten fractions were counted on a Coulter Counter model ZM to obtain feeding rates by difference. Fry maintained on reduced rations were fed a daily average of 2,983 prey/day (± 270, n = 7) during the exposure period, while the daily average for all other fry groups was 25,457 prey/day (± 249, n = 35) during the exposure period.

The prey were contaminated with oil by soaking for 24 hours in the WSF of Cook Inlet crude oil (Moles et al. in press). Brine shrimp were exposed to 5%, 25%, and 50% dilutions of WSF to produce three dose levels of OCP (see Table 6). The WSF dilutions and range of hydrocarbon concentrations in OCP were reduced from those used in the previous chapter because of the highly significant decrease in fry feeding and growth rates previously observed between the fish fed 0.6 ppm OCP and controls. The high dose OCP (6.5 ppm) tested in the previous chapter was eliminated because changes in feeding and growth between fry fed 3.2 ppm and 6.5 ppm OCP were not significantly

Table 6. Average aromatic hydrocarbon concentrations of WSF-exposed brine shrimp prey (n = 3 prey rations). Parentheses contain the standard error of the mean.

	ppm (µg/g) Aromatic Hydrocarbons		
Compound	low dose	medium dose[1]	high dose
Toluene	0.01(.01)	0.04(0.0)	0.08(.01)
Ethyl Benzene	0.02(.01)	0.06(.01)	0.14(.02)
Xylenes	0.11(.02)	0.36(.02)	0.82(.01)
Trimethyl Benzenes	0.09(.02)	0.29(.03)	0.57(.01)
Naphthalene	0.06(.02)	0.23(.08)	0.46(.02)
Methyl Naphthalenes	0.07(.01)	0.36(.01)	0.85(.01)
Dimethyl Naphthalenes	0.03(.01)	0.38(.22)	0.63(.02)
Total	0.39	1.72	3.55

[1] n = 2 prey rations

different. WSF dilutions below 5% were unstable, and could not reliably produce even lower hydrocarbon concentrations of OCP.

Absorption efficiency.

Percent absorption efficiency of ingested food was calculated for the fish using Conover's ratio (Conover 1966; see Appendix D) from the percent ash and organic matter in food and feces. The feces were collected daily on 333 µm plankton netting suspended below the outflow tube and above the 100 µm plankton netting used to collect uneaten prey. Food and feces were rinsed with ammonium formate (0.9% w/v) onto glass fiber filters, dried at 60 °C for 24 hours and weighed, then baked at 450 °C for 24 hours and reweighed to determine percent ash. Percent organic matter was obtained by difference.

The average percent ash content of the brine shrimp prey was 20% (± .04%, n = 10). There was no significant difference in percent ash between control and high dose oil-contaminated brine shrimp. Therefore, a value of 20% was used to calculate absorption efficiencies for all fry groups including the fish receiving low and medium dose OCP.

Oxygen consumption.

The amount of oxygen consumed by the fish was determined daily from the difference in partial pressure of oxygen between the inflowing and outflowing seawater to each chamber. Seawater samples were injected into a temperature controlled oxygen electrode connected to a

Radiometer PHM-71 acid-base analyzer. Differences in oxygen partial pressure were converted to micrograms (Strickland and Parsons 1968) to calculate pink fry oxygen consumption rates. Fry oxygen consumption was measured six hours after the fish were fed in order to avoid a rapid increase and decrease in oxygen consumption that occurred while the fish were feeding.

Ammonia excretion.

Seawater flowing through each chamber was assayed daily for ammonia content by the method of Solorzano (1969). Preliminary experiments found that ammonia excretion rose sharply within two hours after the fish were fed, then rapidly declined and were constant approximately five hours after the fish were fed. Brett and Zala (1975) observed a similar increase and decrease in ammonia excretion while feeding juvenile sockeye salmon, Oncorhynchus nerka, and concluded that the sharp rise and fall in ammonia excretion resulted from the break down of dietary protein. Therefore, both oxygen consumption and ammonia excretion were measured six hours after feeding in order to avoid the increase in metabolic activity during and immediately after the fish were fed. O:N ratios for the fish were obtained daily from the simultaneous oxygen consumption and ammonia excretion measurements.

Statistical analysis.

A two-way analysis of variance (Sokal and Rohlf 1969) was used to compare the energetics of control fry with fish exposed to OCP, fish exposed to WSF, and fish fed reduced rations. Comparisons with control fry were made during the exposure and post-exposure periods.

Comparisons between all fry groups were also determined by two-way ANOVA for three days before the experiment began. All energetic parameters were not significantly different between groups before the experiment began. There was also no significant difference in the number of prey added to each flow-through chamber during the experiment, but pre-exposure feeding rates were significantly different between groups on the basis of dry weight ($p < .01$). Pre-exposure feeding rates, however, were not significantly different on a per fish basis. The reason for the difference in statistical significance between feeding rates on a per fish and dry weight basis is not clear, but probably involved behavioral factors controlling feeding activity that were not weight dependent. It was decided to express feeding rates on the basis of dry weight so that they would be compatible with other energetic parameters.

Results

Feeding rate.

Fry exposed to all three dose levels of oil-contaminated prey (OCP) ate significantly less prey than controls (Figure 6a and Table 7). Average feeding rates for control fry and fish exposed to low, medium, and high dose OCP were, respectively, 11,196 (±160), 9,066 (±262), 7,237 (±480), and 5,743 (±187) prey/day/gm dry weight. Feeding rates of fry fed OCP were also significantly less than controls during the seven day post-exposure period (Table 7), but appeared to increase towards the last day of the post-exposure period.

Fry exposed to WSF also ate significantly less prey than controls (Figure 6b and Table 7). Fish exposed to WSF ate 69% less prey than controls during the exposure period. The feeding rates of WSF exposed fish immediately began to increase during the post-exposure period, and were not significantly different from controls during the post-exposure period.

Fry fed reduced rations were fed only 12% of the number of prey fed to the other fry groups, and therefore ate significantly less than controls (Figure 6b and Table 7). The fish fed reduced rations immediately began feeding at control levels during the post-exposure period when prey rations were the same for both groups of fish.

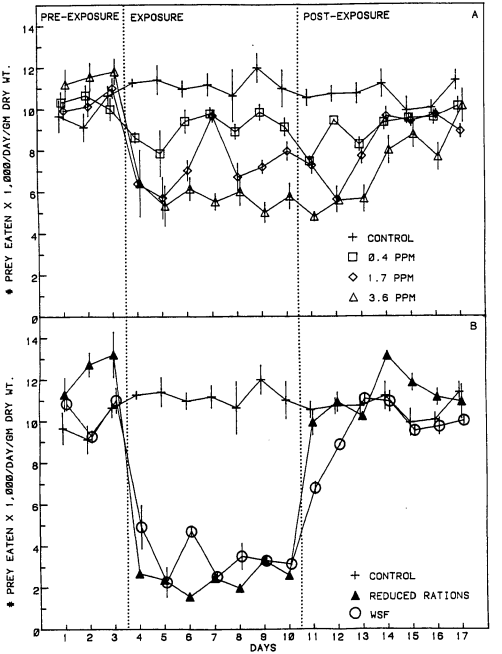

Figure 6. Average daily feeding rate of control fry, fry fed oil-contaminated prey, fry exposed to 0.7 ppm WSF, and fry fed reduced rations (± 1 SE).

Table 7. Two-way ANOVA tables comparing fry feeding rates during the exposure and post-exposure periods. A = time; B = treatment.

Source of Variation	S.S.	d.f.	M.S.	F-ratio
Exposure				
Control & Low Dose				
A	6.92×10^{6}	6	1.15×10^{6}	0.906 ns
B	4.76×10^{7}	1	4.76×10^{7}	37.425 ***
AxB	4.98×10^{6}	6	8.30×10^{5}	0.688 ns
error	3.56×10^{7}	28	1.27×10^{6}	
Control & Medium Dose				
A	1.53×10^{7}	6	2.56×10^{6}	2.032 ns
B	1.65×10^{8}	1	1.65×10^{8}	120.761 ***
AxB	1.69×10^{7}	6	2.82×10^{6}	2.243 ns
error	3.52×10^{7}	28	1.26×10^{6}	
Control & High Dose				
A	1.20×10^{6}	6	2.00×10^{5}	0.106 ns
B	3.12×10^{8}	1	3.12×10^{8}	165.319 ***
AxB	6.42×10^{6}	6	1.07×10^{6}	0.567 ns
error	5.29×10^{7}	28	1.89×10^{6}	
Control & WSF				
A	9.26×10^{6}	6	1.54×10^{6}	1.212 ns
B	6.25×10^{8}	1	6.25×10^{8}	490.674 ***
AxB	1.22×10^{7}	6	2.04×10^{6}	1.602 ns
error	3.57×10^{7}	28	1.27×10^{6}	
Control & Reduced Ration				
A	7.69×10^{6}	6	1.28×10^{6}	1.559 ns
B	8.08×10^{8}	1	8.08×10^{8}	981.919 ***
AxB	9.26×10^{5}	6	1.54×10^{5}	0.188 ns
error	2.30×10^{7}	28	8.23×10^{5}	

Table 7. (continued)

Source of Variation	S.S.	d.f.	M.S.	F-ratio
Post-exposure				
Control & Low Dose				
A	1.10×10^{7}	6	1.83×10^{6}	3.589 **
B	2.47×10^{7}	1	2.47×10^{7}	16.358 **
AxB	9.09×10^{6}	6	1.51×10^{6}	2.970 *
error	1.43×10^{7}	28	5.10×10^{5}	
Control & Medium Dose				
A	2.20×10^{7}	6	3.67×10^{6}	5.975 ***
B	5.70×10^{7}	1	5.70×10^{7}	13.988 **
AxB	2.55×10^{7}	6	4.25×10^{6}	6.915 ***
error	1.72x107	28	6.16×10^{5}	
Control & High Dose				
A	4.05×10^{7}	6	6.74×10^{6}	7.653 ***
B	1.23×10^{8}	1	1.23×10^{8}	22.276 **
AxB	3.32×10^{7}	6	5.54×10^{6}	6.285 ***
error	2.47×10^{7}	28	8.81×10^{5}	
Control & WSF				
A	2.58×10^{7}	6	4.30×10^{6}	8.373 ***
B	1.23×10^{7}	1	1.23×10^{7}	4.256 ns
AxB	1.73×10^{7}	6	2.89×10^{6}	5.622 ***
error	1.44×10^{7}	28	5.14×10^{5}	
Control & Reduced Rations				
A	1.43×10^{7}	6	2.39×10^{6}	3.262 *
B	2.72×10^{6}	1	2.72×10^{6}	1.424 ns
AxB	1.14×10^{7}	6	1.91×10^{6}	2.605 *
error	2.05×10^{7}	28	7.33×10^{5}	

* = $p < .05$; ** = $p < .01$; *** = $p < .001$: ns = not significant

Absorption efficiency.

The percent absorption efficiencies of fry fed medium and high dose OCP were significantly less than controls during the exposure period (Figure 7a and Table 8), and percent absorption efficiencies of fry fed at all three dose levels of OCP were significantly less than controls during the post-exposure period (Table 8). This indicated that the fish exposed to OCP were acquiring less digestible organic matter than controls, both during and after the exposure period. Fry fed low dose OCP, however, appeared to be recovering over the last two days of the post-exposure period since at that time their percent absorption efficiency was the same as controls.

In contrast to the fry fed OCP, the percent absorption efficiency of the fish exposed to WSF was significantly greater than controls during the exposure period (Figure 7b and Table 8). However, the percent absorption efficiency of fry exposed to WSF decreased when uncontaminated seawater replaced WSF. There was no significant difference in percent absorption efficiency between fry exposed to WSF and controls during the post-exposure period.

The fish fed reduced rations had the highest percent absorption efficiency of all fry groups tested, which was also significantly different than controls (Figure 7b and Table 8). However, the percent absorption efficiency of fish fed reduced rations decreased and was not significantly different from controls when prey rations were increased during the post-exposure period.

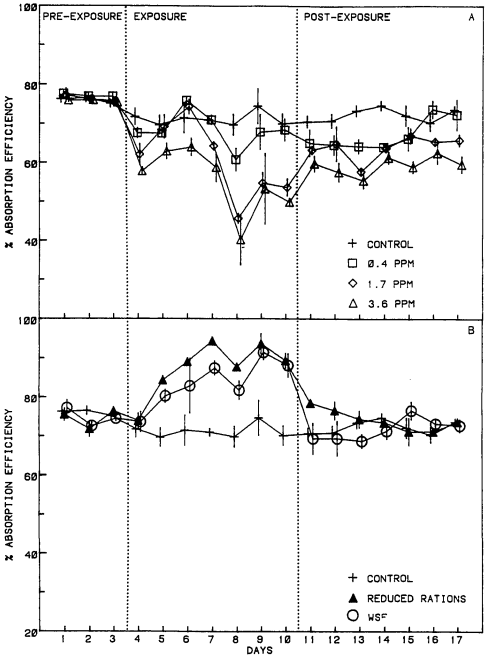

Figure 7. Average daily absorption efficiency of control fry, fry fed oil-contaminated prey, fry exposed to 0.7 ppm WSF, and fry fed reduced rations (± 1 SE).

Table 8. Two-way ANOVA tables comparing fry absorption efficiencies during the exposure and post-exposure periods. A = time; B = treatment.

Source of Variation	S.S.	d.f.	M.S.	F-ratio
Exposure				
Control & Low Dose				
A	319.73	6	53.29	2.683 *
B	35.11	1	35.11	1.768 ns
AxB	248.88	6	41.48	2.089 ns
error	556.11	28	19.86	
Control & Medium Dose				
A	789.36	6	164.893	8.895 ***
B	1246.42	1	1246.42	9.147 *
AxB	817.59	6	136.26	7.350 ***
error	519.07	28	18.54	
Control & High Dose				
A	740.86	6	123.48	2.878 *
B	2755.62	1	2755.62	64.221 ***
AxB	558.09	6	93.01	2.168 ns
error	1201.44	28	42.91	
Control & WSF				
A	407.046	6	67.841	3.685 **
B	1542.149	1	1542.149	32.014 ***
AxB	289.026	6	48.171	2.617 *
error	515.440	28	18.409	
Control & Reduced Ration				
A	512.048	6	85.341	5.790 ***
B	2680.006	1	2680.006	39.396 ***
AxB	408.162	6	68.627	4.615 **
error	412.733	28	14.741	

Table 8. (continued)

Source of Variation	S.S.	d.f.	M.S.	F-ratio
Post-exposure				
Control & Low Dose				
A	145.58	6	24.26	1.564 ns
B	267.02	1	267.02	17.216 ***
AxB	200.08	6	33.35	2.15 ns
error	434.29	28	15.51	
Control & Medium Dose				
A	84.62	6	14.10	1.35 ns
B	711.77	1	711.77	67.862 ***
AxB	132.20	6	22.03	2.101 ns
error	293.27	28	10.49	
Control & High Dose				
A	62.63	6	10.44	0.965 ns
B	1747.31	1	1747.31	161.471 ***
AxB	84.18	6	14.03	1.297 ns
error	302.99	28	10.82	
Control & WSF				
A	99.147	6	16.524	1.047 ns
B	4.275	1	4.275	0.271 ns
AxB	94.451	6	15.742	0.998 ns
error	441.847	28	15.780	
Control & Reduced Ration				
A	71.515	6	11.919	1.213 ns
B	36.214	1	36.214	3.686 ns
AxB	111.242	6	18.540	1.887 ns
error	275.073	28	9.824	

* = $p < .05$; ** = $p < .01$; *** = $p < .001$; ns = not significant

Oxygen consumption.

Fry exposed to all three dose levels of OCP consumed significantly less oxygen than controls during the exposure period (Figure 8a and Table 9). Average oxygen consumption rates for control fry and fry fed low, medium, and high dose OCP were, respectively, 44.8 (± 0.6), 41.9 (± 0.8), 41.2 (± 1.2), and 38.2 (± 2.0) μg O_2/min/gm dry weight. There was no significant correlation between oxygen consumption rates and dose level, but a significant correlation between oxygen consumption and feeding rates (Pearson's $r = 0.972$, $p < .05$). This suggested that the changes in oxygen consumption with exposure to OCP resulted from a decrease in metabolic activity that occurred when feeding rates decreased. The oxygen consumption rates of fry fed high dose OCP were also significantly less than control rates during the post-exposure period (Table 9), but the oxygen consumption rates of fish fed low and medium dose OCP were not significantly different from controls. There was no significant correlation between oxygen consumption and feeding rates during the post-exposure period.

In contrast to the fish fed OCP, fry exposed to WSF consumed significantly more oxygen than controls during the exposure period (Figure 8b and Table 9), even though feeding rates of fry exposed to WSF were lower than the feeding rates of fish exposed to high dose OCP. However, oxygen consumption rates of the fish exposed to WSF rapidly decreased and were not significantly different from controls when uncontaminated seawater replaced WSF during the post-exposure period.

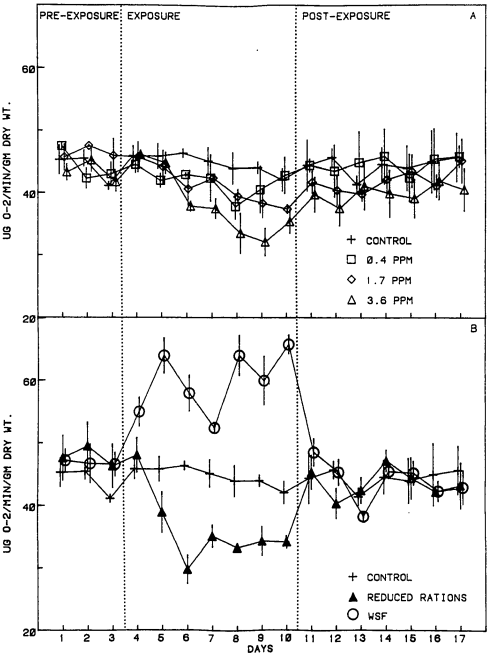

Figure 8. Average daily oxygen consumption rate of control fry, fry fed oil-contaminated prey, fry exposed to 0.7 ppm WSF, and fry fed reduced rations (± 1 SE).

Table 9. Two-way ANOVA tables comparing fry oxygen consumption rates during the exposure and post-exposure periods. A = time; B = treatment.

Source of Variation	S.S	d.f.	M.S.	F-ratio
Exposure				
Control & Low Dose				
A	85.82	6	14.30	1.016 ns
B	88.31	1	88.31	6.270 *
AxB	40.37	6	6.73	0.478 ns
error	394.35	28	14.08	
Control & Medium Dose				
A	172.93	6	28.82	2.787 *
B	135.00	1	135.00	13.055 **
AxB	39.58	6	6.60	0.638 ns
error	289.55	28	10.34	
Control & High Dose				
A	378.80	6	63.13	6.209 **
B	453.43	1	453.43	14.783 **
AxB	184.04	6	30.67	3.017 *
error	284.71	28	10.17	
Control & WSF				
A	172.282	6	28.714	1.864 ns
B	2403.174	1	2403.174	44.008 ***
AxB	327.648	6	54.608	3.545 **
error	431.307	28	15.404	
Control & Reduced Ration				
A	383.171	6	63.862	5.299 ***
B	774.002	1	774.002	16.094 ***
AxB	288.563	6	48.094	3.990 **
error	337.480±	28	12.0529	

Table 9. (continued)

Source of Variation	S.S	d.f.	M.S.	F-ratio
Post-exposure				
Control & Low Dose				
A	33.14	6	5.52	0.139 ns
B	0.83	1	0.83	0.021 ns
AxB	29.22	6	4.87	0.123 ns
error	1109.17	28	39.61	
Control & Medium Dose				
A	71.41	6	11.90	0.466 ns
B	61.69	1	61.69	2.413 ns
AxB	27.84	6	4.64	0.182 ns
error	715.91	28	25.57	
Control & High Dose				
A	23.35	6	3.89	0.117 ns
B	205.93	1	205.93	6.168 *
AxB	54.30	6	9.05	0.271 ns
error	934.87	28	33.39	
Control & WSF				
A	163.747	6	27.291	1.208 ns
B	1.680	1	1.680	0.074 ns
AxB	64.613	6	10.769	0.477 ns
error	632.440	28	22.587	
Control & Reduced Ration				
A	60.680	6	10.113	0.377 ns
B	6.962	1	6.962	0.260 ns
AxB	71.966	6	11.994	0.448 ns
error	750.400	28	26.800	

* = $p < .05$; ** = $p < .01$; *** = $p < .001$; ns = not significant

The fry fed reduced rations consumed significantly less oxygen than controls (Figure 8b and Table 9). The oxygen consumption rate of fry fed reduced rations decreased by 38% during the first three days of food deprivation but did not significantly change for the remainder of the exposure period. There was no significant difference in oxygen consumption rates during the post-exposure period between fry fed reduced rations and controls.

Ammonia excretion.

Fry fed OCP at all three dose levels excreted significantly less ammonia than controls during the exposure period (Figure 9a and Table 10). Average ammonia excretion rates for control fry and fry fed low, medium, and high dose OCP were, respectively, 3.3 (± 0.1), 3.1 (± 0.1), 2.9 (± 0.1), and 2.5 (± 0.1) μg NH_4^+-N/min/gm dry weight. There was no significant correlation between ammonia excretion and dose level, but a significant correlation between ammonia excretion rates and feeding rates (Pearson's $r = 0.951$, $p < .05$), and between ammonia excretion and oxygen consumption rates Pearson's $r = 0.983$, $p < .05$). This suggested that the decreases in ammonia excretion resulted from a decrease in metabolic activity that occurred with the reduction in food intake. Fish fed medium and high dose OCP also excreted significantly less ammonia than controls during the post-exposure period (Table 10). There was no significant correlation between ammonia excretion rates and feeding rates during the post-exposure period, but a significant

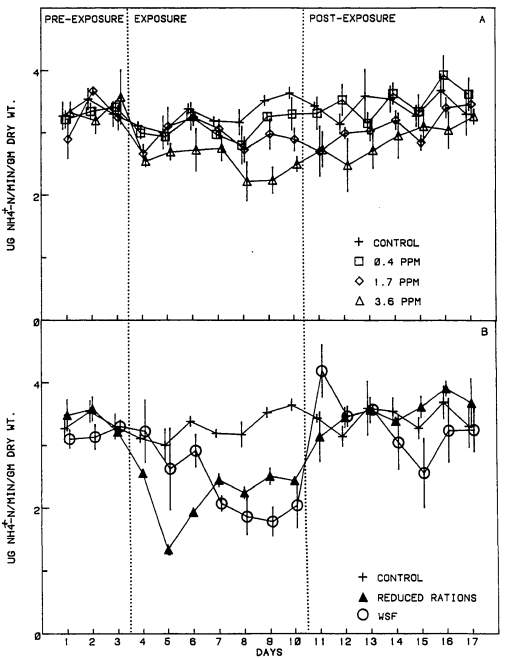

Figure 9. Average daily ammonia excretion rate for control fry, fry fed oil-contaminated prey, fry exposed to 0.7 ppm WSF, and fry fed reduced rations (± 1 SE).

Table 10. Two-way ANOVA tables comparing fry ammonia excretion rates during the exposure and post-exposure periods. A = time; B = treatment.

Source of Variation	S.S.	d.f.	M.S.	F-ratio
Exposure				
Control & Low Dose				
A	1.53	6	0.25	3.384 *
B	0.47	1	0.47	6.298 *
AxB	0.12	6	0.02	0.268 ns
error	2.11	28	0.08	
Control & Medium Dose				
A	0.96	6	0.16	1.798 ns
B	1.16	1	1.16	12.979 **
AxB	0.77	6	0.09	1.437 ns
error	2.50	28	0.09	
Control & High Dose				
A	0.63	6	0.11	1.062 ns
B	6.10	1	6.10	61.435 ***
AxB	1.20	6	0.20	2.016 ns
error	2.78	28	0.10	
Control & WSF				
A	2.293	6	0.382	1.494 ns
B	9.035	1	9.035	12.638 *
AxB	4.289	6	0.715	2.794 *
error	7.164	28	0.256	
Control & Reduced Ration				
A	3.063	6	0.511	12.832 ***
B	12.258	1	12.258	54.287 ***
AxB	1.355	6	0.226	5.675 ***
error	1.114	28	0.040	

Table 10. (continuted)

Source of Variation	S.S.	d.f.	M.S.	F-ratio
Post-exposure				
Control & Low Dose				
A	1.15	6	0.19	1.007 ns
B	0.07	1	0.07	0.354 ns
AxB	0.73	6	0.12	0.638 ns
error	5.31	28	0.19	
Control & Medium Dose				
A	1.36	6	0.23	1.379 ns
B	1.16	1	1.16	7.027 *
AxB	0.73	6	0.12	0.742 ns
error	4.61	28	0.16	
Control & High Dose				
A	1.16	6	0.19	0.811 ns
B	2.86	1	2.86	12.007 **
AxB	0.80	6	0.13	0.557 ns
error	6.67	28	0.24	
Control & WSF				
A	1.849	6	0.308	1.864 ns
B	0.020	1	0.020	0.122 ns
AxB	1.713	6	0.286	1.726 ns
error	4.631	28	0.165	
Control & Reduced Ration				
A	1.099	6	0.183	0.956 ns
B	0.117	1	0.117	0.612 ns
AxB	0.630	6	0.105	0.548 ns
error	5.367	28	0.192	

* = $p < .05$; ** = $p < .01$; *** = $p < .001$; ns = not significant

correlation between ammonia excretion and oxygen consumption rates (Pearson's $r = 0.993$, $p < .01$).

Fry exposed to WSF also excreted less ammonia than controls during the exposure period (Figure 9b and Table 10). The ammonia excretion rate for the WSF exposed fish was near control levels during the first two days of exposure, but then decreased below control levels for the remainder of the exposure period. There was no significant difference in ammonia excretion rates between WSF exposed fry and controls during the post-exposure period.

The ammonia excretion rate for fry fed reduced rations significantly decreased from 3.2 µg NH_4^+-N/min/gm dry weight before prey rations were reduced to 1.3 µg NH_4^+-N/min/gm dry weight within the first two days of food deprivation (Figure 9b and Table 10). The fish maintained on reduced rations continued to excrete less ammonia than controls for the duration of the exposure period. However, the ammonia excretion rate for fry fed reduced rations was not significantly different than controls during the post-exposure period.

O:N ratio.

The daily O:N ratio for control pink salmon fry was within the range of O:N ratios calculated from the oxygen consumption and ammonia excretion rates for juvenile sockeye salmon, Oncorhynchus nerka, obtained by Brett and Zala (1975) over a 24 hr period. The O:N ratio of fry fed high dose OCP was significantly higher than controls during the exposure period (Figure 10a and Table 11). The O:N ratios for fry

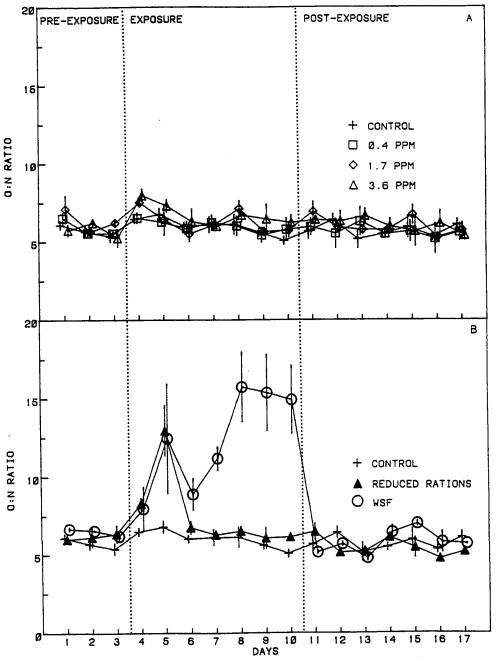

Figure 10. Average daily O:N ratio of control fry, fry fed oil-contaminated prey, fry exposed to 0.7 ppm WSF, and fry fed reduced rations (± 1 SE).

Table 11. Two-way ANOVA tables comparing O:N ratios of fry during the exposure and post-exposure periods. A = time; B = treatment.

Source of Variation	S.S.	d.f.	M.S.	F-ratio
Exposure				
Control & Low Dose				
A	6.67	6	1.11	1.927 ns
B	0.0003	1	0.0003	0.001 ns
AxB	1.28	6	0.21	0.371 ns
error	16.15	28	0.58	
Control & Medium Dose				
A	12.58	6	2.10	3.440 *
B	0.77	1	0.77	1.269 ns
AxB	3.61	6	0.60	0.987 ns
error	17.07	28	0.61	
Control & High Dose				
A	11.70	6	1.95	2.790 *
B	5.00	1	5.00	7.150 *
AxB	2.60	6	0.43	0.621 ns
error	19.58	28	0.70	
Control & WSF				
A	76.852	6	12.809	1.839 ns
B	419.458	1	419.458	23.198 **
AxB	108.491	6	18.082	2.596 *
error	195.061	28	6.967	
Control & Reduced Ration				
A	76.560	6	12.760	13.381 ***
B	24.503	1	24.503	3.667 ns
AxB	40.095	6	6.683	7.008 ***
error	26.701	28	0.954	

Table 11. (continued)

Source of Variation	S.S.	d.f.	M.S.	F-ratio
Post-exposure				
Control & Low Dose				
A	2.13	6	0.35	0.319 ns
B	0.02	1	0.02	0.022 ns
AxB	3.66	6	0.61	0.549 ns
error	31.10	28	1.11	
Control & Medium Dose				
A	6.10	6	1.03	1.784 ns
B	0.89	1	0.89	1.564 ns
AxB	3.60	6	0.60	1.053 ns
error	15.96	28	0.57	
Control & High Dose				
A	1.83	6	0.31	0.349
B	1.37	1	1.37	1.564
AxB	4.98	6	0.83	0.949
error	24.50	28	0.88	
Control & WSF				
A	8.383	6	1.397	3.037 *
B	0.034	1	0.034	0.075 ns
AxB	4.875	6	0.812	1.766 ns
error	12.880	28	0.460	
Control & Reduced Ration				
A	4.680	6	0.780	1.642 ns
B	0.731	1	0.731	1.539 ns
AxB	5.379	6	0.897	1.888 ns
error	13.299	28	0.475	

* = $p < .05$; ** = $p < .01$; *** = $p < .001$; ns = not significant

fed all three dose levels of OCP were not significantly different from controls during the post-exposure period.

The O:N ratio for fry exposed to WSF was also significantly higher than controls during the exposure period (Figure 10b and Table 11). The O:N ratio of WSF exposed fish began to increase on the first day of exposure, and eventually was more than double the O:N ratio of control fry. However, there was no significant difference in O:N ratio between control and WSF exposed fry during the post-exposure period.

The O:N ratio for the fish fed reduced rations increased from 6.3 before prey rations were reduced, to 12.9 after two days of food deprivation (Figure 10b). The O:N ratio of fry fed reduced rations subsequently decreased to near control levels, and over the entire seven-day exposure period was not significantly different from controls (Table 11). There was no significant difference in O:N ratios between fry fed reduced rations and controls during the post-exposure period.

Discussion

Exposure to oil-contaminated prey (OCP) did not increase fry metabolic rate. Fry metabolic rate declined with reductions in food intake, which has been documented for other fish species fed reduced amounts of food (Warren and Davis 1967). However, fry metabolic rate did not correlate with feeding rate during the post-exposure period. The fish became metabolically more active when oil was removed from the

diet. The increase in activity may have been caused by an increase in feeding activity that occurred when OCP were replaced with uncontaminated prey. The feeding activity of fishes, which includes additional energy expenditures for locating, capturing, and competing for food can increase to more than four times routine metabolism (Brett and Groves 1979). It is possible that some fish in each chamber may have responded earlier to uncontaminated prey than others, and stimulated an overall increase in activity among fish in each chamber that was not entirely explained by the number of ingested prey.

The fry exposed to WSF ate less prey than fish exposed to OCP, and were the only group of fish that had an increase in metabolic activity during the exposure period. Although the hydrocarbon concentration in OCP was as much as five times greater than the WSF hydrocarbon concentration, a larger amount of hydrocarbons was available to the fry exposed to WSF than to the fry fed OCP. Therefore, exposure to WSF had a greater effect on fry feeding and metabolic rates than OCP. The results of this study, and the results of the growth rate study in the previous chapter, support the earlier finding of Moles and Rice (1983) that reductions in feeding rate and an increase in metabolic activity, are responsible for reducing the growth rate of fry exposed to WSF.

Fry exposed to OCP or WSF did not excrete more ammonia than controls, but instead excreted decreasing amounts of ammonia with reductions in food intake. Unlike mammals, fish normally exploit a portion of their protein as an energy source (Brett and Groves 1979). Cowey and Sargent (1972) explained that exogenous (dietary) and

endogenous protein contribute to a common pool of amino acids from which energy requirements for maintenance and growth can be met. It is reasonable to assume, therefore, that the fish in the present study were exploiting some endogenous protein for energy, but the amount of endogenous protein being catabolized and excreted as ammonia was insignificant when compared to the amount of ammonia excreted from the breakdown of dietary protein.

The increase in O:N ratio of fry fed high dose OCP indicated that these fish were utilizing proportionately more lipid and/or carbohydrate than protein, but the increase was relatively small and probably resulted from a decrease in feeding rate and not directly as a result of exposure to OCP. For example, the fry fed reduced rations immediately began consuming proportionately more non-nitrogenous substrates, and their O:N ratio increased. However, after two days of food deprivation, the O:N ratio of fry fed reduced rations was again near the O:N ratio of control fry that were consuming five times more food. For the fish exposed to high dose OCP, the highest daily O:N ratios also occurred on the first and second day of the exposure period, but subsequently declined to near control levels. In fact, if the first two days of the exposure period are ommitted from a two-way ANOVA comparing O:N ratios of fry fed high dose OCP and controls there is no significant difference between these two fry groups. The increase in O:N ratio of fry fed high dose OCP, therefore, was similar to the change in O:N ratio of fry fed reduced rations, and suggests

that the increase in O:N ratio of fry fed high dose OCP resulted from a decrease in feeding rate.

The O:N ratio of fry exposed to WSF, however, significantly increased because fry oxygen consumption rates increased while ammonia excretion rates decreased, and indicated that the fish were utilizing proportionately more lipid and/or carbohydrate than protein. Under this condition of abnormal stress when the fish ingested reduced amounts of food but required additional energy, it was probably more advantageous for the fry to conserve protein and instead utilize other energy sources, such as fat tissue, since more energy is obtained from the breakdown of lipids and carbohydrates than protein (Elliott and Davison 1975), and because salmonids are capable of utilizing dietary carbohydrates to meet energy needs (Phillips 1969). Non-protein energy reserves would eventually become depleted, which could result in the breakdown of more endogenous protein. However, by the time energy reserves are depleted and additional protein is broken down and excreted, the fry exposed to WSF would probably be near death from starvation. This effect was in contrast to invertebrate species exposed to oil that excrete proportionately more ammonia, and thereby decreases O:N ratio (Capuzzo and Lancaster 1981).

The absorption efficiency of pink salmon fry decreased with exposure to OCP. Several studies of fish energetics reported that food utilization efficiency by various fish species increases when food rations decrease (Paloheimo and Dickie 1966; Warren and Davis 1967; Solomon and Brafield 1972; Elliott 1976), which also was observed with

the fry fed reduced rations in the present study. However, a decrease in absorption efficiency by fry fed OCP indicated that these fish were absorbing less organic matter, which represented a loss of available food energy. Fish also excrete nitrogenous waste products in their feces resulting from the breakdown of endogenous protein (Elliott 1976). Fecal nitrogen content was not determined in the present study, but it is possible that the feces of fry fed OCP could have included waste products resulting from the breakdown of endogenous protein that were not taken into account by ammonia excretion rates.

Reduced absorption efficiencies of fry fed OCP probably resulted from damage to gut tissues that were in contact with petroleum hydrocarbons. Prolonged reductions in absorption efficiency during the post-exposure period suggest that damage to gut tissue was not reversible except in those fish exposed to low dose OCP. Hawkes et al. (1980) found consistent patterns of alteration with the morphology of gut tissue of juvenile chinook salmon, Oncorhynchus tshwaytsha, fed a mixture of 5 ppm aromatic hydrocarbons. The cells below the intestinal mucosa of the juvenile chinook salmon contained foreign flocculent material either produced by the fish or acquired in the diet. Alterations in cellular morphology of juvenile chinook salmon gut tissues were the same after 14 and 28 days of exposure to oil, and persisted unchanged thereafter for 21 days of feeding on clean food. Rainbow trout fed 0.1 ppm of Prudhoe Bay crude oil over two months had depleted energy reserves in their liver, and 25% slower growth rates (Hawkes 1977). Absorption efficiencies of juvenile chinook salmon and

rainbow trout were not measured. However, trout energy stores were probably depleted because the fish were unable to absorb all their food energy, and changes in cellular morphology of juvenile chinook salmon gut tissues suggest that impaired food uptake and digestion could have resulted from exposure to oil in the diet. Fry gut tissues were not examined in the present study. However, reductions in absorption efficiency indicated a loss of available food energy that probably occurred when fry gut tissues came into contact with oil.

A decrease in the absorption efficiency of fry fed OCP could have contributed to the reduction in fry percent dry weight that occurred during the growth rate study in the previous chapter. During the growth rate study, the percent dry weight of the fry fed 3.2 ppm and 6.5 ppm OCP was significantly less than controls after prolonged exposure to OCP. The decrease in percent dry weight indicated that the fry were consuming body tissues faster than they could be replaced by the incorporation of ingested food. The results from the present study suggest that exposure to OCP could have sufficiently reduced the uptake of food energy to the extent that the fry chronically exposed to OCP were utilizing stored energy, such as fat tissue, and possibly were catabolizing other body tissues.

In contrast to the fry fed OCP, fry exposed to WSF had higher absorption efficiencies than controls. The increase in absorption efficiency probably resulted from an increase in metabolic activity. Eldridge et al. (1977) reported that the food utilization efficiency of herring larvae, Clupea harengus, increased with exposure to benzene in

seawater which could have resulted from an increase in metabolic activity, because Struhsaker et al. (1974) previously reported that the oxygen consumption rates of herring larvae increased when the larvae were exposed to benzene in seawater.

The results of this study found distinct differences in the energetics of pink salmon fry exposed to WSF when compared to the energetics of fry exposed to OCP. Exposure to WSF had a greater effect on depleting fry of energy than exposure to OCP because of a reduction in feeding rate compounded by an additional energy burden as indicated by an increase in oxygen consumption rates of fry exposed to WSF. Exposure to WSF, however, did not reduce fry absorption efficiency. On the other hand, exposure to OCP did not effect fry metabolic rate, but did impair the normal uptake of food energy. Although the effect of WSF on reducing fry feeding rate and increasing metabolic rate was reversible when WSF was later removed from seawater, the effect of OCP on reducing fry feeding rate and absorption efficiency when the fish were later offered uncontaminated prey was not.

CHAPTER 4

SUMMARY OF EXPERIMENTAL RESULTS

This study found that oil-contaminated prey (OCP) were not acutely toxic to pink salmon fry. However, changes in feeding rate, growth rate, and additional energy losses indicate that prolonged exposure to OCP can reduce fry survival.

Fry feeding rate was reduced by more than 40% when hydrocarbon concentrations of contaminated prey exceeded 3.5 ppm. Hydrocarbon concentrations as low as 0.4 ppm significantly reduced the number of prey eaten by the fish. Feeding rates remained below control levels for several days after exposure to OCP.

Exposure to the water-soluble fraction (WSF) of Cook Inlet crude oil in seawater had a greater effect on reducing fry feeding rates than exposure to OCP. Hydrocarbon concentrations in OCP were as much as five times greater than the WSF concentration, but greater amounts of hydrocarbons were available to the fry from WSF than from OCP. In contrast to the prolonged reductions in feeding rates of fry exposed to OCP, however, the feeding rates of WSF exposed fish quickly increased to control levels following exposure.

Exposure to OCP reduced fry growth rate, and there was a significant correlation between reductions in feeding and growth rates over a large range of OCP dose levels. Prolonged exposure to OCP

resulted in a decrease in fry percent dry weight, possibly because some body tissue was utilized as an energy source. Exposure to WSF had a greater effect on reducing fry growth than did OCP. Although the fish exposed to WSF increased in wet weight over fifty days of exposure, dry weight decreased and was lower than at the start of exposure. The percent dry weight of fish exposed to WSF was significantly lower than controls by the end of the exposure period.

Exposure to OCP did not affect fry metabolic rate. However, reduced oxygen consumption and ammonia excretion rates resulted from reductions in feeding rate. Fry food absorption efficiency was affected by OCP. Fry food absorption efficiency decreased with continued exposure to OCP, indicating a loss of food energy available to the fish. The absorption efficiency of fry fed OCP was also less than controls during a seven day post-exposure period. It is possible that the prolonged reduction in absorption efficiency of fry fed OCP resulted from damage to gut tissues in contact with oil, because previous experiments found that gut tissues of juvenile chinook salmon remained morphologically altered for several weeks after exposure to petroleum hydrocarbons (Hawkes et al. 1980). Chronic exposure to OCP in the present study may have contributed to reducing fry percent dry weight by impairing normal uptake of food energy.

In contrast to the decrease in metabolic rate of fry fed OCP, fry metabolic rate increased from exposure to WSF. Lipid and/or carbohydrate was utilized to meet the increased demand for energy instead of protein. The absorption efficiency of fry exposed to WSF

was higher than controls probably because of an increase in metabolic activity. The metabolic rate and absorption efficiency of fry exposed to WSF returned to control levels within one day following exposure.

CHAPTER 5

IMPLICATIONS OF RESULTS

Higher concentrations of petroleum hydrocarbons in invertebrate prey than those used in the present study can result from an oil spill and be available to the diet of juvenile salmon. The effect of oil-contaminated prey (OCP) on the survival of pink salmon fry, therefore, could be greater than indicated by the results of the present study, which probably represent a conservative estimate. Oil has been found in pelagic copepods following an oil spill (Conover 1971), and aromatic hydrocarbon concentrations in zooplankton species as high as 175 ppm have been reported as far away as 45 km from the site of a major oil spill (Mackie et al. 1978). Because invertebrate prey species included in the diet of juvenile salmon, such as copepods, can accumulate petroleum hydrocarbons in excess of an order of magnitude more than the surrounding seawater (Corner 1975; Corner et al. 1976; Harris et al. 1977), OCP organisms chronically exposed to low concentrations of hydrocarbons in seawater, such as in polluted urban areas, could contain hydrocarbon concentrations similar to those used in the present study. Although pink salmon fry can avoid hydrocarbon concentrations in seawater as low as 1.16 ppm (Rice 1973), the fry could remain in oil-contaminated areas containing hydrocarbon

concentrations lower than 1.16 ppm, and still be exposed to prey organisms containing higher concentrations of petroleum hydrocarbons.

Other sources of petroleum for prey organisms available to juvenile salmon can come from hydrocarbons in bottom sediments. Cooney et al. (1981) reported that pink salmon fry ate benthic harpacticoid copepods in addition to pelagic zooplankton species. Other instances of harpacticoids in the diet of juvenile salmon have been reported in the Northwest Pacific (Kacysinski et al. 1973; Healy 1979), and a long-term source of contaminated prey organisms could be available from benthic prey species in contact with oil-contaminated bottom sediments. Oil in sediments dissipates more slowly than oil in seawater. Hydrocarbon concentrations in seawater could decrease below an avoidance threshold, such as below 1.16 ppm for pink salmon fry (Rice 1973), while benthic organisms continue to provide a long-term source of contaminated prey.

Although the results of this study indicate that exposure to oil in seawater has a greater effect on reducing the feeding and growth of fry than exposure to OCP, the latter organism could have a greater impact on fry survival than oil in seawater when the results are applied to field conditions. Juvenile salmon can detect and avoid oil in seawater (Rice 1973; Maynard and Weber 1981), and it has been suggested that an avoidance response to oil could cause the fish to leave biologically productive areas, and possibly migrate to other nearshore areas less abundant in prey organisms. This seems unlikely because juvenile salmon are opportunistic predators that are capable of

finding sufficient amounts of food, even when zooplankton standing stock measurements would indicate to the contrary (Cooney et al. 1981). However, pink salmon fry did not completely avoid OCP when allowed to choose between contaminated and uncontaminated prey. Furthermore, juvenile salmon depend on their sight and not chemoreception when searching for food (Hoar 1958). Pink salmon fry, therefore, would probably remain in an area containing OCP so long as there is an abundant supply of prey organisms. However, the fry could detect and avoid oil in seawater and thereby avoid its harmful effects.

Since the growth rate of control fry in the present study was approximately half the growth rate of fry feeding on existing zooplankton (Cooney et al. 1981), the effect of OCP on reducing fry growth could be greater for pink salmon fry in the marine environment. The reduction in growth rate of control fry suggests that the fish were exposed to stress that normally would not be encountered in the marine environment, and probably resulted from the fry feeding on brine shrimp nauplii instead of zooplankton prey species. Without the additional stress of an inadequate diet, it is possible that the effect of OCP on reducing fry growth could become apparent in half the time observed in the present study, because the growth rate of fry in the marine environment can be twice the growth rate of control fry in this study.

It is difficult to assess the impact of OCP on fry survival to adulthood without also examining the relationship between fry growth and survival. The fry must grow rapidly within four months while feeding in coastal waters. Only the larger fish will survive and

migrate offshore to develop into adults. The smaller fish are more likely to be outcompeted for food, and are easy prey for larger fishes (Parker 1971). Bilton et al. (1982) conducted an experiment to determine the optimum release time and size of hatchery reared coho salmon, Oncorhynchus kisutch, by releasing three different size classes of smolts once every month for four months (April through July) when the fish normally inhabit nearshore feeding areas. They found that the time of release had a significant effect on the number of adults returning to the hatchery. The smolts released in June had a higher return than those released in April. However, with each time of release the pattern of returning adults from smolts of different sizes was the same; smaller fish produced significantly fewer returning adults than larger fish. A 25% difference in smolt weight could produce as much as a 20% difference in the number of returning adults. Wertheimer et al. (1983) reported a 57% decrease in returning adult sockeye salmon, O. nerka, from a 37% decrease in smolt weight, although the time of release effected the percent return of adults because the larger smolts were released six weeks later that smaller smolts, and yielded higher returns. Since survival of juvenile salmon is to a significant extent dependent on size, pink salmon fry exposed to OCP are probably less likely to survive than fry feeding on uncontaminated prey.

The size of pink salmon fry chronically exposed to OCP was reduced by more than one-third in the present study. This suggests that chronic exposure to OCP could cause sufficient reductions in the

survival of hatchery reared fry and result in lower returns for adult pink salmon. Information on the number of returning adult pink salmon that were exposed as fry to OCP is not available at this time. However, the reductions in growth of coho salmon, Oncorhynchus kisutch, smolts reported by Bilton et al. (1982) which produced lower numbers of returning adults suggests that a similar reduction in growth of pink salmon fry described in the present study could produce a 20% decrease in the number of returning adult pink salmon.

Exposure to OCP could have an even greater impact on the survival of wild populations of pink salmon fry, because these fish are not protected from predation. For example, Martin et al. (1981) found that pink salmon fry held for thirty days at a hatchery survived better than fry released immediately after hatching, because the fish were protected from predation when held in floating pens. However, wild populations of juvenile pink salmon could be highly vulnerable to the effects of OCP when they initially enter the marine environment, and must make the transition from freshwater to seawater, and the transformation from yolk dependent larvae to feeding fry. The fish are small at this stage in their life history, and a decrease in growth during early development could have a significant impact on their survival. The results of this study imply that exposure to OCP could also be detrimental to the survival of other juvenile fish species, since zooplankton prey species are the feedstock for many fishes during early sea life.

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APPENDIX A. Average daily feeding rates (number of prey eaten/day/fish) of pink salmon fry corresponding to Figure 1. Parentheses contain the standard error of the mean.

Time (Days)	Control	ppm Oil-Contaminated Prey 0.6	3.2	6.5
Exposure				
1	2,085 (192)	2,142 (296)	1,274 (86)	880 (189)
2	3,799 (274)	1,579 (230)	1,230 (398)	1,326 (58)
3	2,104 (213)	2,174 (123)	919 (493)	1,634 (408)
4	2,567 (276)	1,560 (116)	1,861 (217)	2,070 (98)
5	3,536 (426)	1,256 (261)	2,274 (184)	1,725 (129)
6	3,357 (162)	1,808 (260)	1,599 (194)	1,300 (137)
7	4,036 (432)	2,440 (219)	1,398 (500)	1,453 (162)
8	2,756 (107)	1,850 (54)	2,388 (201)	1,320 (66)
9	3,302 (294)	2,957 (396)	2,432 (394)	1,753 (281)
10	3,973 (498)	1,815 (213)	1,558 (391)	819 (254)

APPENDIX A. (continued)

Time (Days)	Control	ppm Oil-Contaminated Prey 0.6	3.2	6.5
Post-exposure				
11	3,139 (424)	1,332 (112)	1,260 (247)	725 (377)
12	2,849 (436)	2,013 (467)	1,456 (464)	1,290 (200)
13	2,862 (444)	1,558 (312)	1,788 (370)	1,905 (454)
14	3,272 (224)	2,090 (270)	1,498 (138)	1,324 (224)
15	2,836 (165)	2,314 (514)	1,755 (301)	1,227 (82)

APPENDIX B. Average length, wet weight, dry weight and percent dry weight of pink salmon fry. Average length, wet weight and dry weight correspond to day 10, 23, and 36 on Figures 2, 3, and 4, respectively. Sample size for length and wet weight was 30 fish, and sample size for dry weight and percent dry weight was 10 fish. Parentheses contain 95 percent confidence intervals.

		ppm Oil-Contaminated Prey			
	Control	0.6	3.2	6.5	WSF
Day 10					
Length (mm)	34(0.7)	33(0.6)	32(0.5)	33(0.6)	32(0.6)
Wet wt (mg)	307(21)	281(19)	260(16)	287(21)	269(17)
Dry wt (mg)	56(8)	54(7)	46(6)	55(12)	47(5)
% Dry wt	17.6(0.3)	17.2(0.5)	17.1(0.5)	17.5(0.5)	17.1(0.4)
Day 23					
Length (mm)	35(0.6)	34(0.6)	34(0.6)	34(0.6)	32(0.7)
Wet wt (mg)	384(25)	336(23)	333(21)	342(25)	284(23)
Dry wt (mg)	73(8)	57(11)	58(10)	58(10)	51(9)
% Dry wt	17.6(0.3)	17.0(0.5)	17.0(0.6)	17.0(0.3)	16.9(0.7)
Day 36					
Length (mm)	37(1.0)	36(0.8)	35(0.8)	35(0.9)	
Wet wt (mg)	428(41)	392(28)	368(30)	371(33)	
Dry wt (mg)	72(16	74(10)	64(7)	67(15)	
% Dry wt	17.1(0.6)	17.7(0.5)	16.7(0.5)	17.1(0.7)	

APPENDIX C. Average daily feeding rates (number of prey eaten/day/gm dry wt) of pink salmon fry corresponding to Figure 6. Parentheses contain the standard error of the mean.

Time (Days)	Control	ppm Oil-Contaminated Prey 0.4	1.7	3.6	0.7 ppm WSF	Reduced Rations
Pre-exposure						
1	9,660 (773)	10,334 (497)	9,925 (418)	11,196 (713)	10,861 (314)	11,297 (798)
2	9,120 (663)	10,664 (485)	10,137 (474)	11,557 (665)	9,257 (298)	12,722 (596)
3	10,644 (46)	9,990 (541)	11,009 (473)	11,807 (609)	11,006 (606)	13,198 (1,088)
Exposure						
4	11,263 (138)	8,618 (2540	6,398 (156)	6,413 (1,605)	4,917 (1,044)	2,686 (120)
5	11,391 (697)	7,853 (1,101)	5,715 (999)	5,329 (967)	2,273 (708)	2,377 (95)
6	10,956 (342)	9,397 (566)	7,037 (494)	6,145 (548)	4,710 (275)	1,580 (66)
7	11,160 (537)	9,763 (310)	9,653 (295)	5,527 (397)	2,533 (176)	2,457 (37)
8	10,624 (1,257)	8,882 (359)	6,713 (661)	6,011 (670)	3,511 (626)	1,982 (104)
9	11,978 (700)	9,831 (387)	7,193 (324)	4,995 (480)	3,293 (272)	3,313 (106)
10	11,001 (893)	9,117 (436)	7,952 (447)	5,781 (621)	3,133 (47)	2,578 (102)

APPENDIX C. (continued)

Time (Days)	Control	ppm Oil-Contaminated Prey 0.4	1.7	3.6	0.7 ppm WSF	Reduced Rations
Post-exposure						
11	10,555 (390)	7,476 (225)	7,265 (395)	4,803 (224)	6,803 (300)	9,950 (598)
12	10,733 (384)	9,433 (44)	5,630 (622)	5,579 (61)	8,892 (47)	10,933 (469)
13	10,749 (497)	8,276 (324)	7,723 (379)	5,669 (633)	11,095 (283)	10,246 (167)
14	11,229 (664)	9,353 (361)	9,651 (435)	8,013 (655)	10,959 (489)	13,148 (221)
15	9,936 (650)	9,558 (229)	9,425 (283)	8,767 (636)	9,551 (205)	11,857 (408)
16	10,071 (341)	9,606 (358)	9,743 (239)	7,691 (645)	9,755 (404)	11,168 (394)
17	11,395 (473)	10,148 (418)	8,924 (320)	10,156 (810)	10,036 (170)	10,932 (831)

APPENDIX D. Average daily percent absorption efficiencies for pink salmon fry corresponding to Figure 7. Parentheses contain the standard error of the mean.

Time (Days)	Control	ppm Oil-Contaminated Prey 0.4	1.7	3.6	0.7 ppm WSF	Reduced Rations
Pre-exposure						
1	76.3 (0.4)	77.6 (0.6)	77.2 (1.0)	75.9 (0.5)	77.2 (2.1)	75.5 (1.6)
2	76.3 (1.1)	76.9 (1.0)	76.1 (0.6)	76.0 (1.0)	72.6 (0.9)	71.8 (2.0)
3	75.2 (0.8)	76.9 (1.0)	75.7 (1.0)	75.4 (0.2)	74.5 (0.7)	76.4 (1.0)
Exposure						
4	71.8 (2.1)	67.6 (1.3)	62.1 (2.7)	57.8 (0.7)	73.6 (2.6)	73.9 (1.9)
5	69.7 (2.4)	67.5 (0.8)	69.2 (3.0)	62.9 (2.0)	80.2 (1.7)	84.3 (1.1)
6	71.5 (3.9)	75.9 (0.8)	74.3 (1.9)	64.0 (2.2)	83.8 (7.0)	89.0 (1.0)
7	71.0 (0.8)	71.0 (1.2)	64.3 (1.1)	58.8 (3.7)	87.4 (1.8)	94.3 (0.5)
8	69.8 (2.7)	60.8 (2.9)	45.7 (1.3)	40.2 (6.5)	81.7 (2.4)	87.7 (0.8)
9	74.6 (4.5)	67.9 (4.5)	54.8 (2.7)	53.3 (9.1)	91.4 (1.9)	93.6 (2.6)
10	70.1 (2.5)	68.5 (2.8)	53.7 (2.2)	49.9 (0.9)	88.1 (3.0)	89.3 (1.9)

APPENDIX D. (continued)

Time (Days)	Control	ppm Oil-Contaminated Prey 0.4	1.7	3.6	0.7 ppm WSF	Reduced Rations
Post-exposures						
11	70.6 (1.5)	65.1 (3.4)	63.3 (0.7)	59.8 (2.0)	69.3 (3.9)	78.4 (1.0)
12	70.8 (1.8)	64.6 (2.5)	64.9 (4.4)	57.2 (2.4)	69.3 (4.4)	76.5 (2.3)
13	73.4 (1.4)	64.3 (1.8)	57.8 (0.6)	55.5 (2.0)	68.7 (1.8)	74.3 (2.2)
14	74.7 (1.2)	64.1 (1.0)	63.8 (1.1)	61.3 (1.5)	71.3 (2.1)	73.4 (0.7)
15	72.1 (2.6)	66.2 (3.0)	66.9 (1.9)	58.9 (1.5)	76.6 (2.3)	71.1 (3.5)
16	70.3 (2.1)	73.7 (2.2)	65.4 (1.0)	62.5 (2.7)	73.2 (1.1)	71.1 (0.5)
17	73.6 (0.5)	72.3 (3.9)	65.8 (1.5)	59.6 (2.1)	72.7 (1.6)	73.6 (1.0)

Conover's Ratio:

$$\% \text{ Efficiency} = (F - E / (1 - E)F) \times 100$$

where F = fraction of organic matter in food,

and E = fraction of organic matter in feces.

APPENDIX E. Average daily oxygen consumption rates for pink salmon fry (ug O_2/min/gm dry wt) corresponding to Figure 8. Parentheses contain the standard error of the mean.

Time (Days)	Control	ppm Oil-Contaminated Prey 0.4	1.7	3.6	0.7 ppm WSF	Reduced Rations
Pre-exposure						
1	45.3 (2.3)	47.5 (0.5)	45.7 (2.1)	43.3 (1.3)	47.2 (1.8)	47.6 (3.6)
2	45.5 (1.3)	42.3 (1.6)	47.5 (0.2)	45.2 (1.8)	46.7 (3.1)	49.5 (3.8)
3	41.1 (0.6)	43.0 (1.8)	45.9 (2.7)	41.7 (1.5)	46.6 (1.9)	46.3 (3.5)
Exposure						
4	45.9 (1.6)	44.5 (1.3)	45.7 (2.4)	46.1 (0.7)	55.0 (2.3)	48.1 (2.8)
5	45.9 (2.0)	42.0 (1.1)	44.2 (2.6)	44.8 (1.7)	64.0 (2.8)	38.9 (3.3)
6	46.4 (0.7)	43.0 (0.3)	40.7 (2.6)	37.9 (0.8)	58.0 (2.9)	29.7 (2.3)
7	45.1 (2.2)	42.4 (3.7)	42.4 (0.3)	37.5 (1.6)	52.4 (0.6)	35.0 (1.8)
8	43.9 (2.5)	37.8 (2.0)	39.4 (1.1)	33.5 (3.2)	64.0 (3.2)	33.2 (0.7)
9	44.0 (0.9)	40.5 (3.7)	38.3 (2.3)	32.1 (2.2)	60.0 (3.9)	34.3 (2.3)
10	42.1 (1.8)	42.8 (2.9)	37.4 (0.7)	35.4 (1.9)	65.8 (1.5)	34.1 (1.0)

APPENDIX E. (continued)

Time (Days)	Control	ppm Oil-Contaminated Prey 0.4	1.7	3.6	0.7 ppm WSF	Reduced Rations
Post-exposure						
11	44.4 (4.1)	44.4 (1.0)	41.7 (1.8)	39.7 (2.8)	48.5 (2.2)	45.3 (2.7)
12	45.7 (1.4)	43.5 (4.2)	40.4 (2.4)	37.5 (2.8)	45.3 (2.1)	40.3 (2.4)
13	41.3 (1.4)	44.9 (5.0)	39.8 (1.1)	41.1 (3.8)	38.2 (0.8)	42.4 (2.1)
14	44.6 (2.7)	45.9 (4.4)	42.2 (2.4)	39.9 (3.8)	45.5 (1.1)	47.2 (1.7)
15	44.0 (3.6)	42.9 (3.8)	43.5 (2.6)	39.1 (3.1)	45.2 (1.9)	44.4 (3.3)
16	45.0 (5.0)	45.4 (4.9)	41.1 (1.8)	41.9 (3.0)	42.3 (1.6)	42.2 (0.4)
17	45.7 (3.9)	45.8 (1.7)	45.1 (3.5)	40.5 (3.4)	42.9 (2.7)	43.2 (3.7)

APPENDIX F. Average daily ammonia excretion rates for pink salmon fry ($\mu g\ NH_4^+$-N/min/gm dry wt) corresponding to Figure 9. Parentheses contain the standard error of the mean.

Time (Days)	Control	ppm Oil-Contaminated Prey 0.4	1.7	3.6	0.7 ppm WSF	Reduced Rations
Pre-exposure						
1	3.3 (0.2)	3.2 (0.1)	2.9 (0.3)	3.3 (0.2)	3.1 (0.2)	3.5 (0.3)
2	3.5 (0.2)	3.3 (0.1)	3.7 (0.1)	3.2 (0.2)	3.1 (0.2)	3.6 (0.2)
3	3.4 (0.2)	3.4 (0.1)	3.2 (0.2)	3.6 (0.4)	3.3 (0.1)	3.2 (0.1)
Exposure						
4	3.1 (0.1)	3.0 (0.1)	2.7 (0.1)	2.5 (0.1)	3.2 (0.5)	2.6 (0.1)
5	3.0 (0.3)	2.9 (0.1)	3.1 (0.3)	2.7 (0.1)	2.6 (0.7)	1.3 (0.1)
6	3.4 (0.1)	3.3 (0.2)	3.2 (0.1)	2.7 (0.3)	2.9 (0.3)	1.9 (0.1)
7	3.2 (0.1)	3.0 (0.1)	3.1 (0.2)	2.8 (0.2)	2.1 (0.1)	2.4 (0.1)
8	3.2 (0.2)	2.8 (0.3)	2.7 (0.2)	2.2 (0.3)	1.9 (0.3)	2.2 (0.1)
9	3.5 (0.1)	3.3 (0.1)	3.0 (0.2)	2.2 (0.2)	1.8 (0.2)	2.5 (0.1)
10	3.6 (0.1)	3.3 (0.3)	2.9 (0.2)	2.5 (0.1)	2.0 (0.4)	2.4 (0.1)

APPENDIX F. (Continued)

Time (Days)	Control	ppm Oil-Contaminated Prey 0.4	1.7	3.6	0.7 ppm WSF	Reduced Rations
Post-exposure						
11	3.4 (0.1)	3.3 (0.3)	2.7 (0.4)	2.7 (0.3)	4.1 (0.3)	3.1 (0.4)
12	3.1 (0.2)	3.5 (0.3)	3.0 (0.1)	2.5 (0.4)	3.5 (0.1)	3.4 (0.2)
13	3.6 (0.4)	3.2 (0.2)	3.0 (0.2)	2.7 (0.3)	3.5 (0.1)	3.6 (0.2)
14	3.5 (0.2)	3.6 (0.2)	3.2 (0.2)	3.0 (0.4)	3.4 (0.1)	3.4 (0.1)
15	3.3 (0.2)	3.3 (0.3)	2.8 (0.1)	3.1 (0.3)	2.8 (0.2)	3.6 (0.2)
16	3.7 (0.3)	3.9 (0.3)	3.4 (0.2)	3.0 (0.3)	3.3 (0.3)	3.9 (0.1)
17	3.3 (0.3)	3.6 (0.3)	3.5 (0.2)	3.3 (0.1)	3.3 (0.2)	3.7 (0.4)

APPENDIX G. Average daily O:N ratios for pink salmon fry corresponding to Figure 10. Parentheses contain the standard error of the mean.

Time (Days)	Control	ppm Oil-Contaminated Prey 0.4	1.7	3.6	0.7 ppm WSF	Reduced Rations
Pre-exposure						
1	6.1 (0.3)	6.5 (0.3)	7.1 (0.9)	5.7 (0.4)	6.7 (0.1)	6.0 (0.1)
2	5.7 (0.3)	5.6 (0.2)	5.7 (0.1)	6.2 (0.2)	6.5 (0.1)	6.1 (0.6)
3	5.4 (0.4)	5.5 (0.2)	6.2 (0.2)	5.3 (0.6)	6.2 (0.3)	6.3 (0.6)
Exposure						
4	6.5 (0.1)	6.5 (0.3)	7.5 (0.3)	8.0 (0.4)	7.9 (1.4)	8.3 (0.4)
5	6.8 (0.4)	6.3 (0.3)	6.4 (1.0)	7.3 (0.4)	12.4 (3.5)	12.9 (1.6)
6	6.0 (0.2)	5.9 (0.5)	5.5 (0.5)	6.3 (0.8)	8.9 (1.0)	6.7 (0.4)
7	6.1 (0.5)	6.2 (0.5)	6.1 (0.3)	6.0 (0.3)	11.2 (0.7)	6.3 (0.2)
8	6.1 (0.6)	6.0 (0.6)	7.1 (0.5)	6.7 (0.4)	15.7 (2.2)	6.5 (0.3)
9	5.6 (0.2)	5.4 (0.5)	5.7 (0.3)	6.4 (1.0)	15.4 (2.5)	6.1 (0.7)
10	5.1 (0.2)	5.6 (0.7)	5.7 (0.4)	6.2 (0.5)	14.9 (2.2)	6.1 (0.1)

APPENDIX G. (continued)

Time (Days)	Control	ppm Oil-Contaminated Prey 0.4	1.7	3.6	0.7 ppm WSF	Reduced Rations
Post-exposure						
11	5.7 (0.5)	6.0 (0.5)	6.9 (0.6)	6.4 (0.3)	5.2 (0.1)	6.5 (0.5)
12	6.4 (0.4)	5.5 (0.9)	5.9 (0.2)	6.3 (0.6)	5.7 (0.1)	5.1 (0.1)
13	5.2 (0.6)	6.3 (0.9)	5.8 (0.2)	6.7 (0.5)	4.8 (0.2)	5.2 (0.2)
14	5.5 (0.1)	5.5 (0.3)	5.8 (0.3)	6.0 (0.5)	6.4 (0.4)	6.1 (0.2)
15	6.0 (0.7)	5.7 (0.7)	6.7 (0.6)	5.7 (1.0)	7.0 (0.2)	5.4 (0.6)
16	5.3 (0.2)	5.2 (1.0)	5.3 (0.2)	6.2 (0.8)	5.8 (0.7)	4.7 (0.1)
17	6.1 (0.4)	5.6 (0.6)	5.7 (0.5)	5.4 (0.3)	5.7 (0.1)	5.2 (0.1)