INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each original is also photographed in one exposure and is included in reduced form at the back of the book.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

UMI

A Bell & Howell Information Company
300 North Zeeb Road, Ann Arbor MI 48106-1346 USA
313/761-4700 800/521-0600

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

EFFECTS OF OIL LADEN SEDIMENTS ON BEHAVIOR AND GROWTH OF JUVENILE FLATFISHES

A

THESIS

Presented to the Faculty
of the University of Alaska Fairbanks

In Partial Fulfillment of the Requirements
for the Degree of

DOCTOR OF PHILOSOPHY

By
Adam Moles, M.S.

Juneau, Alaska
December 1996

UMI Number: 9710852

UMI Microform 9710852

UMI
300 North Zeeb Road
Ann Arbor, MI 48103

EFFECTS OF OIL LADEN SEDIMENTS ON BEHAVIOR AND GROWTH OF JUVENILE FLATFISHES

By

Adam Moles

RECOMMENDED:

Stanley D Rice

Thomas C. Shirley

Ronald L. Smith

Robert E Thomas

Lewis J. Haldorson

Brenda L. Norcross

Advisory Committee Chair

Director, Division of Fisheries

APPROVED:

Dean, School of Fisheries and Ocean Sciences

Dean, Graduate School

10-11-96

Date

Abstract

Three species of juvenile Pacific flatfishes: yellowfin sole (Pleuronectes asper), rock sole (P. bilineatus), and Pacific halibut (Hippoglossus stenolepis) were exposed to sediments contaminated with Alaska North Slope crude oil to determine the behavior and growth of juveniles in polluted nursery grounds. Responses were correlated with known biomarkers of toxicant exposure.

In the behavior experiments, fish exhibited a strong preference for fine grained sediments (<500 microns) when presented with eight different sediment types ranging from mud to pebble. Juvenile yellowfin sole showed a preference for mud and mixed mud substrate, rock sole preferred sand substrates and halibut chose both mud and sand sediments. Flatfishes were able to detect and avoid heavily oiled (1400 μg/g total petroleum hydrocarbons-TPH) sediments but did not avoid sediments at oil concentrations of 400 μg/g TPH. Among yellowfin sole and rock sole, sediment preference altered behavioral response to oil whereas halibut did or did not avoid oil irrespective of sediment type.

If flatfish do not avoid oil concentrations of 1600 μg/g and higher on preferred sediment, growth reductions occur. Fish reared on oiled sediment grew slower than controls on non-oiled sediments. Growth reductions in all three species were significant following 30 days of exposure to 1600-1800 μg/g TPH and became more pronounced over time. As the toxicant concentration or the length of exposure increased, growth per day decreased. By 90 days of exposure, fish exposed to 1600-1800 μg/g TPH grew 38-

57% slower than controls. Halibut had the greatest change in growth rate following oil exposure. Exposure of halibut to sand laden with 4700 μg/g total hydrocarbons resulted in an 93% reduction in growth in 30 days. Condition factor was also most reduced in halibut.

Changes in tissues and parasites indicated a reduction in fish health for all three species. There was an increase in fin erosion, liver lipidosis, gill hyperplasia and hypertrophy, and gill ciliate infestation combined with a decline in macrophage aggregates and gut parasites. Chronic marine oil pollution that results in hydrocarbon concentrations of 1600 μg/g in nursery sediments has the potential to reduce growth and health of juvenile flatfishes. Recruitment of juveniles to the fishery would be reduced due to increased susceptibility to predation and slower growth to maturity.

TABLE OF CONTENTS

Page

LIST OF FIGURES

Page

LIST OF TABLES

Acknowledgements

I wish to thank a number of people for their help in developing and creating this dissertation. My advisor, Dr. Brenda Norcross, though hundreds of miles away, provided much needed guidance and advice. Her ability to see the "big picture" kept me focused on my objectives. Drs. Thomas Shirley and Lewis Haldorson provided the bulk of my classroom training and many valuable ecological discussions. Dr. Robert Thomas of CSU Chico provided helpful advice and training for over 20 years and convinced me that physiology could be a powerful tool in fisheries. It was the work of Dr. Ron Smith that inspired my interest in hydrocarbon biomarkers and whose positive and constructive reviews kept me from getting discouraged. Ryan Scott helped me immensely in setting up the experiments and helping with the laborious preliminary observations, counting, and collecting. Numerous staff at the Auke Bay Laboratory assisted in fish collections and advice over the years, in particular Mark Carls. Dr. Jeep Rice, Habitat Program Manager at ABL, has my deepest gratitude. For 25 years, Jeep has been my mentor and supervisor and without his moral, scientific, and financial support this work would not have been possible. Although I did all the work presented in Chapters 2 and 3, I included Brenda and Jeep as co-authors in recognition of their longterm contributions in the development of my research.

CHAPTER ONE

Introduction

Pollutants such as petroleum hydrocarbons present in marine sediments present a persistent challenge to benthic organisms, especially to species such as flatfishes which depend on nearshore sediments for overwintering and predator avoidance (Gibson and Robb 1992). Hydrocarbons are entering estuaries on an ever increasing basis, both from increased land use and from tidal borne offshore events such as oil spills and leaks. There is growing concern about the progressive loss of critical habitats such as estuarine nursery areas needed by juvenile fishes due to human activities (FAO 1995).

Demersal fishes in the nearshore environment are particularly vulnerable to pollution as the hydrocarbons settle out into the fine grained sediments where they can persist for years (Gundlach et. al. 1983, O'Clair et al. 1996). Since the primary route of hydrocarbon uptake is via the skin and gills (Ariese et al. 1993), flatfishes which live in direct contact with the sediments may be constantly exposed to contaminated sediments.

While there is increasing evidence that flatfish health is negatively affected by chronic exposure to low levels of polynuclear aromatic hydrocarbons (PAH) in the sediments, there is little known about the results of exposure on the fish itself. Flatfishes in heavily polluted estuaries have an increased prevalence of disease, parasites, and tissue alterations that have been closely correlated with petroleum hydrocarbons in the sediments (Haensley et al. 1982, Mix 1986, Overstreet 1988, Myers et al. 1991). In contrast, the few laboratory studies that have included ancillary data on growth of oil-exposed adult flatfishes (Fletcher et al. 1981, McCain and Malins 1982, Truscott et al. 1992), have found little or no difference in wet weight between exposed and unexposed fish.

Juvenile flatfishes in the presence of contaminated sediments are faced with three options: to remain in the water column, bury in the contaminated sediment, or search for uncontaminated sediments. To remain in the water column implies increased predator risk whereas prolonged contact with sediments implies potential accumulation of toxicants. If the fish avoid contaminated sediments and seek out uncontaminated sediment, they also avoid both any contaminant effects and find fresh sediments for predator avoidance.

The first part of the present study examines whether juvenile flatfishes can detect and avoid petroleum hydrocarbons in the sediments. Behavioral choices were recorded when flatfishes were presented with 1) eight uncontaminated sediments (Chapter Two), 2) clean and oil contaminated preferred sediment (Chapter Three), and 3) oil contaminated preferred sediment and clean unpreferred sediment (Chapter Three).

As exposure is likely when flatfishes are unable to detect oil or have no alternative sediment, the second part of my study examines the effects of chronic exposure to oil laden sediments on the growth and health of juvenile flatfishes. These results are reported in Chapter Four.

Chapter Four also describes a number of alterations in fish tissue and parasite levels. The biomarkers of fish health chosen for this study have been extensively reported for several species of fishes in the field and laboratory for a variety of toxicants (reviews by Malins 1982, Khan and Thulin 1991). In Chapter Five, I have synthesized the results of my studies on behavior and growth and examined the ecological implications of this research for flatfish in the field.

CHAPTER TWO

Sediment Preference in Juvenile Pacific Flatfishes 1

Abstract

Behavioral preference tests were used to determine if sediment selection played a role in habitat choice. Four species of juvenile pleuronectids were given a choice of eight sediments in a carousel and final choices were recorded after 20 hours. Juvenile flatfishes demonstrated strong selection for sediments less than 500 microns. Juvenile starry flounder (Platichthys stellatus) selected larger particles with increasing fish size. Starry flounder under 25 mm in length chose mud, 50-80 mm fish chose mud and mixed mud sediments and larger juveniles (>150 mm) confined themselves to fine sand. Juvenile halibut (Hippoglossus stenolepis) at 50-80 mm preferred a combination of mud and fine sand and were spatially segregated. Yellowfin sole (Pleuronectes asper) at 50-80 mm showed a slight preference for mud and mixed mud sediments over sand, a selection that became stronger in larger (>150 mm) fish. Juvenile rock sole (Pleuronectes bilineatus) at 50-80 mm preferred substrata of sand and mixed sand nearly 90% of the time. For all species, sediments which were too coarse to allow the flatfishes to bury themselves, such as granular or pebble substrata were seldom selected. The results of these laboratory studies can be used to predict the distribution of juvenile flatfishes in a nursery area.

1. Moles, A. and B.L. Norcross, 1995. Sediment preference in juvenile Pacific flatfishes.-Netherlands Journal of Sea Research 34: 177-182.

1.0 INTRODUCTION

The primary nurseries for many species of juvenile flatfishes on the Pacific coast of North America are located in inshore coastal areas (Krygier and Pearcy, 1986; Gunderson et al., 1990; Kramer, 1991). Flatfishes are thought to settle onto fine grained sediments (Wyanski 1990, Tanda 1990, Keefe and Able 1994) and the character of the sediment in concert with food availability may be a significant factor in recruitment to nurseries. Juvenile flatfishes may be limited in their ability to exploit many bottom types because they can't exert enough energy to bury (Gibson and Robb 1992). Adult and late juvenile flatfishes may therefore have a larger choice of acceptable substrata in which they can bury.

Variations in recruitment of juvenile flatfishes to the fishery may be partially explained by the quality of microhabitat available to settling flatfish larvae (Hoss and Thayer 1993, Gibson 1994). Understanding what bottom types are preferred by settling juveniles is important in analyzing variations in distribution and recruitment. Juveniles growing in an area rich in preferred bottom type are likely to prosper while juveniles in an area depauperate of suitable substratum are not likely to grow as well (Gibson 1994). The evidence for selection of fine grained sediments for initial settlement is from trawl surveys of juvenile flatfish abundance which report nursery substrata as silt, mud and fine to coarse sand (Poxton et al. 1982; Wyanski, 1990; Kramer, 1991).

Field collections from Kodiak, Alaska (Norcross et al. 1995) demonstrate that substratum preference is species specific for many juvenile Pacific flatfishes. Juvenile flatfishes were rarely found on pebble or cobble substrata but different species were associated with different bottom types. Rock sole are often found on sand substrata, yellowfin sole on mixed mud/sand substrata and halibut on a mixed sand substrata. Laboratory studies can be used to assess the relative preferences of juvenile flatfish over a range of grain sizes and to confirm the field data.

In this study, I investigated the habitat preference of four species of juvenile Alaskan flatfishes, starry flounder (Platichthys stellatus), Pacific halibut (Hippoglossus stenolepis), yellowfin sole (Pleuronectes asper) and rock sole (Pleuronectes bilineatus). The starry flounder is caught in a sport fishery and the other three species are the three primary flatfishes caught commercially in the northeast Pacific (FAO 1990). In 1990, 233,488 metric tons of flatfish were landed in the NE Pacific - 20% of the world's flatfish catch. Over 155 metric tons of yellowfin sole were landed along with 34,000 metric tons of halibut and 38 metric tons of rock sole. Juveniles of all these species are found along the NE Pacific coast with variations in location, depth, and bottom type (Norcross et al. 1995). Each of the four species of juvenile flatfishes was offered a variety of sediment choices in the laboratory to determine their preference. I examined the role of intraspecific interactions and size in this preference.

2.0 METHODS

2.1 Collection of Animals

Rock sole and yellowfin sole were obtained from Auke Bay, Alaska by 6 mm mesh beach seine between May and July of 1992. Halibut were collected in 30-70 m depths in Sitkinak Strait and Ugak Bay off Kodiak Island, Alaska by plumb staff beam trawl (Norcross et al. 1995) in August, 1992. All specimens were transported alive to the National Marine Fisheries Service (NMFS) laboratory at Auke Bay, Alaska and held in flowthrough seawater tanks. Fishes were fed a diet of Tubifex bloodworms to satiation every day. The fishes were held in rectangular (80 cm x 20 cm x 20 cm) tanks with sediment that was a mixture of equal parts granule, sand and mud by volume. The fishes were categorized in three length groups: small (10-25 mm), medium (50-80 mm) and large (150-250 mm). I tested newly settled starry flounder in the small category, age zero starry flounder, halibut and rock sole and age one yellowfin sole in the medium category and age one starry flounder, yellowfin sole and rock sole in the large category.

2.2. Pilot Tests

An initial experiment tested the feasibility of using 20 hrs as the observational endpoint and to determine the effect of endogenous diel and tidal rhythms on changes in substratum preference within a 24 hour period. Ten flatfish of each species were observed hourly over two 24 hour periods. During the first period, the maximum high tides coincided with noon and midnight. During the second period, noon and midnight were midway between high and low tide for that day. Each fish was in a separate testing tank and observations included both sediment choice and whether the fish was buried or swimming.

Additional trials were conducted to ensure that the flatfishes did not become so acclimated to the holding sediment that they selected it out of habit. Ten tanks were constructed with half of each tank containing the holding substratum (equal parts granule, coarse sand and mud) and the other half containing either mud (5 tanks) or sand (5 tanks). Ten trials with each species were run on each tank and habitat choice was recorded after 20 hrs.

2.3. Sediment Preparation

Eight sediment types were tested: mud, sandy mud, muddy sand, fine sand, coarse sand, angular granule, spherical granule, and pebble (Table 1). Each was defined according to its particle size distribution using the Wentworth scale (Sheppard 1973) with mud (<63 microns) as the smallest grain size and pebble (4-64 mm) as the largest. Mud was defined as a combination of silts and clays with a grain size below 63 microns. The Wentworth scale is the standard measure of grain size of sediment and imposes finite increments to convert analyses of sediment into discrete series given the continuous distribution of particle sizes within sediments.

Each sediment was sieved prior to testing to eliminate particles too coarse or fine for that category. Pebble, and sand were obtained from the local sand and gravel

Table 1. Grain sizes of tested sediments according to the Wentworth scale (Sheppard 1973)

Name	microns	
Pebble	4,000-64000	
Granule	2,000-4000	
Flat		
Round		
Coarse sand	500-2000	
Fine sand	62-500	
Muddy Sand	<500	1/3 mud - 2/3 fine sand
Sandy Mud	<500	2/3 mud - 1/3 fine sand
Mud	<62	Silt plus clay fractions

pit and were sieved. Mud and granule were taken from beaches at low tide. Muddy sand was created by mixing two parts sand with one part mud and sandy mud was created by mixing two parts mud with one part sand. One Wentworth category (granule) was represented by two different substrata: flat and round. Both kinds of granule were selected from areas in which the rock sole and yellowfin sole were captured in Auke Bay. In these areas, the flat granule was primarily shale and the round granule was a mixture of igneous types. Because these two granular shapes are common in benthic samples and are seldom present together, each was tested as a different category. All sediments were frozen, washed and sieved to remove meiofauna and to create a homogeneous sample.

Twelve test chambers were used, each consisting of a circular fiberglass tank 120 cm in diameter with a 100 cm depth and a central standpipe. The eight test sediments were arrange in a carousel on the bottom to give eight equal sectors radiating from the central standpipe. This avoided the problem of the fish choosing one end or another of the tank (Noakes and Baylis 1990). The bottom substratum was 6 cm deep and allowed the flatfishes to burrow without contacting the tank bottom but avoided the sediments becoming anoxic. Water depth in each tank was 20 cm. The same sediment types were used in each of the 12 tanks but the relative positions of the sediments were varied randomly to minimize tank and other orientation effects.

2.4 Test Protocol

Habitat preference of the flatfishes was tested using the methods of Aziz and

Greenwood (1982) and Keefe and Able (1994). Fish were fed just before they were added to the tanks to eliminate hunger as a variable in sediment choice. At 1100 hrs each day one fish was added to the center of each tank. After 20 hrs, final sediment choice was recorded. The fish was removed, length recorded, and replaced by a new fish. The total number of trials per species ranged from 50-80, depending on the total number of fish available. No fish were reused.

The number of fish choosing each substratum was compared with the predicted number if the choice was random using a Chi-square test (Siegal, 1956). The null hypothesis was that all substrata would be chosen equally. If the substratum type was chosen more often than predicted by the null hypothesis ($P<0.05$), it was defined as moderate selection for that substratum. Selection was considered strong if differences were significant at the ($P<0.01$) level. To test the possibility of intraspecific interaction between fish, the trials were repeated with two fish of the same species in each tank. A Chi-square test determined if significant differences in sediment preference existed between the trials with a single fish and trials with two fish per tank.

3.0 RESULTS

3.1. Pilot Tests

Pilot tests run in August of 1993 verified the experimental protocol. Hourly observations on all four species confirmed that almost any time could have been chosen for observing final sediment choices. The 20 hour observation period recommended by

Aziz and Greenwood (1982) permitted time to make assessments and add new fish. If the juveniles were well fed prior to testing, they buried within minutes of introduction and remained there for the duration of the tests.

The sediment in which the flatfishes were held prior to testing had no effect on sediment choice. When offered a choice of their preferred substratum and the holding substratum, juvenile flatfish significantly ($P<0.001$) selected the preferred substratum (starry flounder:sand, halibut:muddy sand, yellowfin sole:mud, rock sole: sand).

3.2 Sediment Preference of Juvenile Flatfishes

Four species of juvenile Alaskan flatfishes seem to prefer various combinations of mud and sand (Table 2). Sediment with particle sizes in excess of 500 microns, such as coarse sand, granule and pebble, were seldom selected by test fish (20 mm to 250 mm). Instead, mud, sand, or some combination of both were selected to varying degrees. The smaller flatfishes selected smaller grain sizes more often than did the larger flatfishes. Among medium flatfishes, starry flounder and yellowfin sole preferred muddy sediments in contrast to the preference of halibut and rock sole for sandy sediments. Larger flatfishes preferred sand over mud, although large yellowfin sole retained some selection for mud.

3.3. Size Specific Sediment Preference

3.3.1 Starry Flounder

Starry flounder selected fine grained sediments <500 microns and never selected

Table 2. Percent selection of selected sediment types by four species of juvenile Pacific flatfishes. Sediment types: M=mud, sM= sandy mud, mS=muddy sand, fS=fine sand, cS=coarse sand, fG=flat granule, rG=round granule, P= pebble. Density is the number of fish per trial, N= number of trials. Significance is indicated by asterisks: *** = (P<0.001), ** = (P<0.01), * = (P<0.05) by Chi-squared test for goodness of fit.

Species	Size(mm)	Sediment Type								Density	N
		M	sM	mS	fS	cS	fG	rG	P		
Starry Flounder											
	<25	100***	0	0	0	0	0	0	0	1	50
	<25	100***	0	0	0	0	0	0	0	2	50
	50-80	40**	6	18*	24*	0	12	0	0	1	50
	50-80	39**	7	13	30*	0	11	0	0	2	46
	150-250	0	0	0	100***	0	0	0	0	1	54
	150-250	0	0	0	100***	0	0	0	0	2	54
Pacific Halibut											
	50-80	0	0	100***	0	0	0	0	0	1	50
	50-80	0	44**	56**	0	0	0	0	0	2	25
Yellowfin Sole											
	50-80	40**	10	25*	25*	1	0	0	0	1	80
	50-80	35**	5	28*	27*	2	1	1	0	2	81
	150-250	23*	18*	45**	9	0	4	0	0	1	50
Rock sole											
	50-80	3	15	33**	45**	1	1	2	0	1	61
	50-80	22*	24*	6	32**	14	0	2	0	2	63
	150-250	6	21*	14	54**	5	0	0	0	1	52
	150-250	8	32**	22*	24*	8	6	0	0	2	50

round granules, coarse sand or pebble in any of the trials. As fish length increased from 25 mm to 250 mm, the preferred grain size shifted from <62 microns to 200 microns. The newly settled (<25 mm) starry flounder selected only mud (100%). In 50 trials, these juvenile flatfishes never chose sandy mud, muddy sand, fine sand, coarse sand, round or flat granule, or pebble.

Medium sized (50-80 mm) starry flounder tested with one fish per tank chose sediments with grain sizes less than 500 microns (Table 2). Mud was selected by 40% of the fish ($P<0.01$), fine sand was selected by 24% and muddy sand by 18% ($P<0.05$). Flat granule was selected by 12% of the fish which would have been predicted by a random choice. Three substrates were never selected. When two medium starry flounders were tested in the same tank, the differences in selection were not significant. The same sediments were selected in nearly the same proportions, and sediment choice did not alter by more than 6% in any category. Mud ($P<0.01$) and fine sand ($P<0.05$) were again selected. Large (150-250 mm) starry flounder, singly or with two fish per tank, chose only fine sand ($P<0.01$) selecting it in all 54 trials (100%).

3.3.2. Pacific halibut

Medium sized Pacific halibut tested with one fish per tank were always found on muddy sand (Table 2). When a second halibut was added, 56% of the fish tested chose muddy sand and 44% chose sandy mud. These two substrata were strongly selected ($P<0.01$). Distribution among sediments was statistically different between tests with one fish per tank and tests with two fish per tank ($P<0.001$). Most often (94%) this reflected one choosing one substratum and the other halibut choosing an alternate substratum. No aggressive behavior was noted.

3.3.3. Yellowfin sole

Medium sized (50-80 mm) yellowfin sole tested with one fish per tank preferred mud or mixed mud substratum (Table 2) over non-mud substrata in 75% of the trials. Mud was selected in 40% of the tests ($P<0.01$) whereas muddy sand and fine sand (both 25%) were moderately selected ($P<0.05$). Grain sizes over 500 microns were not selected. When two medium yellowfin sole were tested in the same tank, sediment choice was not significantly different from that observed with a single fish. Single large yellowfin sole had a strong preference ($P<0.01$) for muddy sand (40%). In combination with sandy mud (18%) and mud (23%), 86% of the selection was for sediments containing mud. Overall the large yellowfin sole selected mud based sediments 11% more than the medium yellowfin sole; this difference was significant ($P<0.05$).

3.3.4. Rock Sole

Single medium sized rock sole preferred sediments containing fine sand over non-sand substrata in 93% of the trials. Although other substrata were also selected, only fine sand (45%) and muddy sand (33%) were strongly selected ($P<0.01$) (Table 2). When two medium rock sole were tested in the same tank the pattern altered significantly ($P<0.05$). Mud based sediments were utilized more by two rock sole than by a single rock sole. Rock sole continued to select muddy sand (24%) but fewer fish selected fine sand (32%) although the selection was still strongly significant ($P<0.01$). Mud, a sediment seldom selected by single rock sole, was significantly ($P<0.05$) selected (22%). Distribution differences between tests with one fish or two fish per test were significant ($P<0.01$) for medium rock sole. Large rock sole had a strong preference ($P<0.01$) for fine sand (Table 2), selecting it in 54% of the trials when tested singly. Sand and mixed sand substrata were selected in 88% of the trials. Sandy mud was moderately selected ($P<0.05$). Other sediments were seldom selected. Large rock sole selected sand and mixed sand sediments 11% more than the medium rock sole; this difference was significant ($P<0.05$). The presence of a second large rock sole altered the sediment selection significantly ($P<0.05$) (Table 2). Sandy mud was strongly selected (32%, $P<0.01$), and fine sand (24%) and muddy sand (22%) were moderately selected ($P<0.05$).

4.0 DISCUSSION

Many juvenile flatfishes prefer fine grained sediments (Wyanski 1990, Rogers 1992) which permit easier access to prey items than larger sediments as well as facilitating the burying behavior critical to predator escape (Burke et al. 1991, Gibson and Robb 1992). Newly metamorphosed plaice (Pleuronectes platessa) were unable to bury in grain sizes larger than 500 microns (Riley et al. 1981) and trawl surveys of plaice (Poxton and Nadir 1985) and several species of Alaskan flatfish (Norcross et al. 1995) distribution confirm that newly settled juveniles are seldom present on granular and larger grained substrates.

The ability to bury in sediment has survival advantages and flatfish select such sediments when offered (Jager et al. 1993, Keefe and Able 1994). Juvenile bastard halibut (Paralichthys olivaceus) were less vulnerable to predators when they were able to bury themselves (Tanda 1990). Burrowing has also been related to reduction of metabolic rates to resting phase (Howell and Canario 1987) and avoidance of current effects (Cook 1985). Observation of flatfishes in my tests showed that were unable to bury in granule or pebble but could quickly bury in coarse sand and smaller particles. If the ability to burrow into sediment was the only criterion for choice, coarse sand should have been strongly selected as the smaller grain sizes. Laboratory studies have examined the relationship between ability to bury in sediment, fish body size and grain size of the sediment (Tanda 1990, Gibson and Robb 1992). Larger fish are able to exert more force and utilize coarser sediments than smaller fish. This relationship was true for the starry flounder in my study which selected larger grain sizes with larger body size. Small flounders (10-25 mm) may be unable to exert sufficient force to bury in coarser sediments. The ability to utilize coaser sediments increases with size and smaller particle-sized sediments were no longer utilized by larger fish.

If sediment selection was a simple matter of matching the size of the fish with the size of the particles, all the species in a given size range should choose the same sediments. Juvenile flatfish species, while sharing an affinity for mud/sand habitat, differ in their preferences. Plaice prefer sandy sediments (Jager et al. 1993) whereas newly settled flounder (Paralichthys flesus) of the same size seem to prefer muddy sediments

(Veer et al. 1991). I noted specialization and habitat separation as the fish grew. Large starry flounder chose sand exclusively while large yellowfin sole selected mud. Large rock sole chose sand more often than yellowfin sole but less often than starry flounder. Juvenile halibut and rock sole may have some form of intraspecific avoidance. Habitat preference was statistically altered by the presence of conspecifics in the tank whereas sediment preference in yellowfin sole and starry flounder was unaltered by another fish. There was no evidence of aggressive behavior in any of the trials.

My laboratory data agree with field data on distribution of juvenile flatfish on various substrata in Alaska (Norcross et al.1995). Most juvenile flatfish captured in the Kodiak region were found on sand/mud substrata. Rock sole were found on sand and Pacific halibut were found on sand mixed with some mud and granule. Other species, such as arrowtooth flounder (Atheresthes stomias) were found on mud mixed with sand and flathead sole (Hippoglossoides elassodon) were found on mud or mud-based sediments. Based on my laboratory data, it would be unlikely to find juvenile flatfish on pebble or granular sediments, however, this may be difficult to field test.

Grain size is probably only one of the factors operating in sediment selection by juvenile flatfishes. Jager et al. (1993) cite the presence of flounder at only a few sites despite the presence of its preferred sediment throughout the Ems estuary. Burke et al. (1991) observed that summer flounder (Paralichthys dentatus) selected for sand when prey was present in both mud and sand tanks but showed no preference when prey was absent from the substrata. Salinity (Burke et al. 1991) and depth (Norcross et al. 1995) can be overriding factors in sediment selection of some species of flatfishes. While I controlled or eliminated these variables in my laboratory experiments, they are certainly important in the field.

Along with depth, salinity and food, bottom type may determine where flatfishes settle and rear. The knowledge of the location of fine grained substrates could be useful in predicting the patterns of settling and rearing of juvenile flatfishes within an estuary or in nearshore areas. Additionally, knowledge of the differences in sediment preference between species and size groups may be useful in predicting distributional shifts in abundance.

Identification of juvenile flatfish habitat is an excellent tool to reduce the inadvertent capture and destruction of juveniles. Knowledge of habitat preferences can allow us to identify potential nursery grounds to be avoided in fishing. Such knowledge may also allow development of recruitment indices so that exploitation rates can be calculated for use in earlier forecasting of populations.

5.0 REFERENCES

Aziz, K.A. and J.G. Greenwood, 1982. Response of juvenile Metapenaeus bennettae Racek and Dall 1965 (Decapoda, Penaeidae) to sediments of differing particle size.-- Crustaceana 43(2): 121-126.

Burke, J.S., J.M. Miller and D.E. Hoss, 1991. Immigration and settlement pattern of Paralichthys dentatus and P. lethostigma in an estuarine nursery ground, North Carolina, USA.--Netherlands Journal of Sea Research 27: 393-405.

Cook, P.H., 1985. The behavior of the plaice (Pleuronectes platessa L.) in relation to bottom current and sediment type. PhD thesis, University of East Anglia, U.K.: 1-136.

FAO, 1990. Fishery Statistics, Volume 70.--United Nations Food and Agriculture Organization, Rome: 1-647.

Gibson, R.N., 1994. Does habitat quality and quantity affect recruitment in the juvenile stage of flatfishes.--Netherlands Journal of Sea Research 32: 191-206.

Gibson, R.N. and L. Robb, 1992. The relationship between body size, sediment grain size and the burying ability of juvenile plaice, Pleuronectes platessa L.-- Journal of Fish Biology 40: 771-778.

Gunderson, D.R., D.A. Armstrong and Y.-B. Shi, 1990. Patterns of estuarine use of juvenile English sole (Parophrys vetulus) and Dungeness crab (Cancer magister).--Estuaries 13: 59-71.

Hoss, D.E. and G.W. Thayer, 1993. The importance of habitat to the early life history of estuarine dependent fishes.--American Fisheries Society Symposium 14: 147-158.

Howell, B.R. and A.V.M. Canario, 1987. The influence of sand on the estimation of resting metabolic rate of juvenile sole, Solea solea (L.)-- Journal of Fish Biology 31: 277-280.

Jager, Z., H.L. Kleef and P. Tydeman, 1993. The distribution of 0-group flatfish in relation to abiotic factors on the tidal flats in the brackish Dollard (Ems estuary, Wadden Sea).-- Journal of Fish Biology 43A: 31-43.

Keefe, M.L. and K.W. Able, 1994. Contributions of abiotic and biotic factors on settlement in summer flounder, Paralichthys dentatus.--Copeia 1994(2):458-465.

Kramer, S.H., 1991. Growth, mortality, and movements of juvenile California halibut Paralichthys californicus, in shallow coastal and bay habitats of San Diego County, California. Fishery Bulletin (United States) 84: 119-132.

Krygier, E.E. and W.G. Pearcy, 1986. The role of estuarine and offshore nursery areas for young English sole, Parophrys vetulus Girard of Oregon.--Fishery Bulletin (United States) 84: 119-132.

Noakes, D.L.G., and J.R. Baylis, 1990. Behavior. In: C.B. Schreck and P.B. Moyle, editors. Methods for Fishery Biology. American Fisheries Society, Bethesda, MD. pg. 555-584.

Norcross, B.L., B.A. Holladay and F.J. Muter, 1995. Comparisons of nursery area characteristics of Pleuronectids in coastal Alaska, USA.--Netherlands Journal of Sea Research 34: 161-175.

Poxton, M.G., A. Eleftheriou and A.D. McIntyre, 1982. The population dynamics of 0-group flatfish on nursery grounds in the Clyde Sea area.--Estuarine and Coastal Shelf Science 14:265-282.

Poxton, M.G. and N.A. Nasir. 1985, The distribution and population dynamics of 0-group plaice on nursery grounds in the Firth of Forth.--Esturaine and Coastal Shelf Science 21: 845-857.

Riley, J.D., D.J. Symonds, and L. Woolner, 1981. On the factors influencing the distribution of 0-group fish in coastal waters.--Rapports et Proces-verbaux des Reunions Conseil International pour l'Exploration de la Mer 178: 223-228.

Rogers, S.I., 1992. Environmental factors affecting the distribution of sole (Solea solea) within a nursery area.-- Netherlands Journal of Sea Research 29: 153-161.

Sheppard, F.M., 1973. Submarine Geology. 3rd ed. Harper and Row, New York: 1-517.

Siegel, S., 1956. Nonparametric Statistics for the Behavioral Sciences. McGraw-Hill Book Company, New York: 1-312.

Tanda, M., 1990. Studies on burying ability in sand and selection to the gain size for hatchery reared marbled sole
and Japanese flounder.--Japanese Society for Grassland Sciences 56: 1543-1548.

Van der Veer, H.W., M.J.N. Bergman. R. Dapper, and J.I. Witte, 1991. Population dynamics of an intertidal 0-group flounder Paralichthys flesus population in the western Dutch Wadden Sea.--Marine Ecology Progress Series 73: 141-148.

Wyanski, D.M., 1990. Patterns of habitat utilization in 0-age summer flounder Paralichthys dentatus. College of William and Mary, MS thesis: 1-54.

Ziljstra, J.J., R. Dapper, and J.IJ. Witte, 1982. Settlement, growth, and mortality of post-larval plaice Pleuronectes platessa in the western Wadden Sea. Netherlands Journal of Sea Research 15: 250-272.

CHAPTER THREE

Avoidance of Hydrocarbon Laden Sediments by Juvenile Flatfishes

Abstract

Behavioural tests were used to determine whether juvenile flatfishes were capable of detecting and avoiding sediment containing various concentrations of petroleum hydrocarbons. Three species of juvenile Alaskan flatfishes: rock sole (Pleuronectes bilineatus), yellowfin sole, (P. asper), and Pacific halibut (Hippoglossus stenolepsis) were tested in laboratory chambers containing contaminated mud or sand offered in combination with clean mud, sand or granule. The flatfishes were able to detect and avoid heavily oiled (2%) sediment, but they did not avoid lower concentrations of oiled sediment (0.05%). Oil concentrations that would not be avoided if the substrate choices were the same, were significantly avoided if the preferred sediment was oiled and unpreferred sediment was unoiled. If unpreferred sediment was oiled and preferred sediment alternative unoiled, there was strong avoidance of oil at all concentrations. This latter avoidance was not signficant since selection of sediments was not altered by the presence of oil. Oiled sand or mud were always preferred over unoiled granule. The observed lack of avoidance at concentrations <400 $\mu g/g$ may lead to longterm exposure to contaminated sediment following a spill. Recruitment of juveniles may be affected if the exposure to oil is long enough to affect growth and survival.

1.0 INTRODUCTION

The development of the oil industry throughout the world and the increasing numbers of large oil spills has resulted in increased interest in the effects of oil hydrocarbons on fishery resources. Changes in fish survival, pathology, and physiology are well documented but behaviour of fish in the presence of oil has received little attention. Fish avoid polluted waters (NAS 1985), but only a few researchers have examined oil avoidance in fish (Syazuki 1964, Rice 1973, Maynard and Weber 1981, Weber et al. 1981). These studies were all with salmonid fishes and demonstrated the ability of fish to detect oil. There is little evidence to demonstrate that fishes avoid oil by making a behavioural decision, although it is widely assumed that they do. In this study, the behavioural response of flatfish to oiled sediment is investigated.

Benthic sediments act as a final sink for petroleum hydrocarbons in the marine environment. Oil may be introduced directly via submarine spills or indirectly from surface spills (Karinen 1980). Hydrocarbons in the marine sediments can persist for years (Teal et al. 1978). Oil adsorbed onto suspended particulate materials in the intertidal zone is also transported into deeper waters (Sale et al. 1996). Petroleum was present subtidally at 11 out of 20 sampled sites in Prince William Sound in 1989 and persisted for several years following the *Exxon Valdez* oil spill (O'Clair et al. 1996). Surface sediments sampled following the spill were contaminated at seven sites at a depth of 20m and in two heavily contaminated bays at a depth of 100 m (O'Clair et al. 1996).

Flatfishes should be particularly vulnerable to oil exposure given the close interaction of these species with the sediment. The proximity of their habitat to the shore increases their vulnerability to shore-based pollutants such as oil. Juveniles bury themselves in the top layer of sediment and ingest sediment when feeding (Levings 1972, Fletcher et al. 1981). As obligatory residents of benthic sediments, flatfishes would be exposed to oil through direct substrate contact as well as ingestion of benthic prey. Studies on the effects of oil on juvenile flatfish, particularly behaviour, are lacking.

Exposure of adult flatfishes to oil in the field has been correlated with reproductive impairment (Spies et al. 1985, Johnson et al. 1988), pathological changes (McCain et al. 1978, Haensly et al. 1982). Liver damage (Fletcher et al. 1982), and reductions in feeding and growth (Fletcher et al. 1981) have been noted in oil exposed flatfishes in the laboratory.

Inshore nursery areas are particularly vulnerable to hydrocarbon pollution. Flatfishes are hypothesized to settle out onto fine grained sediments (Wyanski 1990, Tanda 1990, Gibson and Robb 1992, Keefe and Able 1994) which have the potential for holding more oil for longer periods than coarser substrates. Initial selection of substrata by flatfishes is a function of grain size (Cook 1985, Tanda 1990) because the finer grained sediments allow small juveniles to bury more completely (Gibson and Robb 1992) as well as providing the preferred prey. The primary nursery areas for juvenile flatfishes are believed to be the inshore coastal areas (Hogue and Carey 1982, Krygier and Pearcy 1986, de Ben et al. 1990). Sediment in the inshore nursery areas is particularly vulnerable as oil washes up on beaches. Wave action can continually resuspend oil/sand particles from the beaches making it available for settling in subtidal sediments (Sale et al. 1992).

The purpose of this investigation is to determine if juvenile flatfishes are able to detect and if they will avoid crude oil in mud and sand sediments. I tested three species of commercially important flatfishes: rock sole (Pleuronectes bilineatus), yellowfin sole (Pleuronectes asper), and Pacific halibut (Hippoglossus stenolepis) on oiled mud and sand to evaluate the relative importance of substrate type and oil concentration in the behavioural response. I chose these three species because of their differences in sediment preference. Rock sole prefer sand substrate, yellowfin prefer mud, and halibut prefer a mud/sand mixture (Moles and Norcross 1995).

2.0 METHODS

2.1 Animal Collection

Juvenile rock sole and yellowfin sole were obtained from Auke Bay, Alaska by 6 mm mesh beach seine between May and July 1992. Halibut were collected in 30-70 m depths in Sitkinak Strait and Ugak Bay off Kodiak Island, Alaska by plumb staff beam trawl (4 mm mesh) in August 1992. All specimens were transported live to the National Marine Fisheries Service (NMFS) laboratory at Auke Bay and held in flowthrough seawater tanks. Fishes were fed a diet of Tubifex bloodworms and Mysis mysids to satiation every day. Fish 50-80 mm were used in the tests. The fishes were held in rectangular (80 cm x 20 cm x 20 cm) tanks with sediment that was a mixture of equal volumes of granule, sand and mud.

2.2. Sediment Preparation

Mud, defined as sediment smaller than 63 microns (Holmes and McIntyre 1984), was gathered intertidally, frozen and thawed three times to kill any organisms present. It was then washed with seawater and filtered through 63 micron mesh to remove larger particles as well as any dead prey items. The primary prey of the three test species are larger than 63 microns (Sturdevant 1987, Holladay and Norcross1995) and would be removed by sieving. Sand (64-249 microns) and granule (2000-4000 microns) were obtained from the local sand and gravel yard and also frozen, seived and washed.

Oiled sand or mud was prepared by mixing a volume of Alaska North Slope crude oil corresponding to 2%, 1% and 0.5% of the sediment volume with the sediment in a polyethylene tub. The upper concentration (2%) was the maximum amount of oil that could be reasonably expected to be held in sediment (Karinen et al. 1985). The lower concentration (0.05%) was chosen to simulate the levels found in sediments following an oil spill (O'Clair et al. 1996). Control sediment was prepared identically, but without the addition of oil. The oiled sediments were placed in the test tanks and clean running seawater was supplied to each tank for 48 hours prior to adding fish to flush any unmixed oil off the sediments.

Water samples were taken just prior to the addition of fish to verify that leaching of oil from the sediments was negligible. The actual concentrations of total hydrocarbons in the sediments were determined by chemists at the Auke Bay Laboratory using an High Pressure Liquid Chromatography (HPLC)/Flourescent detection method (Krahn et al. 1993). Concentrations are reported in parts per billion per wet weight of sediment at the phenanthrene (260-380) excitation/emmission wavelenghts. Water concentrations were analyzed using ultraviolet absorbance of hexane extracts at 240 nm (Larsen et al. 1994).

2.3. Avoidance Test Protocol

Oiled sediments to be tested were mud and sand; clean sediments were mud, sand or spherical granule. The seven sediment combinations tested were 1) unoiled mud with unoiled sand, 2) oiled mud with unoiled mud, 3) oiled sand with unoiled sand, 4) oiled mud with unoiled sand, 5) oiled sand with unoiled mud, 6) oiled mud with unoiled granule and 7) oiled sand with unoiled granule. Each of the 24 test chambers was divided into two equal portions - one containing the oiled and the other containing the unoiled sediment. Control tanks had unoiled sediment on both halves. There was no barrier separating the portions, thus enabling test fish to swim freely between the halves. The unoiled sediments were sampled for hydrocarbons to confirm that no cross contamination occurred.

Mud and sand were the preferred substrates for the species to be tested based on the results of the sediment preference work (Moles and Norcross, 1995). Granules were chosen as an unoiled substrate to determine if avoidance of oil was strong enough to force fish off their preferred sediment onto a sediment type that would be actively avoided in the absence of oil.

Avoidance tests with individual flatfish were conducted in flowthrough fiberglass tanks measuring 200 x 50 x 48 cm. Water entered at one end and the drain was situated at the other end. The middle of each tank was divided into three 50 x 50 cm chambers using screens. Water height was 20 cm. The chamber farthest from the drain contained the lowest concentration of oil , the middle chamber had a higher concentration of oil, and the final chamber had the highest concentration (Figure 1). This reduced the possibility of oil in an adjacent tank flowing to a lower concentration. Seawater flow rates were set at one liter per minute for each tank. Ambient salinity was constant at 28ppt and temperature varied between 7 °C and 8 °C over the duration of the tests.

All three species of juvenile flatfish were tested daily for preference between oiled and unoiled sediments. One fish was placed in each chamber on a neutral (without sediment) raised platform in the center of the chamber and allowed to acclimate for five minutes before being released from the restraining plastic screen. Twenty replicate trials were run in each of the three tanks for each experimental condition. No indvidual fish was used more than once. The trials took 60 days and used the original oiled sediment. Oil concentrations in the sediments were measured at the beginning and end of those 60 days.

Standard avoidance methods for pelagic fishes, i.e., one minute incremental observations over a one hour testing period (Rice 1973), are impractical with flatfish because the fish immediately bury themselves and remain immobile for hours (Keefe and Able 1994). Each fish was allowed 20 hours to choose a sediment, providing ample

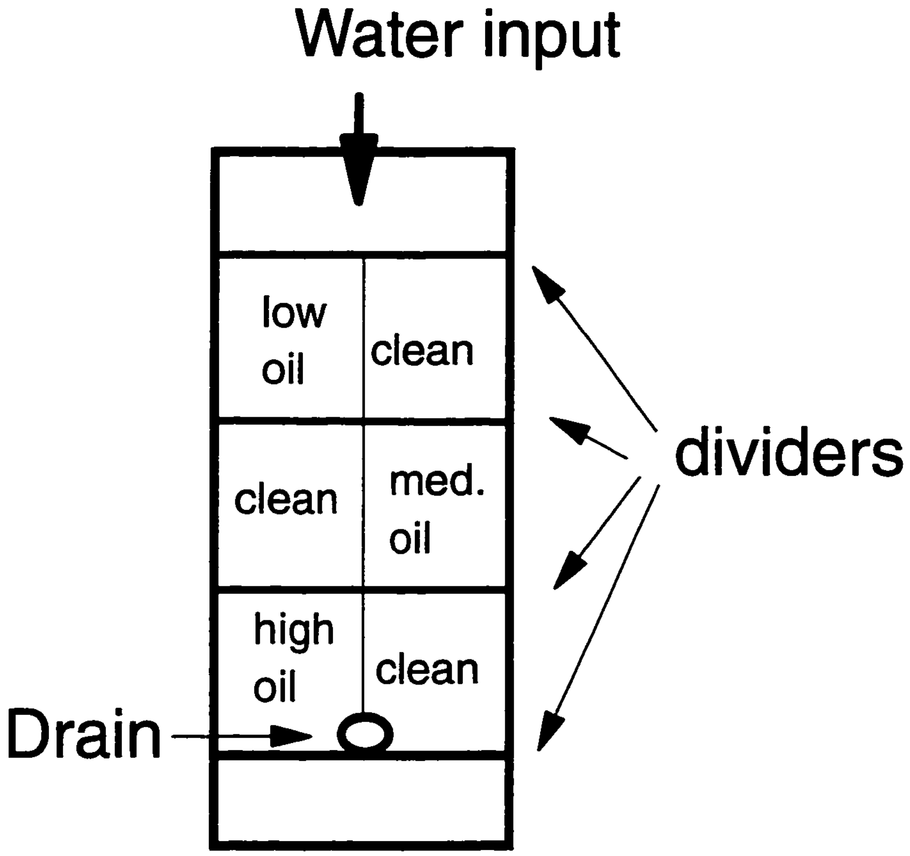

Figure 1. Diagram of the two-choice preference tanks. Relative positioning of oiled and unoiled portions and concentrations were randomized except as noted in text.

opportunity for the fish to contact the test strata during both day- and night-time hours (Aziz and Greenwood 1982). The distribution of flatfishes in the treatment groups (one half of the chamber oiled, the other half unoiled) and their corresponding controls (both halves unoiled) were analyzed using a chi-square test (Siegal 1956). In the controls, one half was designated as treatment and the other as untreated, despite the absence of oil in either half. When substrates were the same for both halves, the treatment half was the same side of the tank as in the oiled groups. When the substrates differed, the treatment half of the control was the half containing the same substrate as in the oiled portions.

3.0 RESULTS

3.1 Sediment Preference Without Oil

Sediment type was an important criterion in the behaviour of flatfish in these tests. The preference of rock sole and yellowfin sole for a particular sediment type was heavily skewed (Figure 2). When offered a choice between unoiled mud and unoiled sand, 100% of the rock sole selected sand. In contrast, 95% of the yellowfin sole selected mud over sand. Halibut, offered the same choice had no significant preference for sand or mud. Mud was selected in 9 of the 20 trials (45%) and sand was selected in 11 of the trials (55%)

3.2 Oil Chemistry

Initial concentrations of total hydrocarbons in the sand sediments were 1420 μg/g, 820 μg/g, 495 μg/g and 0 μg/g for 2%, 1%, 0.5% and control doses. The oiled sand lost

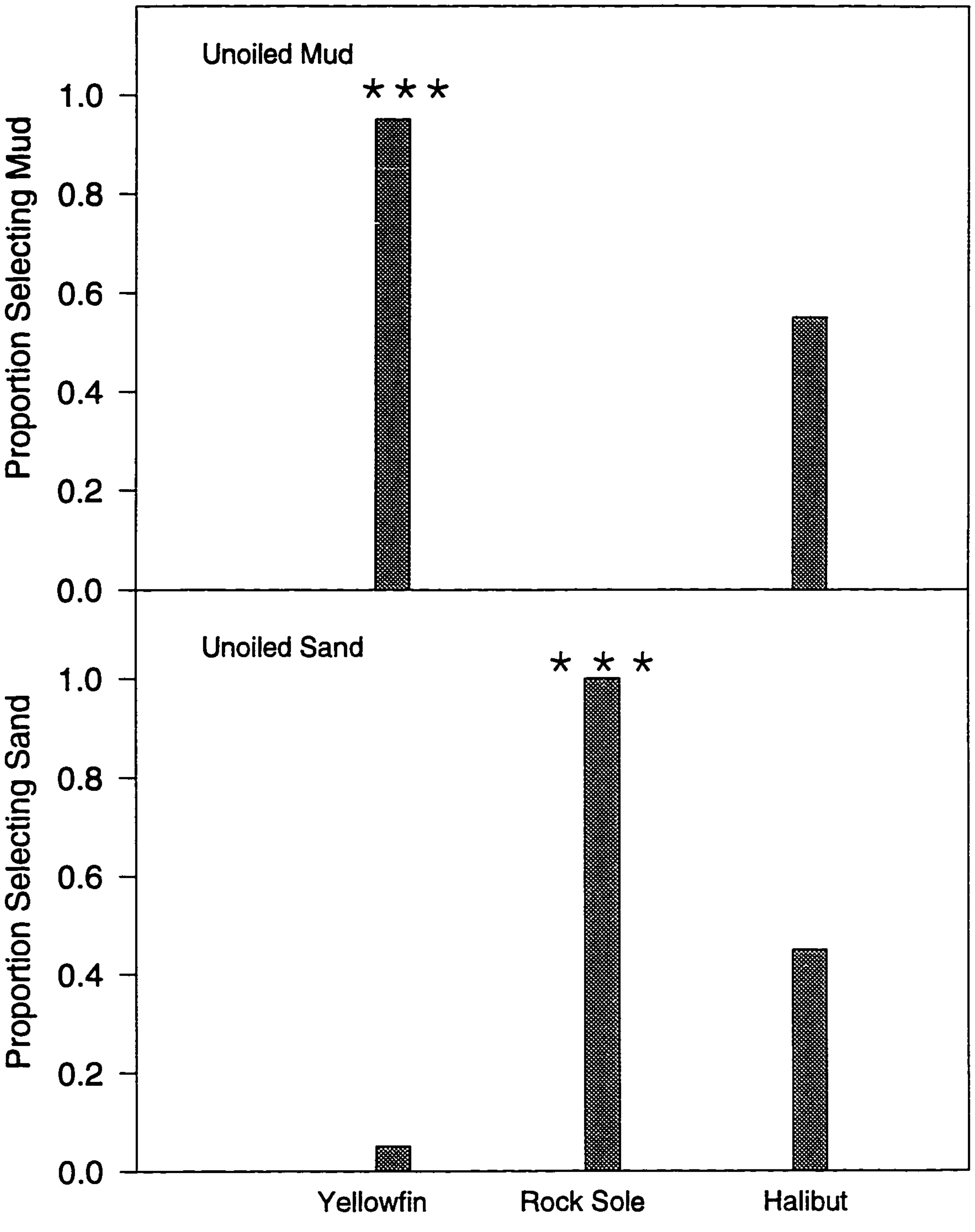

Figure 2. Different substrates without oil: proportion of fish selecting unoiled mud or sand in a two-choice preference tank. Asterisks denote significant greater selection than random (0.5) by chi-square(*p<0.001).

13-23% of its hydrocarbon load over the 60 day duration of the experiment for final concentrations of 1230 μg/g, 681 μg/g, 381 μg/g and 0 μg/g. Mud concentrations were 1448 μg/g, 406 μg/g, 141 μg/g and 0 μg/g for 2%, 1%, 0.5% and control doses respectively. The oiled mud lost 2-17% of its hydrocarbon load over the duration of the experiment for final concentrations of 1418 μg/g, 335 μg/g, 554 μg/g, and 0 μg/g. The increase in concentration in the 0.5% mud dose is probably an error. Hydrocarbons were not detectable by HPLC/flourescence measurement in the control sediments nor by ultraviolet spectrophotometry in the water column overlaying the sediments, suggesting that leaching of oiled sediments onto unoiled sediments was not a factor in selection.

3.3 Oil Avoidance

Most of the juvenile flatfishes avoided high concentrations of oiled sediment but not lower concentrations (Figure 3). When the only difference between two halves of the experimental chambers was the presence or absence of oil, five of the 18 test groups avoided oil. Yellowfin sole did not avoid any concentration of oiled mud but avoided the medium concentration of oiled sand (1%). Sixty percent of the rock sole in 2% oiled mud avoided the oiled side ($P<0.05$) while 58% of the rock sole avoided 1% oiled sand. Other concentrations, including 2% oil in sand, were not significantly avoided. Halibut significantly avoided both 2% oiled mud ($P<0.05$) and 2% oiled sand ($P<0.01$). All other choices were non-significant for all three species.

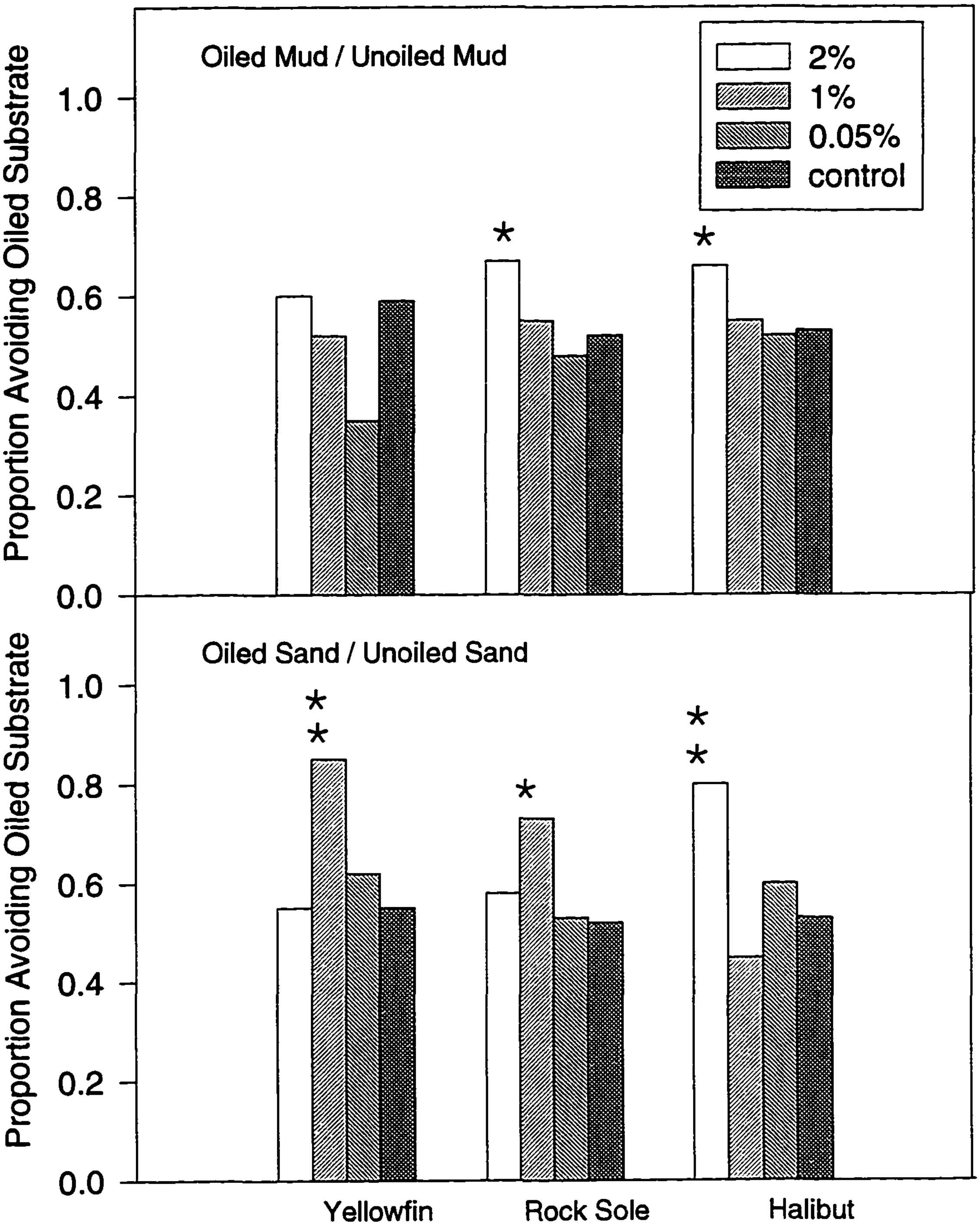

Figure 3. Similar substrates, with and without oil: proportion of fish avoiding oiled side in a two-choice preference tank. Control response is proportion avoiding the same side of an unoiled tank as the fish in an oiled tank. Asterisks denote significant differences from control selection by chi-square (*p<0.05,**p<0.01

3.4. Interaction of Oil and Sediment Type

The response of the fish to oil was altered by the type of substrates involved for yellowfin sole and rock sole but less for halibut (Figure 4). When the preferred substrate was unoiled, nearly all yellowfin sole and rock sole chose the preferred unoiled sediments over the oiled but less preferred sediments. Even though there was strong avoidance of oiled unpreferred sediments (85-100%), these responses were not due to oil but to sediment preference alone. If the preferred sediment was also the unoiled sediment, all comparisons between treatment (tanks with oil) and control (no oil on either side) were non-significant by chi-square analysis.

If the choice was between oiled/preferred substrate and unoiled/unpreferred substrate, the presence of oil reduced the number of yellowfin sole and rock sole chosing the preferred substate (Figure 4). Fewer yellowfin sole selected mud over sand if the mud was oiled than they did if the mud was unoiled. For yellowfin sole, oil significantly reduced the preference for mud at all three concentrations when compared to the response yellowfin sole to a choice of unoiled mud or unoiled sand (control). Similarly, the presence of oil in the sand reduced the natural preference of rock sole for sand. If sand was oiled, fish in the 2% and 1% concentration selected unoiled mud instead. While fewer than half of the yellowfin sole and rock sole avoided oiled/preferred sediments, the effect of oil in altering the natural choice of preferred sediment was significant. Oil concentrations that would not be avoided if the oiled and unoiled substrates were the

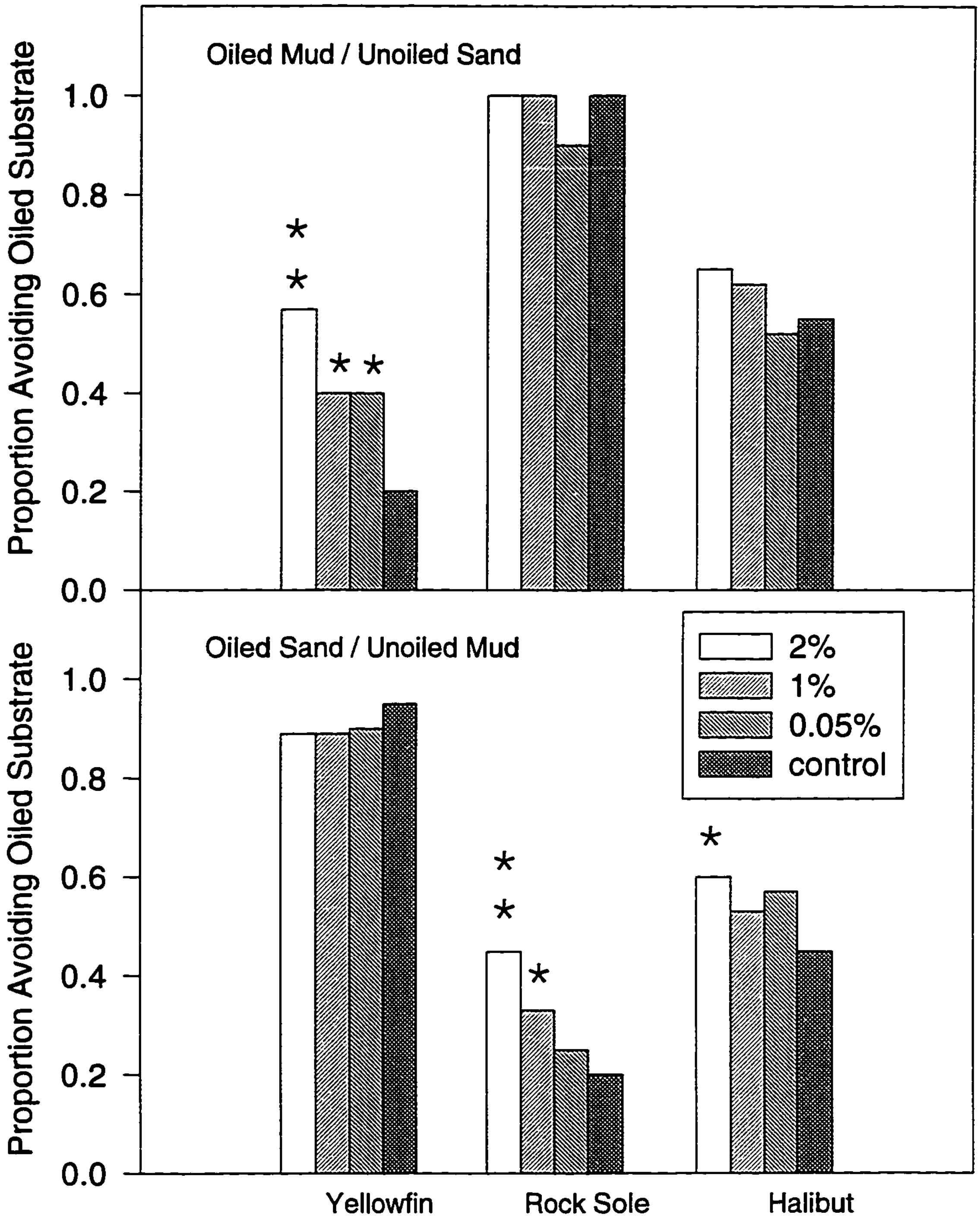

Figure 4. Different substrates, with and without oil: proportion of fish avoiding oiled side in a two-choice preference tank.Control response is proportion of fish in unoiled tank avoiding the same substrate as fish in oiled tank. Asterisks denote significant differences from control selection by chi-square (*p<0.05,**p<0.01)

same, were significantly avoided if the preferred sediment was oiled and unpreferred sediment was unoiled.

Halibut, which did not have a preferred sediment, avoided 2% oiled sand just as they had when the sediment types were the same. Given a choice between oiled mud and unoiled sand, 65% of the fish avoided oiled mud at 2% oil-similar to the 60% that avoided that concentration when unoiled mud was the alternative. If unoiled mud and oiled sand were the options, the same number of halibut avoided 2% oiled sediment (60%) as when unoiled sand was the alternative (Figure 4). The apparent preference for unoiled substrates was non-significant in all other cases.

Unoiled granules were strongly rejected by all three species in favor of mud or sand ($P<0.001$), regardless of the hydrocarbon concentration (Figure 5). Ninety to 100% of the flatfish selected oiled mud or sand over unoiled granule. Fish given a choice between unoiled mud or sand and unoiled granule also selected mud or sand. Oil, therefore, had no effect on sediment choice between mud/sand and granule since mud or sand was chosen regardless of whether it was oiled or unoiled.

4.0 DISCUSSION

Juvenile Pacific flatfishes are likely to remain on oiled sediments except at very high concentrations (1600 μg/g TPH) unless the available sediment alternatives are a more preferred sediment. Exposure of yellowfin sole and rock sole to concentrations as low as 141 μg/g TPH, however, significantly reduced selection of preferred sediments.

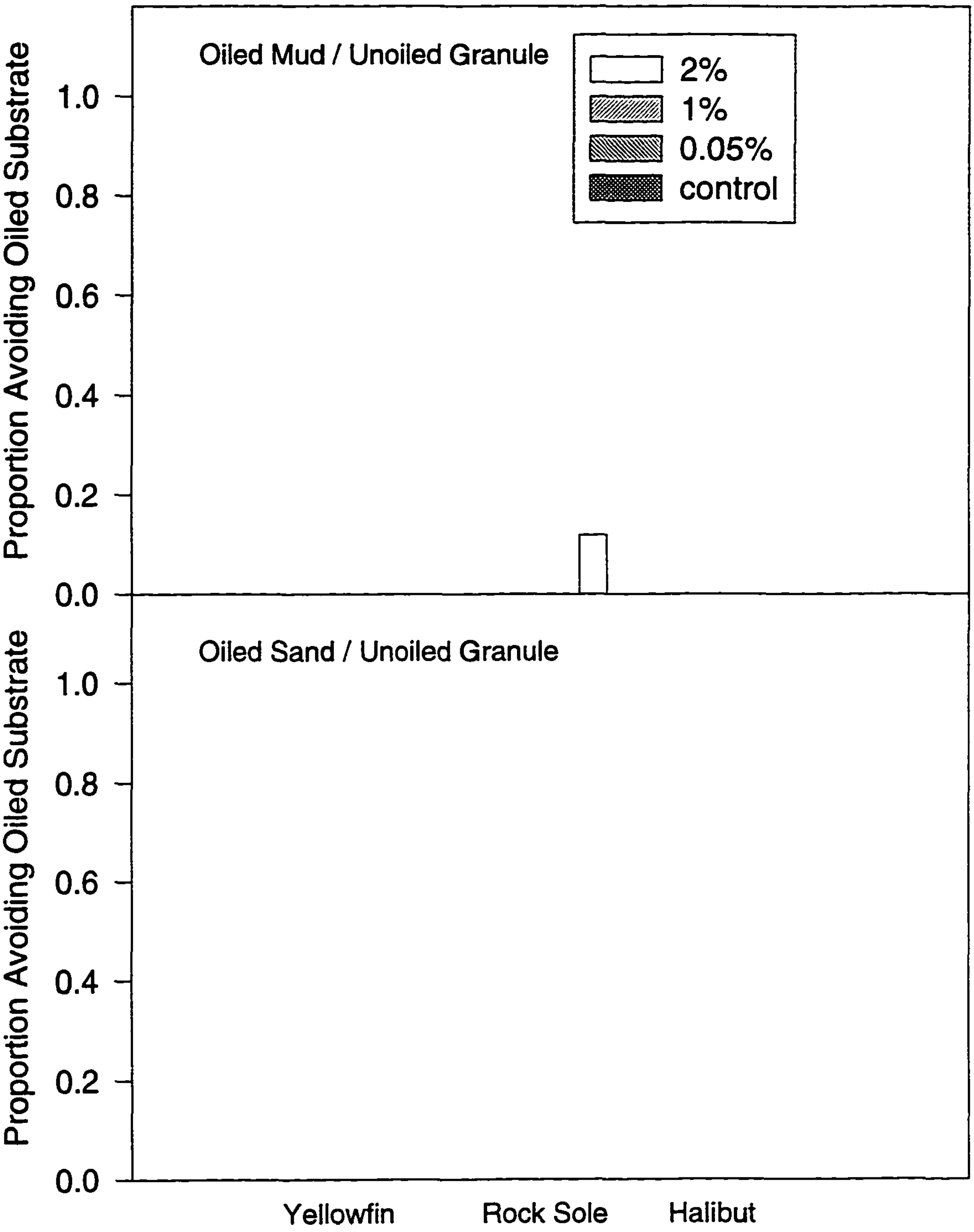

Figure 5. Unoiled granule versus oiled mud or sand: proportion of fish avoiding oiled side in a two-choice preference tank. Selection did not differ from fish offered unoiled sand or mud versus unoiled gravel (control).

Thus, whether flatfish avoid oiled sediments is a function of oil concentration, the type of oiled sediment, and the type of unoiled sediment available as an alternative. Rock sole and yellowfin sole, while able to discriminate between oiled and unoiled habitat, are likely to remain on oiled sediment if that sediment is the favored sediment, except at high concentrations. This preference for select sediments is reduced by the presence of oil but not eliminated. Halibut appear to be much more adaptable, and do not select oiled sediments due to sediment type. Thus, they are less likely to be affected by an oil spill. All three species preferred oiled sediment under 250 μm over the larger grained (2000 - 4000 μm) unoiled substrate.

The effects of remaining on oiled sediment will vary depending on the bioavailability of oil from that sediment to the flatfish. This in turn is a function of the dynamics of oil in the sediment. The fine grained sediments preferred by juvenile flatfish retain far more oil than coarser grained sediments (O'Clair et al. 1996). How much of the retained oil in sediments is actually available or toxic to the fish that choose to inhabit oiled sediment is unknown.

Concentrations of oil avoided by flatfish in this study, while fairly high (1400 μg/g) have been detected in the upper subtidal (O'Clair et al. 1996) and intertidal zones (Babcock et al. 1996) following a large oil spill. In the oilspill following the wreck of T/V *Exxon Valdez*, Babcock et al. (1996) detected concentrations as high as 62,000 μg/g TPH in intertidal sediments three years later. If flatfish do not avoid oil at concentrations lower than 400 μg/g and remain in oil laden sediments if it is the preferred sediment, the potential for sublethal effects is reason for concern. The close contact of flatfish with sediments, particularly when they bury, is likely to lead to far greater bioavailability through contact than would be measured by simple leaching.

The non-avoidance of oiled sediments at concentrations likely to be found following an oil spill makes it likely that long term effects on growth, reproduction and pathology will occur (Fletcher et al. 1982, Haensly et al. 1982, Johnson et al. 1988). The high incidence of environmentally induced neoplasms (McCain et al. 1978) noted in flatfish from polluted estuaries may be the result of non-avoidance of pollutants by these species. Halibut, which do avoid oiled substrates, are less likely to have longterm damage from spilled oil. Whether rock sole and yellowfin sole can detect low concentrations of oil and chose not to avoid them or whether they can only detect high concentrations is unclear.

Although the effect of prolonged contact with oiled sediments on juvenile flatfishes is unknown, oil exposure of juvenile salmonids (Moles and Rice 1983, Vignier et al. 1992) and adult flatfishes (Fletcher et al. 1981) results in reduced growth. Predation is greatest among the smallest juveniles (Van der Veer and Bergman 1987), so reduced growth of juvenile flatfishes would result in increased predation and a reduction in the number or size of flatfishes recruiting to the fishery (Van der Veer et al. 1994).

No food was present in the test sediments and was not a factor in sediment choice among laboratory animals. This is not the case in natural sediments. The presence of benthic food items are themselves likely to be affected by the presence of sediment bound oil. The effect of sediment choice in juvenile flatfishes will be influenced by prey density. Long-term exposure to oiled sediment is most likely if the benthic food supply is unaffected.

5.0 REFERENCES

Aziz, K.A. and J.G. Greenwood, 1982. Response of juvenile Metapenaeus bennettae Racek and Dall 1965 (Decapoda, Penaeidae) to sediments of differing particle size.-- Crustaceana 43(2): 121-126.

Babcock, M.M., G. Irvine, P.M. Harris, J.A. Cusick, and S.D. Rice, 1996. Persistence of oiling in mussel beds three and four years after the *Exxon Valdez* oil spill.-- American Fisheries Society Symposium 18: 286-297.

Cook, P.H., 1985. The behaviour of the plaice Pleuronectes platessa L. in relation to bottom current and sediment type. Ph.D. thesis, University of East Anglia, U.K.: 1-136.

De Ben, W.A., W.D. Clothier, G.R. Ditsworth, D.J. Baumgartner, 1990. Spatio-temporal fluctuations in the distribution and abundance of demersal fish and epibenthic crustaceans in Yaquina Bay, Oregon--Estuaries 14: 469-478.

Fletcher, G.L., J.W. Kiceniuk, and U.P. Williams, 1981. Effects of oiled sediments on mortality, feeding and growth of winter flounder Pseudopleuronectes americanus.-- Marine Ecology Progress Series 4: 91-96.

Fletcher, G.L., M.J. King, J.W. Kiceniuk, and R.F. Addison, 1982. Liver hypertrophy in winter flounder following exposure to experimentally oiled sediments.--Comparative Biochemistry and Physiology 73C: 457-462.

Gibson, R.N. and L. Robb, 1992. The relationship between body size, grain size and the burying ability of juvenile plaice, Pleuronectes platessa L.-- Journal of Fish Biology 40: 771-778.

Haensly, W.E., J.M. Neff, J.R. Sharp, A.C. Morris, M.F. Bedgood and P.D. Boem, 1982. Histopathology of Pleuronectes platessa L. from Aber Wrac'h and Aber Benoit, Brittany, France: long-term effects of the Amomo Cadiz crude oil spill.-- Journal of Fish Diseases 5: 365-391.

Hogue, E.W. and A.G. Carey, 1982. Feeding ecology of 0-age flatfishes at a nursery ground on the Oregon coast.--Fisheries Bulletin (United States) 80: 555-565.

Holladay, B.A. and B.L. Norcross, 1995. Diet diversity as a mechanism for partitioning nursery grounds of Pleuronectids. Proceedings of the International Symposium on North Pacific Flatfish, Alaska Sea Grant College Program, AK-SG-95-04.

Holmes, N.A. and A.D. McIntyre, 1984. Methods For the Study of Marine Benthos. Blackwell Scientific Publications, Oxford: 1-134.

Johnson, L.L., E. Casillas, T.K. Collier, B.B. McCain, and U. Varanasi, 1988. Contaminant effects on ovarian development in English sole (Parophrys vetulus) from Puget Sound, Washington.--Canadian Journal of Fisheries and Aquatic Sciences 45: 2133-2146.

Karinen, J.F., 1980. Petroleum in the deep sea environment: potential for damage to biota.--Environment International 3: 135-144.

Karinen, J.F., S.D. Rice and M.M. Babcock, 1985. Reproductive success in Dungeness crab Cancer magister during longterm exposures to oil-contaminated sediments. Final Report Outer Continental Shelf Environmental Assessment Program, April 1985: 435-461.

Keefe, M.L. and K.W. Able, 1994. Contributions of abiotic and biotic factors on settlement in summer flounder, Paralichthys dentatus.--Copeia 1994(2):458-465.

Krahn, M.M, G.M. Ylitalo, J. Buzitis, S.-L. Chan, U. Varanasi, T.L. Wade, T.J. Jackson, J.M. Brooks, D.A. Wolfe, and C.-A. Manen. 1993. Comparison of high-performance liquid chromatography/fluorescence screening and gas chromatography/mass spectrometry analysis for aromatic compounds in sediments after the *Exxon Valdez* oil spill.--Environmental Science and Technology 27: 699-708.

Krygier, E.E. and W.G. Pearcy, 1986. The role of estuarine and offshore nursery areas for young English sole, Parophrys vetulus Girard of Oregon.--Fishery Bulletin (United States) 84: 119-132.

Levings, C.D., 1972. A study of temporal change in a marine benthic community, with particular reference to predation by Pseudopleuronectes americanus (Walbaum) (Pisces: Pleuronectidae). Ph.D. thesis. Dalhousie University, Halifax, Nova Scotia, Canada.

Maynard, D.J. and D.D. Weber, 1981. Avoidance reactions of juvenile coho salmon (Oncorhynchus kisutch) to monocyclic aromatics.--Canadian Journal of Fisheries and Aquatic Sciences 38:772-778.

McCain, B.B., H.O. Hodgins, W.D. Gronlund, J.W. Hawkes, D.W. Brown, M.S. Myers and J.H. Vandermeulen, 1978. Bioavailability of crude oil from experimentally oiled sediments to English sole (Parophrys vetulus, and pathological consequences.--Journal of the Fisheries Research Board of Canada 35:657-664.

Moles, A. and B.L. Norcross, 1995. Sediment preference in juvenile Pacific flatfishes.--Netherlands Journal of Sea Research 33:361-367.

Moles, A. and S.D. Rice, 1983. Effects of crude oil and naphthalene on growth, caloric content, and fat content of pink salmon juveniles in seawater.--Transactions of the American Fisheries Society 112:205-211.

National Academy of Sciences (NAS), 1985. Oil in the Sea: Input, Fates and Effects. National Academy Press, Washington, D.C: 1-601.

O'Clair, C.E., J.W. Short and S.D. Rice, 1996. Contamination of intertidal and subtidal sediments by oil from the *Exxon Valdez* in Prince William Sound, Alaska.--American Fisheries Society Symposium 18: 61-93.

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

Short, J.W., D.M. Sale, and J. Gibeaut, 1996. Nearshore transport of hydrocarbons and sediments after the *Exxon Valdez* oil spill.--American Fisheries Society Symposium 18: 40-60.

Siegal, S., 1956. Non-parametric Statistics for the Behavioral Sciences. McGraw-Hill, NY.: 1-312.

Spies, R., D.W. Rice, P.A. Montagna and R.R. Ireland, 1985. Reproductive success, xenobiotic contaminants and hepatic mixed function oxidase (MFO) activity in Platichthys stellatus populations from San Francisco Bay.--Marine Environmental Research 17: 117-121.

Sturdevant, M.V. 1987. The role of meiofauna in the diets and feeding ecology of postmetamorphic flatfish. M.S. thesis, University of Alaska Juneau. Juneau, Alaska. 194p.

Syazuki, K., 1964. Studies on the toxic effects of industrial waste on fish and shellfish.--Journal of the Shimonoseki College of Fisheries 13: 157-211.

Tanda, M., 1990. Studies on burying ability in sand and selection to the grain size for hatchery reared marbled sole and Japanese flounder.--Journal of the Japanese Society of Grassland Sciences 56: 1543-1548.

Teal, J.M., K. Burns, and J. Farrington, 1978. Analyses of aromatic hydrocarbons in intertidal sediments resulting from two spills of No. 2 fuel oil in Buzzards Bay, Massachusetts.-- Journal of the Fisheries Research Board of Canada 35: 510-520.

Van der Veer, H.W. and M.J.N. Bergman, 1987. Predation by crustaceans on a newly settled 0-group plaice Pleuronectes platessa population in the western Wadden Sea.--Marine Ecology Progress Series 64: 1-12.

Van der Veer, H.W., R. Berghahn, and A.D. Rijnsdorp. 1994. Effect of juvenile growth on recruitment in flatfish--Netherlands Journal of Sea Research 32: 153-173.

Vignier, V., J.H. Vandermeulen, and A.J. Fraser, 1992. Growth and food conversion by Atlantic salmon parr during 40 days' exposure to crude oil.--Transactions of the American Fisheries Society 121: 322-332.

Weber, D.D., D.J. Maynard, W.D. Gronlund, and V. Konchin, 1981. Avoidance reactions of migrating adult salmon to petroleum hydrocarbons.--Canadian Journal of Fisheries and Aquatic Sciences 38: 779-781.

Wyanski, D.M., 1990. Patterns of habitat utilization in 0-age summer flounder Paralichthys dentatus. M.S. Thesis. College of William and Mary: 1-54.

CHAPTER 4

Contaminant Effects on Growth and Health of Juvenile Flatfish Exposed to Oil Laden Sediment

Abstract

Individually marked juvenile flatfishes, yellowfin sole, rock sole, and halibut (43-111 mm) were exposed for 90 days to sediments containing 0 to 4700 μg/g total petroleum hydrocarbons (TPH) of Alaskan North Slope crude oil. Growth reductions in all three species were significant following 30 days of exposure to 1600-1800 μg/g total oil and became more pronounced over time. Over a 90 period, fish exposed to 1600-1800 μg/g TPH grew at a rate of 0.43-0.57% body weight per day (BWD), depending on species. Fish exposed to 4300-4700 μg/g grew between 0.17 and 0.35% BWD. In contrast, control fish grew at a rate of 0.71-1.18% BWD. Additionally, growth rate was measured every 30 days and compared with control growth rates. These incremental growth rates of oil exposed fish ranged from 13% to 93% below rates for unexposed fish and were mostly significant reductions ($P<0.05$). Halibut and yellowfin sole growth rates were all significantly ($P<0.05$) lower for oil exposed fish.

Tissue and parasite alterations indicated a reduction in fish health. There was an increase in liver lipidosis, gill hyperplasia and hypertrophy, and gill ciliate infestation combined with a decline in macrophage aggregates and gut parasites. Fish exposed to 4300-4700 μg/g TPH for 90 days lost 22% to 69% of their caudal fins to erosion. Fish exposed to the lower concentration had moderate to severe fin erosion. Chronic marine

oil pollution that results in hydrocarbon concentrations of 1600 μg/g in nearshore sediments would have the potential to reduce growth and health of juvenile flatfishes that use these sediments as nursery areas. Recruitment of juveniles to the fishery would be reduced due to increased susceptibility to predation and slower growth to maturity.

1.0 INTRODUCTION

1.1 Rationale for Study

Pollutants such as petroleum hydrocarbons are known to alter the growth of pelagic fishes (Woodward et al. 1981, Moles et al. 1981, Moles and Rice 1983, Vignier et al. 1992). Despite decades of study on the effects of water-borne crude oil on fishes, little is known about the effects of hydrocarbons on growth of demersal fishes, particularly juveniles. Yet the estuarine nursery areas needed by juvenile flatfishes are more vulnerable to loss due to pollution than are the habitats of any other fish (FAO 1995).

Juvenile flatfishes, which reside in nearshore sediments, are particularly vulnerable to effects from contaminated sediments due to direct contact with the pollutants. Juveniles rear in the fine-grained substrates of nearshore bays (Norcross et al. 1995) burying themselves in the top layer of sediment and actively ingest sediment when feeding (Hicks 1984, Truscott et al. 1992). As obligatory residents of benthic sediments, flatfishes would be continuously exposed to oil through direct substrate contact as well as ingestion of benthic prey. Since the primary route of hydrocarbon uptake is via the skin and gills (Ariese et al. 1993), flatfishes which live in direct contact with the sediments may not be able to avoid chronic exposure.

These bottom sediments are now recognized as the final repository of hydrocarbons following an oil spill as the hydrocarbons settle out into the fine grained sediments where they can persist for years (Gundlach et. al. 1983, O'Clair et al. 1996). Following the *Exxon Valdez* oil spill in 1989, hydrocarbons from oil deposited on the shoreline were constantly resuspended and deposited in the subtidal sediments at 20m of depth where they persisted for over three years (O'Clair et al. 1996).

The effects of these hydrocarbon laden sediments on flatfish tissues has received considerable attention while the effects on the fish itself, particularly the juvenile stage, has had scant attention. Petroleum hydrocarbons in polluted urban sediments have been closely correlated with alterations in detoxification enzymes (Monoson and Stegeman 1994, Vignier et al. 1992), tissue abnormalities (McCain et al. 1978, Myers et al. 1991), and reproductive hormones (Spies et al. 1985, Johnson et al. 1988, Truscott et al. 1992) of flatfishes. Unfortunately, the correlation of many of these cellular biomarkers of exposure with changes at the organism level is unknown. Of these organismal effects, growth may be the singly most important factor in recruitment of juvenile fishes to the fishery (van der Veer et al. 1994) as well as the best indicator of fish health (Goede and Barton 1990) since it integrates all cellular changes as well as abiotic variables acting on the organism.

The objective of the present experiment was to determine the amount of time required to produce a significant reduction in growth rates of three species of juvenile flatfishes coupled with recognizable alterations at the tissue level. To do this, I examined the growth rate and condition of juvenile (age 0 and age 1) flatfishes reared on hydrocarbon laden sediments for 90 days and correlated these effects with known biomarkers of fish health. The chosen biomarkers were alterations in parasite load and structure of gill and liver tissue. A secondary objective was to determine if the type of oiled substrate (mud or sand) had an effect on growth rates as it does on avoidance behavior (Moles et al. 1994).

1.2 Selection of Study Animals

For this investigation, I tested similar sized (mean of 70 mm, SE=1.0) juveniles of three species of commercially important flatfishes: age-1 yellowfin sole, Pleuronectes asper, age-0 rock sole, Pleuronectes bilineatus , and age-0 Pacific halibut, Hippoglossus stenolepis. The adults of these three species constitute over half of the flatfish catch in the NE Pacific, totaling nearly 300,000 metric tons in 1992 (FAO 1995). This is the third largest fishery in the NE Pacific, after pollock, Theragra chalcogramma, and salmon, Oncorhynchus sp. (FAO 1995), and constitutes the largest unexploited fishery resource in the area with a potential allowable catch of over a million metric tons in the Bering Sea alone (NPFMC 1993). These three species were also selected because of their vulnerability to hydrocarbon exposure. In the field, all three species share vulnerable (<40 m) nursery areas (Norcross et al. 1995), an area that was coated in the *Exxon Valdez* oil spill.

2.0 METHODS

Growth of juvenile flatfishes on oiled sediment was determined by rearing flatfish on concentrations of oiled sediments similar to the levels of oil detected in heavily oiled sediments following severe oil spills. Substrates tested were mud and fine sand, the preferred sediments of these species (Moles and Norcross 1995). Groups tested were rock sole on sand, rock sole on mud, yellowfin sole on mud, and halibut on sand. Rock sole was tested on both mud and sand to determine the effect of sediment type on growth.

2.1 Collection of Animals

Rock sole and yellowfin sole were obtained from Auke Bay, Alaska by 6 mm mesh beach seine in June of 1994 and 1995. Halibut were collected in 10-30 m water depth off Kodiak Island by plumb staff beam trawl (4 mm mesh) in August of 1994. All specimens were transported live to the National Marine Fisheries Service (NMFS) laboratory at Auke Bay and held in flowthrough seawater tanks on a mixed mud/sand sediment. Only fish 43-111 mm (average 70 mm) were used in the tests. To identify individual fish for repeated size measurements, juveniles were dye marked on the ventral surface (Thedinga and Johnson 1995).

2.2. Sediment Preparation

Oiled and control sediments were prepared using the method of Moles et al. (1994). Concentrations of oiled mud and sand for this experiment were obtained by mixing a volume of Alaska North Slope crude oil (2% and 1% of the sediment volume) with the sediment. This gave total petroleum hydrocarbon (TPH) concentrations of 4316, 1636, and 0 μg/g in mud and 4711, 1840, and 0 μg/g in sand (Table 3). A concentration of 4300-4700 μg/g is near maximum saturation for oil in sediments and 1400-1800 μg/g correspond to concentrations found in oiled intertidal sediments following the *Exxon Valdez* oil spill (Babcock et al. 1996). At day 0 and day 90, sediments from each treatment group (eg, high concentration mud) were pooled into a single hydrocarbon sample to determine total hydrocarbon loss.

All sediment samples were analyzed for total petroleum hydrocarbon (TPH) by ultraviolet fluorescence, as adapted from Krahn et al. (1991,1993). Total petroleum hydrocarbons (TPH) were estimated based on the concentration of phenanthrene in the sample. There is good agreement between ultraviolet fluorescence estimates of total hydrocarbons and data derived from the more expensive gas chromatographic /mass spectroscopic measurements of total aromatics present (Babcock et al. 1996).

2.3 Growth Tests

To determine the effects of oiled sediment on the growth of juvenile flatfishes, I exposed 240 fish (60 for each species/substrate combination) to one of three concentrations of toxicant in either sand or mud for 90 days beginning July 7, 1994. Twenty four rectangular (30 x 60 x 40 cm) experimental tanks were located under translucent panels outdoors and natural light was supplemented with a constant twelve hours of fluorescent lighting per day. Each 70 liter tank received a constant flow of 1.4 l/min of ambient seawater at a salinity of 28 ppt and a temperature of 10°C which are

Table 3. Concentration of total hydrocarbons present in experimentally oiled sediments and changes over time for the 90 days exposure period.

	(μg/g dry weight)		% loss 0-90 d
Treatment Group	0 d	90d	
Mud			
High Concentratiion	4316	3312	22%
Low Concentration	1636	1315	20%
Control	23	10	
Sand			
High Concentration	4711	3751	20%
Low Concentration	1840	1410	23%
Control	1	1	

common salinity and temperature values during the late summer growth phase in nursery areas of southeast Alaska. Temperature was controlled by resistive heaters, mercury switches, and associated relays. Treatment groups were randomly allocated to tanks.

Treatment groups were partitioned among the 24 tanks in a factorial design (4 species/sediment combinations x three concentrations x 2 replicates). Each tank contained 10 marked fish for a total of 240 fish. Fish were bloodworms Tubifex sp. and mysids Mysis sp. *ad libitum* beginning two week prior to the test to insure active feeding. The fish were fed six times per day to reduce size variance and possible territoriality (Brannes and Alanara 1993).

Standard lengths and wet weights of each fish were measured initially and after 30, 60, and 90 days of exposure. I then calculated Fulton's condition factor and specific growth rates for length and weight for each fish (Fonds et al. 1995). Daily length increment (dL, mm/day) was estimated from differences in standard length (L) over time as:

$$dL=(L_{end} - L_{start})/t$$

Where t is time in days. Increments included 0-30 days, 30-60 days, 60-90 days, and 0-90 days to examine both incremental and overall growth effects. The specific growth rate in weight was estimated as:

$$G=(\ln W_{end} - \ln W_{start})/t \times 100$$

where W is body wet weight in mg. Growth was considered inhibited if growth rates in any group of treated fish was significantly less than that of control fish in similar sediment types.

The effect of oil exposure on growth was analyzed using one-way analysis of variance of concentration versus size, condition, or growth rate at day T. I used Dunnett's statistic (Winer 1962) to test the differences between individual treatment means and the control means for weight and length at day T at a significance level of 0.95. Differences in growth rates between species were assessed using one-way analysis of variance followed by Student-Newman-Keuls multiple comparisons at a significance level of 0.95. Analyses of variance on linear regressions of concentration versus mean response at day T were used to indicate relationships between the mean size, condition, or growth rate and concentration of toxicant. Analysis of variance was also used to test for significant differences in initial fish size between the test groups. All analyses used the Kolmogorov-Smirnov test (Stevens 1974) for normality and the Levene Median test (Levene 1960) for homoscedasticity.

2.4 Health Biomarkers

After 90 days of oil exposure, five fish from each tank were sampled for tissue alterations and parasite prevalences, known biomarkers of hydrocarbon exposure. The percentage of caudal fin eroded (necrotic length/caudal length x 100) was calculated for each fish at the end of the experiment (Barker et al. 1994). Skin scrapings were taken from 5 fish with the worst erosion to determine if the cause was bacterial or parasitic. Scrapings were examined microscopically and incubated on trypticase soy agar.

Gill and liver tissue of five fish from each tank were sampled for histology. Tissues were excised from living fish after 90 days of oil exposure and fixed in 10% neutral formalin. The tissues were preserved in 70% ethanol. In the laboratory, tissues were dehydrated in a graded ethanol series, cleared in xylene, and embedded in paraffin. Tissues were sectioned by a commercial firm at 6 μm and stained with hematoxylin and eosin. The resulting sections were examined for the presence of tissue changes (Hinton and Lauren 1990) and for Trichodina, a parasitic gill ciliate. The number of macrophage aggregates in the liver was estimated using ten fields (100x) per section. In addition, the gut tracts from the same fish were examined for the presence of parasitic helminths. Stomach and intestinal contents were examined under a dissecting microscope for trematodes. The contents were subsequently digested in pepsin (Moles et al.1990) and the undigested contents examined for the presence of parasitic nematodes.

The proportion of fish infected with gill ciliates was compared between treatment groups using 2 x 2 contingency tables analysis, applying Fisher's exact test when any expectation was less than 5. The number of fish macrophage aggregates was compared between treatment groups using one-way analysis of variance following by Dunnett's statistic. The data on percentage of fin erosion per fish was normalized by arcsin squared transformation before comparing differences between treatment groups by Kruskal-Wallis ANOVA ranks test.

3.0 RESULTS

3.1 Mortality

The only significant mortality occurred in halibut exposed to 4700 μg/g TPH (Table 4). Eighteen of the 20 halibut in that concentration died between day 60 and day 90. Less than 2% (4/220) of the fish in other tanks died during the 90 days of exposure. Mortality could only be assessed at the monthly sampling intervals as the fish were buried in sediment at other times. Carcasses decomposed in the sediment as well. All but four of the 240 flatfish in the experiment could be accounted for at day 60. The two halibut that remained alive in the high concentration at day 90 had lost all fins as had mortalities in that tank. All data presented for halibut exposed to the high concentration at 90 days represents only two fish rather than 20 as in the other treatment groups and growth intervals.

3.2 Growth Effects

Juvenile flatfishes reared for 90 days on oil laden sediments grew less than control fish (Figure 6). Growth rates calculated as changes in weight for the interval between 0 and 90 days were significantly less than control rates for all oil exposed treatments. Ninety day growth rates in the high concentrations were 0.23, 0.21, 0.35 and 0.17 BWD for yellowfin sole, rock sole in mud, rock sole in sand, and halibut, respectively. In contrast, control growth rates for the same period were 0.83, 0.71, 0.86 and 1.18 BWD, respectively. This amounted to a reduction of 59% to 86% below fish in unoiled

Table 4. Cumulative mortality (percent) in juvenile flatfishes exposed to oil laden sediments for 30, 60, and 90 days.

	Percent Cumulative Mortality		
	30d	60d	90d
Yellowfin Sole			
4.3 mg/g	0%	0%	0%
1.6 mg/g	10%	10%	10%
Control	0%	0%	0%
Rock Sole / Mud			
4.3 mg/g	0%	5%	5%
1.6 mg/g	0%	0%	0%
Control	0%	0%	0%
Rock Sole / Sand			
4.7 mg/g	0%	0%	0%
1.8 mg/g	5%	5%	5%
Control	0%	0%	0%
Halibut			
4.7 mg/g	0%	0%	90%
1.8 mg/g	0%	0%	0%
Control	0%	0%	0%

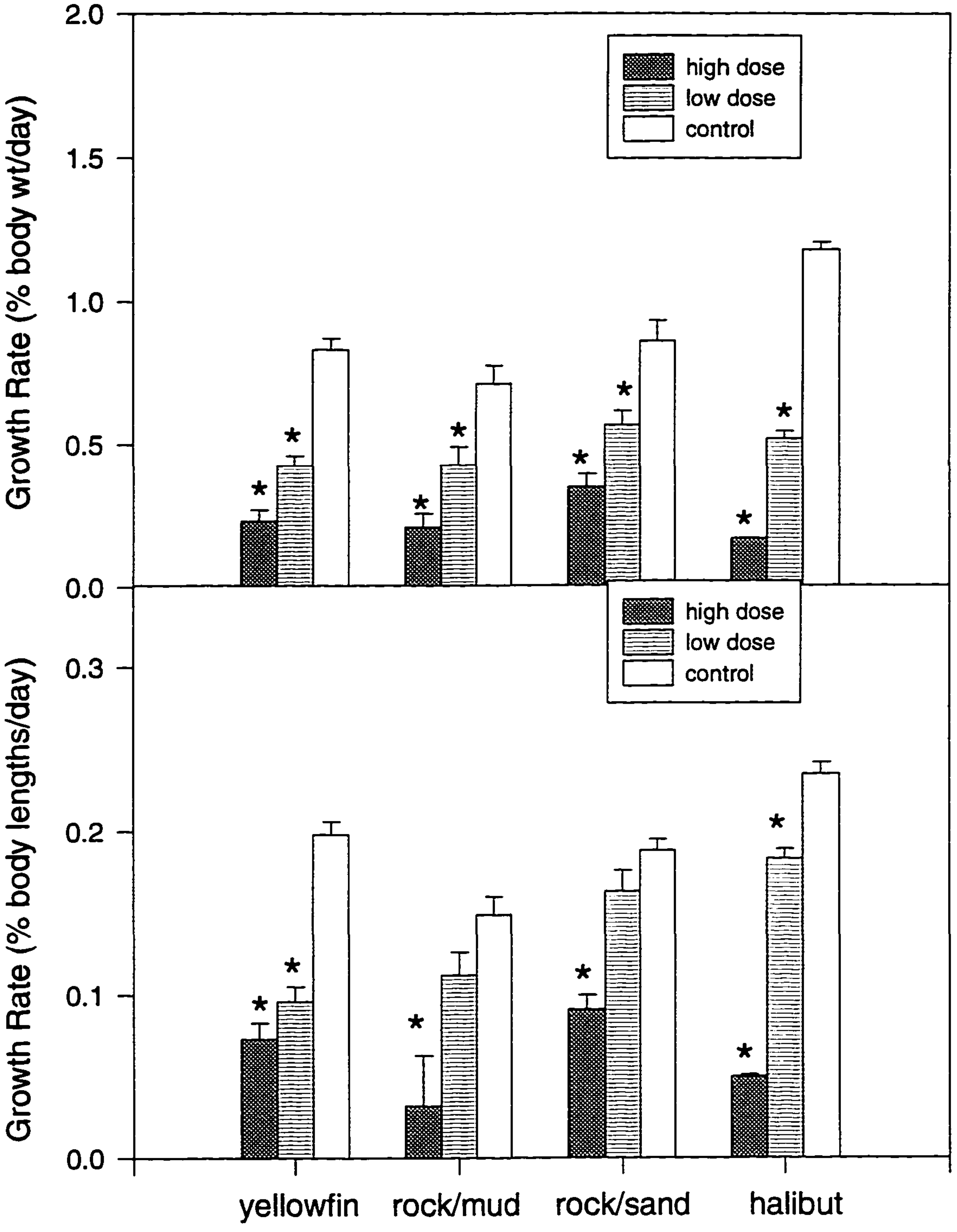

Figure 6. Growth rates over 90 days of four species of juvenile flatfishes exposed to oiled sediments. Asterisks denote significant differences (P<0.05) from control rates by Dunnett's test.

treatments after 90 days. Fish exposed to the low concentration for 90 days grew at a rate 34-56% less than control fish.

Change in weight of fish was a more sensitive indicator of the toxicity of oiled sediment than change in length (Figure 6). Specific growth rates (% body weight per day) had tighter variances and were more often significantly different from control rates than where growth rates measured as daily length increments (mm/day). Specific growth rates were also more sensitive indicators since control weights increased an average of 50% over 90 days whereas control lengths only increased by 20% over 90 days (Table 5). Therefore, all further discussion of growth rates will refer to specific growth rates rather than to daily length increments.

3.2.1 Effect of Oil Concentration on Growth

As the concentration of oil increased, specific growth rates declined. The regression of concentration on growth rate was significant at each 30 day interval in the test for all treatments (Table 6). The effect of oil on growth rate was significant at $P<0.05$ for rock sole and at $P<0.001$ for yellowfin sole and halibut.

Growth rates in most of the oil exposed treatment groups were significantly lower than rates of unexposed fish after 30 days as well (Figure 7). During the first 30 days of exposure, oil concentrations of 4300-4700 μg/g TPH significantly ($P<0.05$) reduced growth rates in all fish. Thirty day growth rates of fish in the high concentration ranged from 0.06 % BWD for halibut to 0.40% BWD for rock sole, a 47-93% reduction below growth rates for unexposed fish. Concentrations of 1600-1800 μg/g significantly ($P<0.05$)

Table 5 Effects of oil laden sediment on mean lengths, wet weights, condition factors, and growth rates (SE in parentheses) of juvenile Pacific flatfishes for 30 day intervals during a 90 day exposure period. Asterisks (*) denote significant difference from the control group at time T by Dunnett's test (P<0.05).

	Exposure Period			
	0d	30d	60d	90d
Treatment Group				
	YELLOWFIN SOLE IN MUD			
High Concentration				
Length (mm)	73 (4)	74 (4)	77 (4)	80 (5)
Weight (g)	4.95 (0.89)	5.26 (0.96)	5.67 (1.03)	6.16(1.15)
Condition. Factor	1.06 (0.02)	1.05 (0.03)	1.02 (0.03)	0.99 (0.03) *
Growth (mm/d)	0	0.06 (0.01) *	0.09 (0.02) *	0.08 (0.01) *
Growth (%BWD)	0	0.19 (0.04) *	0.26 (0.05) *	0.25 (0.04) *
Low Concentration				
Length (mm)	74 (4)	77 (4)	79 (4)	83 (4)
Weight (g)	4.95 (0.85)	5.48 (1.01)	6.15 (1.06)	7.37 (1.23)
Condition Factor	1.06 (0.01)	1.01 (0.02)	1.04 (0.02)	1.11 (0.03)
Growth (mm/d)	0	0.11 (0.02) *	0.09 (0.02) *	0.11 (0.01) *
Growth (%BWD)	0	0.29 (0.07) *	0.47 (0.08) *	0.61 (.06) *
Control				
Length (mm)	71 (4)	75 (4)	83 (4)	89 (4)
Weight (g)	4.38 (0.77)	5.06 (.86)	6.40 (1.01)	8.89 (1.40)
Condition Factor	1.03 (0.03)	1.03 (0.03)	0.99 (0.04)	1.09 (0.03)
Growth (mm/d)	0	0.14 (0.02)	0.26 (.03)	0.21 (0.02)
Growth (%BWD)	0	0.52 (0.06)	0.91 (0.14)	1.08 (0.10)

Table 5 (continued).

Treatment Group	Exposure Period 0d	 30d	 60d	 90d
		ROCK SOLE IN MUD		
High Concentration				
Length (mm)	70 (4)	71 (4)	74 (4)	76 (4)
Weight (g)	4.17 (0.73)	4.37 (0.78)	4.89 (0.84)	5.10 (0.81)
Condition Factor	1.06 (0.01)	1.02 (0.02)	1.02 (0.03)	1.00 (0.03) *
Growth (mm/d)	0	0.06 (0.01) *	0.07 (0.02) *	0.07 (0.02) *
Growth (%BWD)	0	0.14 (0.04) *	0.32 (0.10)	0.26 (0.10) *
Low Concentration				
Length (mm)	69 (4)	72 (5)	75 (5)	79 (5)
Weight (g)	4.49 (1.00)	4.84 (1.01)	5.40 (1.09)	5.90 (1.13)
Condition Factor	1.07 (0.02)	1.05 (0.02)	1.05 (0.02)	1.01 (0.02) *
Growth (mm/d)	0	0.10 (0.02)	0.11 (0.02)	0.13 (0.02) *
Growth (%BWD)	0	0.46 (0.09)	0.45 (0.07)	0.43 (0.08)*
Control				
Length (mm)	66 (4)	69 (4)	73 (4)	79 (4)
Weight (g)	3.76 (0.79)	4.19 (0.73)	4.81 (0.81)	6.20 (0.94)
Condition Factor	1.05 (0.03)	1.05 (0.02)	1.03 (0.02)	1.10 (0.02)
Growth (mm/d)	0	0.13 (0.02)	0.14 (.02)	0.20 (0.02)
Growth (%BWD)	0	0.62 (0.10)	0.63 (0.11)	1.01 (0.10)

Table 5 (continued).

Treatment Group	Exposure Period 0d	30d	60d	90d
	ROCK SOLE IN SAND			
High Concentration				
Length (mm)	64 (2)	67 (2)	70 (3)	72 (3)
Weight (g)	2.96 (0.38)	3.26 (0.38)	3.65 (0.42)	4.06 (0.47) *
Condition Factor	1.06 (0.02)	1.02 (0.03)	0.99 (0.02) *	1.01 (0.01)
Growth (mm/d)	0	0.11 (0.02)	0.11 (0.02) *	0.07 (0.01) *
Growth (%BWD)	0	0.40 (0.08) *	0.37 (0.10) *	0.36 (0.06) *
Low Concentration				
Length (mm)	70 (4)	74 (4)	78 (5)	85 (5)
Weight (g)	4.39 (0.81)	5.09 (0.97)	5.96 (1.15)	7.32 (1.18)
Condition Factor	1.06 (0.02)	1.03 (0.02)	1.00 (0.02) *	1.00 (0.03)
Growth (mm/d)	0	0.10 (0.01)	0.15 (0.02)	0.24 (0.02)
Growth (%BWD)	0	0.38 (0.06) *	0.48 (0.06) *	0.92 (0.13)
Control				
Length (mm)	67 (4.1)	71 (4.2)	76 (4.0)	84 (3.9)
Weight (g)	3.96 (0.85)	4.81 (0.93)	5.79 (0.99)	6.90 (0.92)
Condition Factor	1.02 (0.02)	1.11 (0.04)	1.11 (0.04)	1.07 (0.04)
Growth (mm/d)	0	0.11 (0.01)	0.18 (.02)	0.28 (0.02)
Growth (%BWD)	0	0.75 (0.11)	0.79 (0.11)	1.06 (0.12)

Table 5 (continued).

Treatment Group	Exposure Period 0d	 30d	 60d	 90d
	HALIBUT IN SAND			
High Concentration				
Length (mm)	69 (3)	70 (3)*	72 (3)*	84 (1)
Weight (g)	3.82 (0.41)	3.90 (0.43) *	4.22 (0.45) *	6.51 (0.19) *
Condition Factor	1.07 (0.03)	1.06 (0.04) *	1.05 (0.04) *	1.08 (0.01) *
Growth (mm/d)	0	0.02 (0.00) *	0.07 (0.01) *	0.02 (0.00) *
Growth (%BWD)	0	0.10 (0.02) *	0.27 (0.04) *	0.20 (0.01) *
Low Concentration				
Length (mm)	71 (2)	76 (2)	82 (2)	88 (2)
Weight (g)	4.04 (0.35)	4.68 (0.42) *	5.45 (0.47) *	6.38 (0.53) *
Condition Factor	1.05 (0.02)	1.00 (0.02)	0.95 (0.03)	0.90 (0.03)
Growth (mm/d)	0	0.16 (0.00) *	0.18 (0.01) *	0.21 (0.01) *
Growth (%BWD)	0	0.48 (0.04) *	0.52 (0.04) *	0.56 (0.06) *
Control				
Length (mm)	72 (1.6)	78 (1.5)	85 (1.4)	93 (1.4)
Weight (g)	4.10 (0.29)	6.39 (0.41)	8.88 (0.49)	11.63 (0.62)
Condition Factor	1.06 (0.02)	1.30 (0.02)	1.41 (0.02)	1.41 (0.03)
Growth (mm/d)	0	0.20 (0.01)	0.23 (.01)	0.27 (0.01)
Growth (%BWD)	0	1.51 (0.06)	1.14 (0.05)	0.91 (0.04)

Table 6. Analysis of variance tables for regression of concentration of total hydrocarbons against mean growth rates (%body weight/day) for four experimental groups (species/substrate).

Regression at day	F value	Probability	Power at 0.05
	Yellowfin Sole in Mud		
30	15.8	0.001	0.965
60	18.2	0.001	0.980
90	57.9	0.001	1.000
	Rock Sole / Mud		
30	18.9	0.004	0.984
60	4.21	0.045	0.052
90	26.9	0.001	0.998
	Rock Sole / Sand		
30	6.9	0.012	0.728
60	9.8	0.003	0.856
90	7.5	0.008	0.759
	Halibut / Sand		
30	196.1	0.001	1.000
60	122.1	0.001	1.000
90	34.7	0.001	1.000

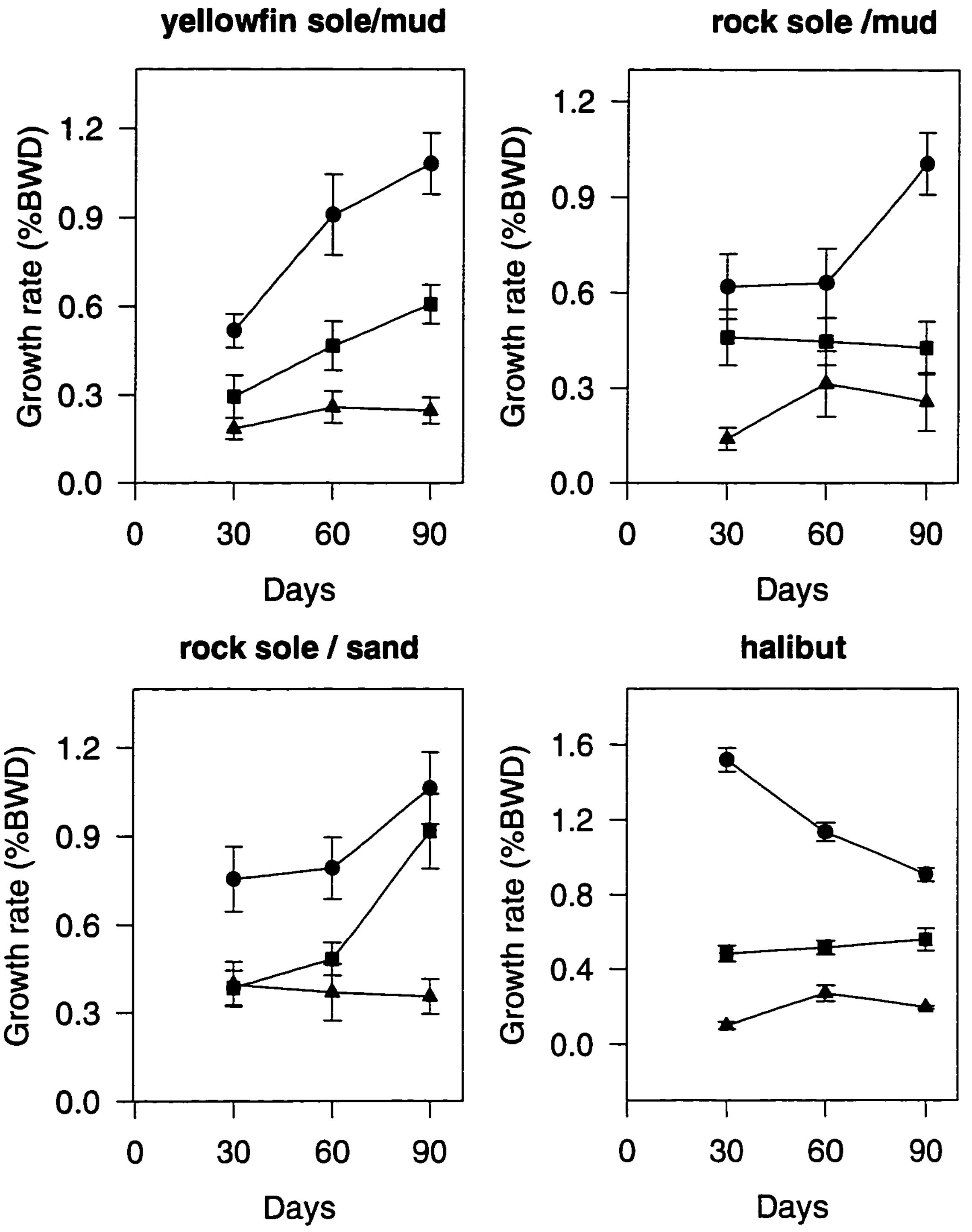

Figure 7. Thirty day growth rates (% BWD) determined at 30, 60, and 90 days for four species of flatfishes exposed to oil laden sediments.
Legend: —●— control, —■— low dose, —▲— high dose

reduced growth rates in all groups except rock sole in mud.. Thirty day growth rates at the low concentration were 0.29, 0.46, 0.38 and 0.48 for yellowfin sole, rock sole in mud, rock sole in sand, and halibut respectively, 25% to 68% below growth rates of fish in unoiled treatments.

During the second 30 days of exposure (30-60 day), growth rates of rock sole in mud increased and were no longer significantly lower than rates for unexposed fish. During the final 30 days of exposure (60-90 day), oil exposed fish had lower growth rates than unexposed fish except for rock sole exposed to 1800 μg/g in sand. This latter group had a 48% increase in growth rate during the last 30 days of exposure, suggesting recovery. There was, however, no similar response among rock sole exposed to a similar concentration in mud. In the high concentrations, growth rates remained at the same reduced level during the last 60 days of the test. Growth rates among fish exposed to the low concentration increased in yellowfin sole and rock sole in mud, remained the same for halibut and declined slightly among rock sole in mud over time.

3.2.2 Species/substrate Differences

Growth rates differed between species but not between substrates. During the first 60 days of the test, unexposed halibut grew significantly faster than yellowfin sole or rock sole (Table 7), despite having similar sizes at day zero. While the incremental growth rate of the other species increased over time, growth rates of unexposed halibut declined over the duration of the test. Growth rates for unexposed halibut fell from a value of 1.51 % BWD during the first 30 days to a value of 0.91 % BWD during the last 30 days, a 40%

Table 7. Growth rate (measured in length and weight) of unexposed (control) juvenile Pacific flatfishes during 90 day experimental period. All values are expressed as means with standard errors in parentheses. Comparisons between species were carried out by a one-way analysis of variance followed by Student-Newman Keuls multiple comparisons. Groups with the same letter at a given exposure period do not differ significantly (P<0.05).

	Time		
	30d	60d	90d
Treatment Group			
	Growth Rate (length)		
Yellowfin Sole	0.14 (0.02) A	0.26 (.03) C	0.21 (0.02)E
Rock Sole / Mud	0.13 (0.02) A	0.14 (.02) D	0.20 (0.02) E
Rock Sole / Sand	0.11 (0.01) A	0.18 (.02) D	0.19 (0.01) E
Halibut	0.20 (0.01) B	0.23 (.01) C	0.27 (0.01) G
	Growth Rate (weight)		
Yellowfin Sole	0.52 (0.06) A	0.91 (0.14) D	1.08 (0.10) E
Rock Sole / Mud	0.62 (0.10) A	0.63 (0.11) D	1.01 (0.10) E
Rock Sole / Sand	0.75 (0.11) A	0.79 (0.11) D	1.06 (0.12) E
Halibut	1.51 (0.06) B	1.14 (0.05) C	0.91 (0.04) E

decline (Figure 7). Unexposed halibut grew 50-66% faster than the other species during the first 30 days and 20-45% faster during the second 60 days. During the final 30 days of the test, growth rates of unexposed halibut did not differ significantly from the growth rates of unexposed yellowfin sole or rock sole (Table 7).

Substrate did not significantly alter growth rates in rock sole, the only species that was reared on both mud and sand. Specific growth rates (%BWD) for unexposed rock sole in mud and on sand did not differ over 90 days(Table 7). Rock sole on sand at all concentrations of oil did grow slightly faster than rock sole reared on mud but the differences were only significant at the high concentration during the first 30 days and the low concentration during the last 30 days.

3.2.3 Condition Factor

Condition factors, a possible measure of fish fitness (Goede and Barton 1990) were less affected by hydrocarbon exposure than were growth rates (Figure 8). Yellowfin sole were the least affected; condition factors for yellowfin sole were not significantly affected except by exposure for the entire 90 days to 4300 μg/g. Condition factors for halibut were likewise only smaller than control values in fish exposed to the high concentration (4700 μg/g) but the effect became significant after 30 days (Table 5). Reduction relative to the controls in condition factors for rock sole in mud were significant at both the high and low concentrations after 90 days. The small reductions in condition factors for rock sole in sand were significant after 60 days of exposure at both concentrations but not after 90 days.

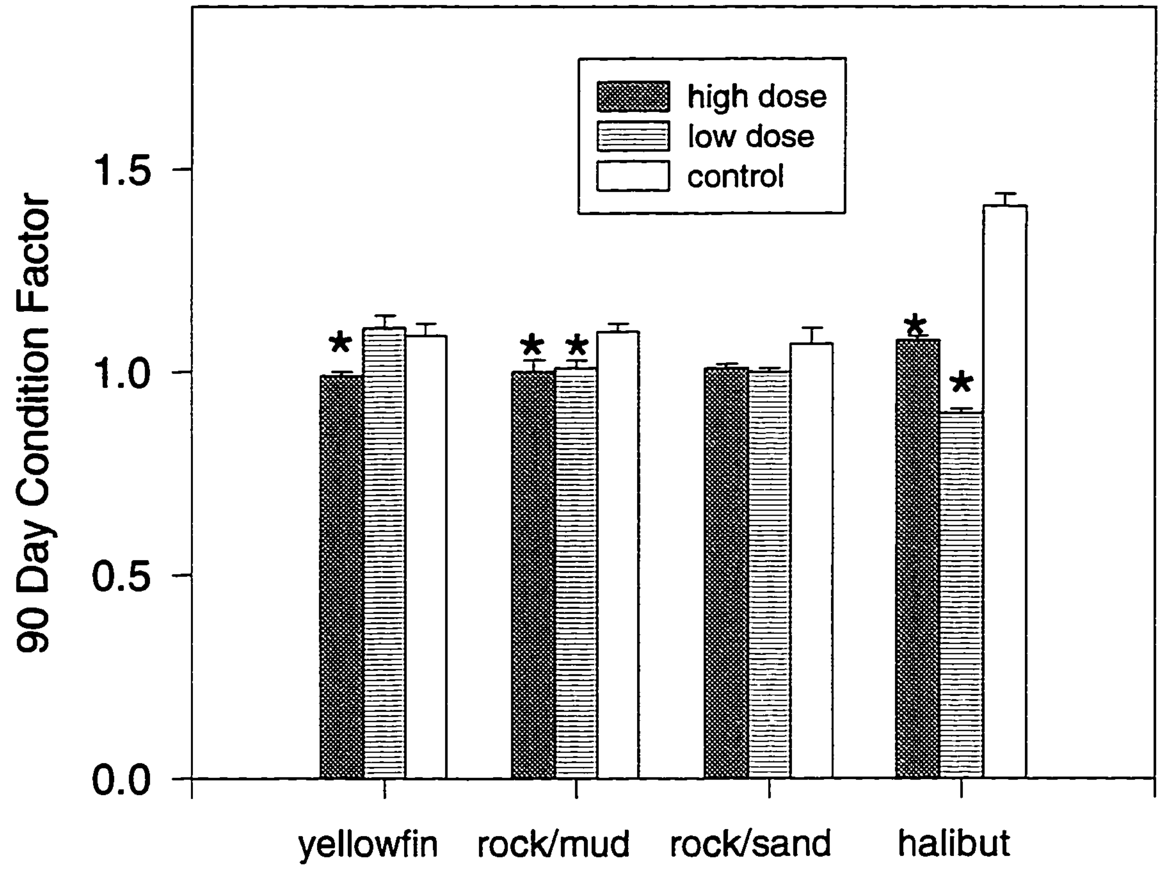

Figure 8. Condition factors of four species of juvenile flatfishes exposed to oiled sediments for 90 days. Asterisks denote significant differences ($P<0.05$) from controls by Dunnett's test.

3.3 Effects on Health Biomarkers

Flatfish exposed to oil had alterations in caudal fin, liver, and gill tissue as well as in parasite prevalences (Table 8). Tissues of juveniles, particularly halibut, were altered by exposure to the high concentration and to a lesser degree by exposure to low concentrations. While halibut had no parasites in either the control or exposed groups, parasite prevalences were altered in yellowfin and rock sole by oil exposure.

Caudal fin erosion occurred in all contaminated tanks but rarely in control tanks and increased in incidence and severity with increased concentration (Table 8). Halibut exposed to oil had the most extreme reactions with total loss of all fins in the highest concentration as early as 60 days. Rock sole and yellowfin sole lost only caudal fin tissue whereas halibut lost dorsal and anal fins as well. Only caudal fin loss after 90 days was quantified. In all cases of erosion, both fin rays and fin tissue was lost. Percent caudal tissue lost ranged from 5% in yellowfin sole at the lowest concentration to 100% in halibut at the high concentration. Erosion was significant for all high concentration exposures as well as for halibut in the low concentration. There were no bacteria or external parasites associated with the eroded areas.

There was little evidence of damage to liver tissues, except in the two specimens of halibut in the high concentration. In the livers of other oil exposed groups, I found only an increase in fat vacuoles or no damage. Livers from halibut in the high concentration stained basophilic with areas of multi-focal coagulative necrosis in the hepatocytes. The necrotic foci had not coalesced but many of the hepatocytes were

Table 8. Effect of 90 day exposure to hydrocarbon contaminated sediment on flatfish health. Parasites are reported as prevalence (no. of fish infected/no. of fish observed), caudal fin erosion as the percent of caudal fin lost ± SE, and macrophage aggregates as the mean number ± SE present in ten fields in liver section.

	Concentration		
	Control	Low	High
Treatment			
	Caudal Fin Erosion		
Yellowfin Sole	0%	5%±2	22%±6 a
Rock Sole / Mud	2%±	5%±2	22%±4 a
Rock Sole / Sand	0%	11%±3	69%±7 a
Halibut	0%	63%±9 a	100%±0 a
	Macrophage Aggregates		
Yellowfin Sole	16.3±0.4	17.6±0.5 b	9.7±0.5 b
Rock Sole / Mud	18.8±0.4	16.9±0.5 b	9.8±0.3 b
Rock Sole / Sand	17.9±0.5	17.3±0.4	11.4±0.4 b
Halibut	19.3±0.4	17.4±0.5 b	6.5±0 b
	Trichodina sp.		
Yellowfin Sole	0/9	10/10 c	8/9 c
Rock Sole / Mud	0/7	5/10 c	4/10
Rock Sole / Sand	1/10	6/8 c	5/9
Halibut	0/10	0/10	0/2
	Digenetic Trematodes		
Yellowfin Sole	4/10	0/10 c	0/10 c
Rock Sole / Mud	7/10	0/10 c	0/10 c
Rock Sole / Sand	5/10	0/10 c	0/10 c
Halibut	0/10	0/10	0/2

a/ significantly different from control by Kruskal-Wallis ANOVA on ranks test
b/ significantly different by Dunnett's test on One-wayANOVA
c/significantlydifferent from control by Fisher's exact test

pyknotic. The majority of the hepatocytes had flattened vacuoles. Livers from halibut exposed to the low concentration as well as livers from rock sole exposed to the high concentration had a few areas of fatty vacuolization but no necrosis. No fatty infiltration of hepatocytes were seen in any other groups. The number of areas of macrophage aggregates was significantly ($P<0.05$) greater in control fish than in oil exposed fish, regardless of species (Table 8). Except for rock sole in sand, the number of aggregates was significantly reduced at the low concentration and was reduced for all groups at the high concentration.

Qualitative examination of histological sections of gill from oil treated fish revealed a consistent pattern of hyperplasia of both primary and secondary lamellae with fusion of the lamellar tips with those of adjacent lamellae in sections taken from all oil exposed fish. There was little evidence of tissue alterations in the control fish with the exception of mild hyperplasia in some sections and there was no separation of respiratory epithelia from underlying support tissue in any fish, oil exposed or control. Hyperplasia was most advanced with more fusion of the distal lamellae in the high concentrations but did not vary between species.

The prevalence of parasites in rock sole and yellowfin sole was significantly altered by oil exposure (Table 8). The percentage of fish infected with the parasitic gill ciliate Trichodina borealis was greater in oil exposed yellowfin sole and rock sole than in the respective unexposed controls, although the increase was not always significant. In contrast, the prevalence of digenetic trematodes was lower in oil exposed fish.

Unexposed yellowfin and rock sole had digenetic trematodes present in the alimentary tract but exposed fish were lacking any parasite fauna in the gut.

4.0 DISCUSSION

Exposure to sediment bound hydrocarbons for as little as 30 days can inhibit growth in juvenile flatfishes. Most of the growth rates for exposed fish were 40-70% of the growth rates for unexposed fish. Exposed fish also had altered gill and liver morphology, increased caudal fin erosion, and changes in parasite prevalences, all evidence of impaired health (Barker et al. 1994).

4.1 Mortality

The mortality of Pacific halibut in the high oil concentration suggests that this species is more sensitive to petroleum hydrocarbons than the other two species. The major loss of fins, reduced growth, liver necrosis, and gill clubbing also point to a higher sensitivity of halibut to oil pollution. In addition, the declining growth rate in the unexposed fish suggests a general decline in halibut health during holding. How much of the observed mortality was due to oil, how much to health problems associated with oil and how much to holding effects is not clear. Mortality would not be expected in short term oil exposures since the flushing of the sediments prior to the addition of animals removes many of the acutely toxic naphthalenes (Paine et al. 1991). However, exposure to oiled sediments is reported to kill both juvenile winter flounder Pleuronectes americanus (Khan 1991) and English sole Parophrys vetulus (McCain and Malins 1982).

4.2 Effects on Growth

The reasons for reduced growth resulting from pollution exposure can range from underfeeding or poor food conversion to disease or increased metabolic demands (Heath 1995). Of these, reductions in feeding have been often cited as the primary reason for reduced growth as a result of exposure to crude oil in: winter flounder (Fletcher et al. 1981), Atlantic cod Gadus morhua (Kiceniuk and Khan 1987), coho salmon Oncorhynchus kisutch adults (Folmar et al. 1981), and pink salmon Oncorhynchus gorbuscha fry (Schwartz 1985). Some of the observed physiological changes accompanying oil exposure of flatfishes such as depleted triglyceride and glycogen levels (Dey et al. 1983) could have resulted from starvation as could some histological changes noted in the present study. Although feeding rate was not measured in my study, the fish appeared to feed actively at all concentrations and stomachs were generally full when sampled for the presence of digenetic trematodes at the end of the study. This, coupled with the robust condition factors suggests that reduced growth cannot be explained entirely by lack of food.

Energetic explanations for depressed growth include reductions in food conversion efficiency (Vignier et al. 1992) or increases in metabolism to detoxify hydrocarbons (Thomas and Rice 1979). Conversion efficiency refers to the percentage of food converted into growth and a decrease implies either reductions in assimilation (gross) or increases in maintenance energy needs (net). If less energy is available, growth will be limited. Winter flounder exposed to sediments containing weathered hydrocarbons experienced loss of lipid stores despite consuming the same amount of food as control fish (Dey et al. 1983). Similarly, growth reductions were noted in oil-exposed pink salmon fry (Carls et al. 1996) and Atlantic salmon Salmo salar (Vignier et al. 1992) despite sustained feeding. Growth rates in these latter two studies were reduced less than 20%. It would be difficult to explain growth reductions of 70% found in the present study by energy loss alone.

The most likely explanation for the growth reductions in this study are a combination of reductions in feeding and conversion coupled with increases in metabolism due to detoxification and impaired health. Without data on feeding and respiration rates, it is impossible to determine the relative importance of each factor.

4.3 Health Biomarkers

Tissue alterations such as fin erosion, liver and gill changes, and decreases in macrophage aggregates were present in flatfish with reduced growth rates due to oil. Severe damage such as total fin loss, liver necrosis, and depletion of macrophage aggregations were associated with moribund halibut. While intriguing, these observations of severe damage in halibut are based on only two animals. As such, they must be viewed with caution as preliminary results. In contrast, partial caudal fin erosion, fat vacuoles in the liver, gill hyperplasia, and declines in macrophage numbers and intestinal parasites were noted in fish with reduced growth. Such tissue changes have been noted for a variety of toxicants in adult fishes and are believed to be a non-specific response to a degraded habitat (Sindermann 1990). The present study supports the hypothesis that the above named alterations are associated with reduced growth in juvenile flatfishes. Whether any of these alterations were responsible for the reduced growth or simply co-occurring is unknown.

The lower growth rates observed in oil exposed fish may have been the direct or indirect result of the observed tissue and parasite alterations. Beyond the obvious loss of fin tissue weight (and by extension total wet weight), tissue damage and increased parasitism can deplete the energy available for growth. For example, fin erosion is thought to be the result of reduced peripheral blood flow (Paine 1988). This, coupled with increased difficulty in swimming, food acquisition, and burial due to fin loss (Sindermann 1990), would require more energy for basic maintenance. It is equally possible that fin loss could result in reduced food intake as well (Murchelano and Ziskkowski 1979) . Additionally, liver vacuolation following hydrocarbon exposure is thought to be the result of depletion of lipid energy reserves needed in synthesizing detoxification enzymes (Dey et al. 1983). The decreased respiratory surface noted in the gill may have contributed to the decreased growth as well. As a major site of hydrocarbon uptake, the hyperplasia and lamellar fusion of the gill noted by several authors (Solangi and Overstreet 1982, Hawkes 1977, Haensly et al. 1982, and Khan and Kiceniuk 1988) would reduce gas and ion exchange and increase stress. These temporary protective mechanisms would result in the expenditure of more energy for maintenance.

Some of these alterations may be more severe in certain species. In the present study, fin erosion was most severe in halibut whereas gill parasitism was not observed in halibut. Some species of flatfishes appear more susceptible than others to fin erosion. Johnson et al. (1988) noted an erosion prevalence of 30% in winter flounder in Boston Harbor whereas Malins et al. 1988) reported lower levels for English sole (0.4%), starry flounder (2.9%) and rock sole (9%) from polluted waterways.

Oil exposure not only reduces growth but affects fish health as well. The increase in gill parasitism and fin erosion, coupled with declines in macrophage aggregations, are all indicators of poor health (Barker et al. 1994). Hydrocarbon exposure results in elevated levels both of cortisol (Pickering 1981) and mixed function oxygenases. Both of these chemicals are capable of suppressing the immune response (Pickering and Pottinger 1989, Hansen et al. 1982, Wojdani and Alfred 1984). Payne and Fancey (1989) hypothesized that low concentrations of oil may activate macrophage activity while higher levels may serve to reduce the number of aggregates as in the present study.

Long-term exposure to crude oil in estuarine sediments is likely to severely inhibit growth and health in juvenile flatfishes. As nearshore residents, juvenile flatfishes are vulnerable to exposure to hydrocarbons. Hydrocarbon concentrations similar to those used in the present study have been reported from the field. Following the *Exxon Valdez* oil spill, intertidal sediment concentrations in 46 of 70 sampled sites were greater than 1600μg/g TPH (Babcock et al. 1996) two years after the spill. Values over 5000μg/g were recorded at 5 beaches. Even low levels of contamination can be of significance if the bioavailability of hydrocarbons is high, as it is for flatfishes (McCain et al. 1978). Additionally, the fish in the present study were fed uncontamined food in the water column but prey items following a spill are likely to be an additional source of hydrocarbons, especially if oiled sediment is also ingested during feeding.

The high vulnerability of flatfish to oiled sediment exposure coupled with their non-avoidance of oil at some concentrations (Moles et al. 1994) makes it likely that juveniles would have reduced growth, survival, and reproduction along with a variety of physical abnormalities following long-term oil exposure. The high incidence of environmentally induced neoplasms (Malins et al. 1988) noted in flatfish from hydrocarbon polluted estuaries may be the result of non-avoidance of pollutants by these species. Flatfish remain buried in the sediment to avoid predators, emerging only to forage, and are likely to remain buried during intervals of low prey availability (Tanda 1990). The lower condition factors observed for oil-exposed flatfishes suggests lower fat reserves. Some species of juvenile flatfishes such as winter flounder spend the late summer and early fall increasing weight with little gain in length as a prelude to the winter non-feeding period (Fletcher et al. 1981). An inability to grow and store enough energy reserves could prove deleterious for survival during intervals when body reserves are not being replenished (Pearson et al. 1984).

In summary, exposure of juvenile flatfishes in the laboratory to concentrations of 1600 μg/g TPH for 30 days has the potential to greatly inhibit growth rates, particularly in Pacific halibut. Such reductions in growth rates were associated with alterations in liver and gill tissues as well as parasite burdens that serve as biomarkers of petroleum exposure. As long as oil is transported, toxic hydrocarbons will be released into the environment. While some researchers feel that oil pollution is not a threat to marine fisheries (McIntyre 1982), the potential for damage to juvenile flatfishes living in polluted sediments is real. Further work is needed to determine the lowest effective concentration that will reduce growth rates in juvenile flatfishes and what biomarkers will best predict this lowest concentration.

5.0 REFERENCES

Ariese, F., S.J. Kok, M. Verkaik, C. Gooijer, N.H.Velthorst, and J.W. Hofstraat, 1993. Synchronous fluorescence spectrometry of fish bile: a rapid screening method for the biomonitoring of PAH exposure.-- Aquatic Toxicolicology 26: 273-286.

Babcock, M.M., G. Irvine, P.M. Harris, J.A. Cusick, and S.D. Rice, 1996. Persistence of oiling in mussel beds three and four years after the *Exxon Valdez* oil spill.--American Fisheries Society 18: 286-297.

Barker, D.E., R.A. Khan and R. Hooper, 1994. Bioindicators of stress in winter flounder, Pleuronectes americanus, captured adjacent to a pulp and paper mill in St. George's Bay, Newfoundland.--Canadian Journal of Fisheries and Aquatic Sciences 51: 2203-2209.

Brannes, E. and A. Alanara, 1993. Monitoring the feeding activity of individual fish with a demand feeding system.-- Journal of Fish Biology 42:209-215.

Carls, M.G., L. Holland, M. Larsen, J.L. Lum, D.G. Mortensen, S.Y. Wang, and A.C. Wertheimer, 1996. Growth, feeding, and survival of pink salmon fry exposed to food contaminated with crude oil. American Fisheries Society Symposium 18: 608-618.

Dey, A.C., J.W. Kiceniuk, U.P. Williams, R.A. , and J.F. Payne, 1983. Long term exposure of marine fish to crude petroleum. I. Studies on liver lipids and fatty acids in cod Gadus morhua and winter flounder Pseudopleuronectes americanus.--Comparative Biochemistry and Physiology 75: 93-101.

FAO, 1995. Review of the State of World Fishery Resources: Marine Fisheries.--FAO Fisheries Circular 884: 1-105.

Fletcher, G.L., J.W. Kiceniuk, and U.P. Williams, 1981. Effects of oiled sediments on mortality, feeding and growth of winter flounder Pseudopleuronectes americanus.-- Marine Ecology Progress Series 4: 91-96.

Folmar, L.C., D.R. Craddock, J.W. Blackwell, G. Joyce, and H.O. Hodgins, 1981. Effects of petroleum exposure on predatory behavior of coho salmon (Oncorhynchus kisutch).-- Bulletin of Environmental Contamination and Toxicology 27: 458-462.

Fonds, M., M. Tanaka, and H.W. van der Veer, 1995. Feeding and growth of juvenile Japanese flounder Paralichthys olivaceus in relation to temperature and food supply.--Netherlands Journal of Sea Research 34: 111-118.

Goede, R.W. and B.A. Barton, 1990, Organismic indices and an autopsy-based assessment as indicators of health and condition of fish--American Fisheries Society Symposium 8: 93-108.

Gundlach, E.R., P.D. Boehm, M. Marchand, R.M. Atlas, D.M. Ward, and D.A. Wolfe, 1983. The fate of Amoco Cadiz oil.--Science (Washington, D.C.) 221: 122-129.

Haensly, W.E., J.M. Neff, J.R. Sharp, A.C. Morris, M.F. Bedgood and P.D. Boem, 1982. Histopathology of Pleuronectes platessa L. from Aber Wrac'h and Aber Benoit, Brittany, France: long-term effects of the *Amoco Cadiz* crude oil spill-- Journal of Fish Diseases 5: 365-391.

Hansen, M.A., G. Ferandes and R.A. Good, 1982. Nutrition and immunity.--Annual Review of Nutrition 2: 151-177.

Hawkes, J.W., 1977. The effects of petroleum hydrocarbon exposure on the structure of fish tissues. In: D.A. Wolfe. Fate and Effects of Petroleum Hydrocarbons in Marine Organisms and Ecosystems. Pergamon Press, NY: 115-128.

Heath, A.G., 1995. Water Pollution and Fish Physiology, Second Edition. Lewis Publishers, Boca Raton, Florida: 1-400.

Hicks, G.R.F., 1984. Spatio-temporal dynamics of a meiobenthic copepod and the impact of predation-disturbance.--Journal of Experimental Marine Biology and Ecology 81: 47-72.

Hinton, D.E. and D.J. Lauren, 1990. Integrative histopathological approaches to detecting effects of environmental stressors on fishes.--American Fisheries Society Symposium 8: 51-66.

Johnson, L.L., E. Casillas, T.K. Collier, B.B. McCain, and U. Varanasi, 1988. Contaminant effects on ovarian development in English sole Parophrys vetulus from Puget Sound, Washington.--Canadian Journal of Fisheries and Aquatic Sciences 45: 2133-2146.

Johnson, L.L., C.M. Stehr, O.P. Olson, M.S. Myers, S.M. Pierce, B.B. McCain, and U. Varanasi, 1992. Histopathology and relationships between lesions and chemical contaminants (1979-89. National Oceanic and Atmospheric Administration Technical Memorandum NMFS-NWFSC-4: 1-96.

Khan, R.A., 1991. Influence of concurrent exposure to crude oil and infection with Trypanosoma murmanensis (Protozoa: Mastigophora) on mortality in winter flounder, Pseudopleuronectes americanus.--Canadian Journal of Zoology 69: 876-880.

Khan, R.A. and J.W. Kiceniuk, 1988. Effect of petroleum aromatic hydrocarbons on monogeneids parasitizing Atlantic cod, Gadus morhua L.--Bulletin of Environmental Contamination and Toxicology 41: 94-100.

Kiceniuk, J.W. and R.A. Khan, 1987. Effect of petroleum hydrocarbons on Atlantic cod, Gadus morhua, following chronic exposure.--Canadian Journal of Zoology 65: 490-494.

Krahn, M.M.,G.M. Ylitalo, J. Buzitis, S.-L. Chan, U. Varanasi, T.L. Wade, T.J. Jackson, J.M. Brooks, D.A. Wolfe, and C.-A. Manen, 1993. Comparison of high-performance liquid chromatography/fluorescence screening and gas chromatography /mass spectrometry analysis for aromatic compounds in sediments after the *Exxon Valdez* oil spill.--Environmental Science and Technology 27: 699-708.

Krahn, M.M., G.M. Ylitalo, J. Joss, and S.-L. Chan, 1991. Rapid, semi-quantitative screening of sediments for aromatic hydrocarbons using sonic extraction and HPLC/fluorescence analysis.--Marine Environmental Research 31: 175-196.

Levene, H., 1960. Robust tests for equality of variances. In: I. Olkins, editor. Contributions to Probability and Statistics. Stanford University Press, Stanford, California: 278-292.

McCain, B.B., H.O. Hodgins, W.D. Gronlund, J.W. Hawkes, D.W. Brown, M.S. Myers and J.H. Vandermeulen, 1978. Bioavailability of crude oil from experimentally oiled sediments to English sole Parophrys vetulus, and pathological consequences.--Journal of the Fisheries Research Board of Canada 35: 657-664.

McCain, B.B., and D.C. Malins, 1982. Effects of petroleum hydrocarbons on selected demersal fishes and crustaceans. Ecological Stress and the New York Bight: Science and Management. United States Department of Commerce, National Technical Information Service, PB84-190909: 315-325.

Malins, D.C., B.B. McCain, J.T. Landahl, M.S. Myers, M.M. Krahn, D.W. Brown, S.-L. Chan, and W.T. Roubal, 1988. Neoplastic and other diseases in fish in relation to toxic chemicals: an overview.--Aquatic Toxicology 11: 43-67.

McIntyre, A.D., 1982. Oil pollution and fisheries.-- Philosophical Transactions of the Royal Society of London B 297: 401-411.

Moles, D.A., S. Bates, S.D. Rice, and S. Korn, 1981. Reduced growth of coho salmon fry exposed to two petroleum components, toluene and naphthalene, in fresh water.-- Transactions of the American Fisheries Society 110:430-436.

Moles, A. and B.L. Norcross, 1995. Sediment preference in juvenile Pacific flatfishes.-- Netherlands Journal of Sea Research 34: 177-182.

Moles, A., P. Rounds, and C. Kondzela, 1990. Use of the brain parasite Myxobolus neurobius in separating mixed stocks of sockeye salmon.-American Fisheries Society Symposium 7: 224-231.

Moles, D.A. and S.D. Rice, 1983. Effects of crude oil and naphthalene on growth, caloric content, and fat content of pink salmon juveniles in seawater.--Transactions of the American Fisheries Society 112: 205-211.

Moles, D.A., S.D. Rice, and B.L. Norcross, 1994. Non-avoidance of hydrocarbon laden sediments by juvenile flatfishes.--Netherlands Journal of Sea Research 32: 361-367.

Monoson, E. and J.J. Stegeman, 1994. Induced cytochrome P4501A in winter flounder, Pleuronectes americanus, from offshore and coastal sites.--Canadian Journal of Fisheries and Aquatic Sciences 51: 933-941.

Murchelano, R.A. and J. Ziskowski. 1979. Fin rot disease in the New York Bight (1973-1977). Ecological Stress and the New York Bight: Science and Management. United States Department of Commerce, National Technical Information Service, PB84-190909: 347-354.

Myers, M.S., J.T. Landahl, M.M. Krahn, and B.B. McCain, 1991. Relationships between hepatic neoplasms and related lesions and exposure to toxic chemicals in marine fish from the U.S. West Coast.--Environmental Health Perspectives 90: 7-15.

NPFMC, 1993. Management Plan for Bering Sea Groundfish. North Pacific Fisheries Management Council, Anchorage, Alaska: 1-76.

Norcross, B.L., B.A. Holladay, and F.J. Muter, 1995. Nursery area characteristics of pleuronectids in coastal Alaska, USA.--Netherlands Journal of Sea Research 34:161-175.

O'Clair, C.E., J.W. Short and S.D. Rice, 1996. Contamination of intertidal and subtidal sediments by oil from the *Exxon Valdez* in Prince William Sound, Alaska.--American Fisheries Society Symposium 18: 61-93.

Paine, J.S., 1988. Fin erosion in fishes.--United States Deptartment of Commerce Technical Memorandum: 88-159.

Paine, M.D., W.C. Leggett, J.K. McRuer, and K.T. Frank, 1991. Effects of incubation in oiled sediment on emergence of capelin Mallotus villosus larvae.--Canadian Journal of Fisheries and Aquatic Sciences 48: 2228-2239.

Payne, J.F. and L.F. Fancey, 1989. Effect of polycyclic aromatic hydrocarbons on immune responses in fish: change in melanomacrophage centers in flounder Pseudopleuronectes americanus exposed to hydrocarbon-contaminated sediments.--Marine Environmental Research 28: 431-435.

Pearson, W.H., D.L. Woodruff, P.C. Sugarman, and B.L. Olla, 1984. The burrowing behavior of sand lance, Ammodytes hexapterus: effects of oil-contaminated sediment.--Marine Environmental Research 11:17-32.

Pickering, A.D., 1981. Stress and Fish. Academic Press, London: 1-367.

Pickering, A.D. and T.G. Pottinger, 1989. Stress responses and disease resistance in salmonid fish: effects of chronic elevation of plasma cortisol levels.--Fish Physiology and Biochemistry 7: 253-258.

Schwartz, J.P., 1985. Effect of oil contaminated prey on the feeding and growth rate of pink salmon fry Oncorhynchus gorbuscha. In: F.J. Vernberg, F.P. Thurberg, A. Calabrese, and W.B. Vernberg. Marine Pollution and Physiology: Recent Advances. University of South Carolina Press, Columbia, South Carolina: 459-476.

Sindermann, C.J., 1990. Principal Diseases of Marine Fish and Shellfish, Vol. I, 2nd Edition. Academic Press, N.Y.: 1-319.

Solangi, M.A. and R.M. Overstreet, 1982. Histopathological changes in two estuarine fishes, Menidia beryllina (Cope)and Trinectes maculatus (Bloch and Schneider) exposed to crude oil and its water-soluble fractions.--Journal of Fish Diseases 5: 13-35.

Spies, R., D.W. Rice, P.A. Montagna and R.R. Ireland, 1985. Reproductive success, xenobiotic contaminants and hepatic mixed function oxidase (MFO) activity in Platichthys stellatus populations from San Francisco Bay.--Marine Environmental Research 17: 117-121.

Stevens, M.A., 1974. EDF statistics for goodness of fit and some comparisons.--Journal of the American Statistical Association 75: 74-81

Tanda, M., 1990. Studies on burying ability in sand and selection to the grain size for hatchery reared marbled sole and Japanese flounder.-Journal of the Japanese Society of Grassland Sciences 56: 1543-1548.

Thedinga, J.F. and S.W. Johnson, 1995. Retention of jet-injected marks on juvenile coho and sockeye salmon.--Transactions of the American Fisheries Society 124: 8782-785.

Thomas, R.E. and S.D. Rice, 1979. The effect of exposure temperatures on oxygen consumption and opercular breathing rates of pink salmon fry exposed to toluene, naphalene, and water-soluble fractions of Cook Inlet crude oil and no.2 fuel oil. In: W.B. Vernberg, A. Calabrese, F.P. Thurberg, and F.J. Vernberg. Marine Pollution: Functional Responses. Academic Press, New York: 39-52.

Truscott, B., D.R. Idler, and G.L. Fletcher, 1992. Alteration of reproductive steroids of male winter flounder Pleuronectes americanus chronically exposed to low levels of crude oil in sediments.--Canadian Journal of Fisheries and Aquatic Sciences 49: 2190-2195.

Van der Veer, H.W., R. Berghahn, and A. Rijnsdorp, 1994. Impact of juvenile growth on recruitment in flatfish.-- Netherlands Journal of Sea Research 32:153-173.

Vignier, V., J.H. Vandermeulen, and A.J. Fraser, 1992. Growth and food conversion by Atlantic salmon parr during 40 days' exposure to crude oil.--Transactions of the American Fisheries Society 121: 322-332.

Winer, B.J., 1962. Statistical principles in experimental design.-McGraw-Hill, New York.

Wojdani, A. and Alfred, L.J., 1984. Alterations in cell-mediated immune functions induced in mouse splenic lymphocytes by polycyclic aromatic hydrocarbons.--Cancer Research 44: 942-945.

Woodward, D.F., P.M. Mehrle, Jr., and W.L. Mauck, 1981. Accumulation and sublethal effects of a Wyoming crude oil in cutthroat trout.--Transactions of the American Fisheries Society 110: 437-445.

CHAPTER 5

Conclusions

The extent to which juvenile flatfishes exposed to oiled sediment in the nearshore environment would be affected depends on the species, substrate and concentration. Unlike pelagic fishes which actively avoid hydrocarbons in the water column and are readily affected by oil if confined in an oiled environment, flatfishes bury in oiled sediments and do not avoid low concentration. Flatfishes will, in fact, select oiled sediments if the sediment is of a more preferred grain size than the unoiled sediment. Once exposed, juvenile flatfishes have the potential for large reductions in growth rate and impairments in health.

The foregoing results demonstrate that juvenile Pacific flatfishes actively select certain grain sizes for burying, often in spite of the presence of oil. Despite the many substrates present in the bays and estuaries of Alaska, mud and sand are consistently selected. While field sediments are not likely to be of a single grain size, the pattern of selected fine grain sediments is clear. The reasons for this selection could include ease of burial or presence of preferred prey (Gibson and Robb 1992).

This preferred sediment selection plays a strong role in the response of flatfish to oil. Flatfishes choose to avoid oiled sediments and bury in clean sediments of the same type. If the preferred sediment is oiled and a less preferred but clean sediment is the alternative, flatfish choose the preferred sediment. Only at concentrations of oil near maximum saturation was avoidance of preferred sediment noted. Thus, flatfish are not apt to avoid oiled sediments and would be subjected to chronic exposure.

If oiled sediments are selected due to grain size or simply the lack of alternative habitat, growth rate reductions coupled with alterations in tissue structure and parasite prevalence are likely to occur. While more work is necessary to establish the lowest effective concentration that will affect growth, the presence of tissue alterations indicates the potential for other physiological effects as well. The high incidence of environmentally induced neoplasms (Malins et al. 1988) noted in flatfish from polluted estuaries may be the result of non-avoidance of pollutants by these species over many years.

Halibut are likely to be much more affected by oil exposure than are either yellowfin sole or rock sole. Not only were they killed by oil but they had the strongest inhibition of growth as well. Halibut, however, are less likely to selected oiled sediment because of substrate type. If unoiled substrate less than 500 μm in diameter is available, halibut are likely to choose it over heavily oiled sediment. Halibut did not avoid oil at most concentrations tested. Because yellowfin sole and rock sole are likely to select oiled habitat if the sediment type is right, these species are quite likely to be subject to reduced growth and tissue alterations as a result of exposure.

Prolonged contact with oiled sediments, especially over winter, could result in smaller individuals with lower energy reserves in the spring (Pearson et al. 1984).

Conversely, the relative allocation of burial and foraging time during the spring and summer months is likely to be affected by reduced energy reserves (Olla et al. 1980). Growth during the initial year following settlement is likely to be critical to subsequent survival (Gibson 1994). Pacific flatfishes experience their most rapid growth during the juvenile phase (Paul et al. 1994, Smith et al. 1995) and any reduction during juvenile growth would prolong the length of the juvenile stage. Mortality during this phase as well as the onset of maturation are directly determined by fish size (Zilstra et al. 1982, Rijnsdorp 1993). Predation decreases with increasing fish size, thus survival is directly related to growth (Witting and Able 1993, van der Veer et al. 1994). If exposure to oil inhibits their growth, these fish would be more susceptible to predation and might compete less successfully for food than larger fish. Slow growing plaice reach maturity at a higher age than fast growing plaice, suggesting later recruitment to the reproductive pool (Rijnsdorp 1993).

5.0 REFERENCES

FAO, 1995. Review of the State of World Fishery Resources: Marine Fisheries.--FAO Fisheries Circular 884, 1-105.

Fletcher, G.L., J.W. Kiceniuk, and U.P. Williams, 1981. Effects of oiled sediments on mortality, feeding and growth of winter flounder Pseudopleuronectes americanus.--Marine Ecology Progress Series 4: 91-96.

Gibson, R.N., 1994. Impact of habitat quality and quantity on the recruitment of juvenile flatfishes.--Netherlands Journal of Sea Research 32:191-206.

Gibson, R.N. and L. Robb, 1992. The relationship between body size, grain size, and the burying ability of juvenile plaice, Pleuronectes platessa L.--Journal of Fish Biology 40: 771-778.

Gundlach, E.R., P.D. Boehm, M. Marchand, R.M. Atlas, D.M. Ward, and D.A. Wolfe, 1983. The fate of Amoco Cadiz oil.--Science (Washington, D.C.) 221: 122-129.

Haensly, W.E., J.M. Neff, J.R. Sharp, A.C. Morris, M.F. Bedgood and P.D. Boem, 1982. Histopathology of Pleuronectes platessa L. from Aber Wrac'h and Aber Benoit, Brittany, France: long-term effects of the Amoco Cadiz crude oil spill.--Journal of Fish Diseases 5: 365-391.

Khan, R.A. and J. Thulin, 1991. Influence of pollution on parasites of aquatic animals.--Advances in Parasitology 30: 201-238.

Malins, D.C., 1982. Alterations in the cellular and subcellular structure of marine teleosts and invertebrates exposed to petroleum in the laboratory and field: a critical review.--Canadian Journal of Fish and Aquatic Sciences 39: 877-889.

Malins, D.C., B.B. McCain, J.T. Landahl, M.S. Myers, M.M. Krahn, D.W. Brown, S.-L. Chan, and W.T. Roubal, 1988. Neoplastic and other diseases in fish in relation to toxic chemicals: an overview.--Aquatic Toxicology 11: 43-67.

McCain, B.B., and D.C. Malins, 1982. Effects of petroleum hydrocarbons on selected demersal fishes and crustaceans. Ecological Stress and the New York Bight: Science and Management.--United States Department of Commerce, National Technical Information Service PB84-190909: 315-325.

Mix, M.C., 1986. Cancerous diseases in aquatic animals and their association with environmental pollutants: a critical literature review.--Marine Environmental Research 20: 1-141.

Myers, M.S., J.T. Landahl, M.M. Krahn, and B.B. McCain, 1991. Relationships between hepatic neoplasms and related lesions and exposure to toxic chemicals in marine fish from the U.S. West Coast.--Environmental Health Perspectives. 90:7-15.

O'Clair, C.E., J.W. Short and S.D. Rice, 1996. Contamination of subtidal sediments by oil from the *Exxon Valdez* in Prince William Sound, Alaska.--American Fisheries Society 18: 61-93.

Olla, B.L., W.H. Pearson, and A.L. Studholme, 1980. Applicability of behavioral measures in environmental stress assessment.--Rapports et Proces-verbaux des Reunions Conseil International pour l'Exploration de la Mer 179: 162-173.

Overstreet, R.M., 1988. Aquatic pollution problems, southeastern U.S. coasts: histopathological indicators.-- Aquatic Toxicology 11: 213-239.

Paul, A.J., J.M. Paul, and R.L. Smith, 1994. Energy and ration requirements of juvenile Pacific halibut Hippoglossus stenolepis based on energy consumption and growth rates.-- Journal of Fish Biology 44:1023-1031.

Pearson, W.H., D.L. Woodruff, P.C. Sugarman, and B.L. Olla, 1984. The burrowing behavior of sand lance, Ammodytes hexapterus: effects of oil-contaminated sediment.-- Marine Environmental Research 11:17-32.

Rijnsdorp, A.D., 1993. Relation between juvenile growth and the onset of sexual maturity of female North Sea plaice, Pleuronectes platessa L.--Canadian Journal of Fisheries and Aquatic Sciences 50: 1617-1631.

Smith, R.L., A.J. Paul, and J.M. Paul, 1995. Minimal food requirements for yellowfin sole in Alaska: estimates from laboratory bioenergetics.--Proceedings of the International

Symposium on North Pacific Flatfish, Alaska Sea Grant College Program, AK-SG-95-04: 285-295.

Truscott, B., D.R. Idler, and G.L. Fletcher, 1992. Alteration of reproductive steroids of male winter flounder Pleuronectes americanus chronically exposed to low levels of crude oil in sediments.--Canadian Journal of Fisheries and Aquatic Sciences 49: 2190-2195.

Van der Veer, H.W., R. Berghahn, and A. Rijnsdorp, 1994. Impact of juvenile growth on recruitment in flatfish.--Netherlands Journal of Sea Research 32:153-173.

Witting, D.A. and K.W. Able,1993. Effect of body size on probability of predation for juvenile summer and winter flounder based on laboratory experiments.--Fishery Bulletin(United States) 91: 577-581.

Zilstra, J.J., R. Dapper, and J.IJ. Witte, 1982. Settlement, growth and mortaility of post-larval plaice Pleuronectes platessa L.) in the Western Wadden Sea.--Netherlands Journal of Sea Research 15: 250-272.

Appendix One. Raw Data. Non-avoidance of Oiled Sediment (Chapter Three)

yellow=1 rock=2 halibut=3	high=4 med=3 low=2 cont=1	mud=1 sand=2	number choosing oil	mud-1 sand=2 gravel=3	number choosing unoiled
species	oil level	oiled substrate	number oiled	unoiled substrate	number unoiled
2	4	2	33	1	27
2	3	2	40	1	20
2	2	2	42	1	18
2	1	2	48	1	12
2	4	1	0	2	60
2	3	1	0	2	60
2	2	1	6	2	54
2	1	1	0	2	60
1	4	2	6	1	54
1	3	2	6	1	54
1	2	2	6	1	54
1	1	2	3	1	57
1	4	1	26	2	34
1	3	1	36	2	24
1	2	1	36	2	24
1	1	1	48	2	12
3	4	2	24	1	36
3	3	2	28	1	32
3	2	2	26	1	34
3	1	2	33	1	27
3	4	1	21	2	39
3	3	1	23	2	37
3	2	1	29	2	31
3	1	1	27	2	33
2	4	1	20	1	40
2	3	1	27	1	33
2	2	1	31	1	29
2	1	1	29	1	31
2	4	2	25	2	35
2	3	2	16	2	44
2	2	2	28	2	32
2	1	2	29	2	31
1	4	1	24	1	36
1	3	1	29	1	31
1	2	1	39	1	21
1	1	1	24	1	36
1	4	2	27	2	33
1	3	2	9	2	51
1	2	2	23	2	37
1	1	2	27	2	33
3	4	1	20	1	40
3	3	1	27	1	33
3	2	1	29	1	31
3	1	1	28	1	32
3	4	2	12	2	48
3	3	2	33	2	27
3	2	2	24	2	36
3	1	2	28	2	32
2	4	1	60	3	0
2	3	1	60	3	0

yellow=1 rock=2 halibut=3	high=4 med=3 low=2 cont=1	mud=1 sand=2	number choosing oil	mud-1 sand=2 gravel=3	number choosing unoiled
species	oil level	oiled side substrate	number oiled	unoiled substrate	number unoiled
2	2	1	60	3	0
2	1	1	60	3	0
2	4	2	60	3	0
2	3	2	60	3	0
2	2	2	60	3	0
2	1	2	60	3	0
1	4	1	60	3	0
1	3	1	54	3	6
1	2	1	60	3	0
1	1	1	60	3	0
1	4	2	60	3	0
1	3	2	60	3	0
1	2	2	57	3	3
1	1	2	60	3	0
3	4	1	53	3	7
3	3	1	60	3	0
3	2	1	60	3	0
3	1	1	60	3	0
3	4	2	60	3	0
3	3	2	60	3	0
3	2	2	60	3	0
3	1	2	60	3	0

Appendix Two. Raw Data. Effects of Oiled Sediment on Growth (Chapter Four)

	yellow=1 rock=2 halibut=3	mud=1 sand=2	high=3 lo=2 cont=1	in days	mm	g		growth rate length	growth rate weight
tank no	species	substrate	oil level	time	length	weight	condition	GrowthL	GrowthW
5	1	1	3	0	58	2.19	1.123	0	0
5	1	1	3	0	55	1.88	1.131	0	0
5	1	1	3	0	59	2.00	0.974	0	0
5	1	1	3	0	68	2.76	0.878	0	0
5	1	1	3	0	94	8.39	1.010	0	0
5	1	1	3	0	64	3.13	1.194	0	0
5	1	1	3	0	103	10.57	0.967	0	0
5	1	1	3	0	56	1.78	1.012	0	0
5	1	1	3	0	87	7.20	1.093	0	0
5	1	1	3	0	111	12.92	0.945	0	0
19	1	1	3	0	63	2.44	0.974	0	0
19	1	1	3	0	56	1.95	1.113	0	0
19	1	1	3	0	66	3.19	1.110	0	0
19	1	1	3	0	61	2.57	1.130	0	0
19	1	1	3	0	104	13.37	1.189	0	0
19	1	1	3	0	60	2.27	1.051	0	0
19	1	1	3	0	99	10.67	1.100	0	0
19	1	1	3	0	61	2.63	1.159	0	0
19	1	1	3	0	74	3.99	0.983	0	0
19	1	1	3	0	66	3.11	1.082	0	0
mean					73	4.95	1.061	0	0
variance					353	15.82	0.008	0	0
se					4	0.89	0.020	0	0
14	1	1	2	0	58	2.13	1.093	0	0
14	1	1	2	0	63	2.60	1.041	0	0
14	1	1	2	0	56	1.82	1.035	0	0
14	1	1	2	0	59	2.21	1.078	0	0
14	1	1	2	0	99	9.79	1.008	0	0
14	1	1	2	0	79	5.56	1.128	0	0
14	1	1	2	0	111	12.40	0.907	0	0
14	1	1	2	0	61	2.53	1.116	0	0
14	1	1	2	0	79	5.39	1.092	0	0
14	1	1	2	0	71	3.48	0.971	0	0
22	1	1	2	0	59	2.32	1.129	0	0
22	1	1	2	0	54	1.71	1.084	0	0
22	1	1	2	0	65	2.90	1.056	0	0
22	1	1	2	0	63	2.71	1.084	0	0
22	1	1	2	0	86	6.76	1.062	0	0
22	1	1	2	0	68	3.24	1.029	0	0
22	1	1	2	0	110	14.68	1.103	0	0
22	1	1	2	0	59	2.15	1.045	0	0
22	1	1	2	0	93	9.21	1.145	0	0
22	1	1	2	0	81	5.44	1.024	0	0
mean					74	4.95	1.061	0	0
variance					320	14.31	0.003	0	0
se					4	0.85	0.013	0	0

	yellow=1	mud=1	high=3	in days	mm	mg		growth	growth
	rock=2	sand=2	lo=2					rate	rate
	halibut=3		cont=1					length	weight
tank no	species	substrate	oil level	time	length	weight	condition	GrowthL	GrowthW
9	1	1	1	0	58	1.53	0.783	0	0
9	1	1	1	0	66	2.50	0.870	0	0
9	1	1	1	0	55	1.73	1.037	0	0
9	1	1	1	0	61	2.28	1.003	0	0
9	1	1	1	0	71	3.92	1.094	0	0
9	1	1	1	0	74	3.82	0.941	0	0
9	1	1	1	0	101	11.05	1.073	0	0
9	1	1	1	0	69	3.18	0.967	0	0
9	1	1	1	0	104	10.65	0.947	0	0
9	1	1	1	0	68	3.26	1.037	0	0
20	1	1	1	0	56	1.95	1.112	0	0
20	1	1	1	0	59	2.25	1.097	0	0
20	1	1	1	0	49	1.16	0.982	0	0
20	1	1	1	0	61	1.91	0.841	0	0
20	1	1	1	0	76	4.92	1.121	0	0
20	1	1	1	0	63	3.06	1.223	0	0
20	1	1	1	0	101	11.16	1.083	0	0
20	1	1	1	0	96	10.29	1.163	0	0
20	1	1	1	0	71	4.14	1.156	0	0
20	1	1	1	0	62	2.78	1.165	0	0
mean					71	4.38	1.035	0	0
variance					274	11.72	0.014	0	0
se					4	0.77	0.026	0	0

10	2	1	3	0	49	1.32	1.124	0	0
10	2	1	3	0	57	2.09	1.128	0	0
10	2	1	3	0	59	2.22	1.079	0	0
10	2	1	3	0	61	2.44	1.075	0	0
10	2	1	3	0	64	2.80	1.068	0	0
10	2	1	3	0	101	10.15	0.985	0	0
10	2	1	3	0	91	8.31	1.102	0	0
10	2	1	3	0	60	2.28	1.056	0	0
10	2	1	3	0	71	3.83	1.071	0	0
10	2	1	3	0	70	3.65	1.064	0	0
21	2	1	3	0	59	1.97	0.957	0	0
21	2	1	3	0	58	1.87	0.958	0	0
21	2	1	3	0	48	1.16	1.047	0	0
21	2	1	3	0	58	1.90	0.972	0	0
21	2	1	3	0	72	4.56	1.220	0	0
21	2	1	3	0	87	6.77	1.028	0	0
21	2	1	3	0	80	5.62	1.097	0	0
21	2	1	3	0	66	3.03	1.053	0	0
21	2	1	3	0	110	13.61	1.022	0	0
21	2	1	3	0	71	3.82	1.066	0	0
mean					70	4.17	1.059	0	0
variance					276	10.62	0.004	0	0
se					4	0.73	0.014	0	0

	yellow=1 rock=2 halibut=3	mud=1 sand=2	high=3 lo=2 cont=1	in days	mm	mg		growth rate length	growth rate weight
tank no	species	substrate	oil level	time	length	weight	condition	GrowthL	GrowthW
12	2	1	2	0	51	1.39	1.045	0	0
12	2	1	2	0	55	1.70	1.022	0	0
12	2	1	2	0	55	1.72	1.031	0	0
12	2	1	2	0	51	1.38	1.041	0	0
12	2	1	2	0	59	2.43	1.184	0	0
12	2	1	2	0	63	2.48	0.993	0	0
12	2	1	2	0	68	3.39	1.079	0	0
12	2	1	2	0	76	5.01	1.140	0	0
12	2	1	2	0	110	15.12	1.136	0	0
12	2	1	2	0	82	6.75	1.223	0	0
17	2	1	2	0	56	1.73	0.986	0	0
17	2	1	2	0	61	2.15	0.948	0	0
17	2	1	2	0	51	1.36	1.025	0	0
17	2	1	2	0	58	1.79	0.915	0	0
17	2	1	2	0	100	11.09	1.109	0	0
17	2	1	2	0	106	14.56	1.222	0	0
17	2	1	2	0	59	2.08	1.013	0	0
17	2	1	2	0	56	1.92	1.092	0	0
17	2	1	2	0	96	9.46	1.069	0	0
17	2	1	2	0	60	2.37	1.099	0	0
mean					69	4.49	1.069	0	0
variance					377	19.97	0.007	0	0
se					4	1.00	0.019	0	0
15	2	1	1	0	53	1.42	0.956	0	0
15	2	1	1	0	51	1.34	1.006	0	0
15	2	1	1	0	46	0.91	0.937	0	0
15	2	1	1	0	46	0.92	0.942	0	0
15	2	1	1	0	97	10.70	1.172	0	0
15	2	1	1	0	64	2.95	1.125	0	0
15	2	1	1	0	109	12.79	0.988	0	0
15	2	1	1	0	64	2.88	1.097	0	0
15	2	1	1	0	60	2.29	1.060	0	0
15	2	1	1	0	58	1.94	0.993	0	0
24	2	1	1	0	51	2.00	1.508	0	0
24	2	1	1	0	72	4.16	1.114	0	0
24	2	1	1	0	46	0.96	0.981	0	0
24	2	1	1	0	51	1.22	0.919	0	0
24	2	1	1	0	66	2.95	1.026	0	0
24	2	1	1	0	60	2.17	1.004	0	0
24	2	1	1	0	96	9.13	1.032	0	0
24	2	1	1	0	70	3.40	0.991	0	0
24	2	1	1	0	92	8.60	1.104	0	0
24	2	1	1	0	61	2.51	1.104	0	0
mean					66	3.76	1.053	0	0
variance					350	12.57	0.017	0	0
se					4	0.79	0.029	0	0

	yellow=1 rock=2 halibut=3	mud=1 sand=2	high=3 lo=2 cont=1	in days	mm	mg		growth rate length	growth rate weight
tank no	species	substrate	oil level	time	length	weight	condition	GrowthL	GrowthW
13	2	2	3	0	55	1.66	0.997	0	0
13	2	2	3	0	61	2.34	1.031	0	0
13	2	2	3	0	43	0.87	1.090	0	0
13	2	2	3	0	55	1.71	1.025	0	0
13	2	2	3	0	69	3.16	0.963	0	0
13	2	2	3	0	63	2.71	1.084	0	0
13	2	2	3	0	96	9.16	1.036	0	0
13	2	2	3	0	65	3.08	1.123	0	0
13	2	2	3	0	76	5.05	1.151	0	0
13	2	2	3	0	68	3.43	1.090	0	0
23	2	2	3	0	55	1.58	0.951	0	0
23	2	2	3	0	55	2.08	1.249	0	0
23	2	2	3	0	65	2.74	0.999	0	0
23	2	2	3	0	55	1.60	0.959	0	0
23	2	2	3	0	63	3.11	1.244	0	0
23	2	2	3	0	65	2.71	0.987	0	0
23	2	2	3	0	68	3.23	1.027	0	0
23	2	2	3	0	63	2.65	1.061	0	0
23	2	2	3	0	68	3.11	0.987	0	0
23	2	2	3	0	66	3.25	1.130	0	0
mean					64	2.96	1.059	0	0
variance					111	2.96	0.008	0	0
se					2	0.38	0.019	0	0

6	2	2	2	0	61	2.74	1.208	0	0
6	2	2	2	0	58	1.85	0.950	0	0
6	2	2	2	0	46	0.97	0.997	0	0
6	2	2	2	0	67	2.66	0.884	0	0
6	2	2	2	0	65	3.11	1.133	0	0
6	2	2	2	0	93	7.75	0.963	0	0
6	2	2	2	0	70	3.52	1.027	0	0
6	2	2	2	0	101	10.34	1.003	0	0
6	2	2	2	0	69	3.89	1.184	0	0
6	2	2	2	0	81	6.25	1.176	0	0
18	2	2	2	0	45	0.90	0.989	0	0
18	2	2	2	0	59	2.28	1.111	0	0
18	2	2	2	0	49	1.24	1.057	0	0
18	2	2	2	0	55	1.84	1.106	0	0
18	2	2	2	0	110	13.53	1.017	0	0
18	2	2	2	0	68	3.67	1.168	0	0
18	2	2	2	0	65	2.84	1.036	0	0
18	2	2	2	0	64	2.61	0.994	0	0
18	2	2	2	0	100	11.36	1.136	0	0
18	2	2	2	0	77	4.41	0.965	0	0
mean					70	4.39	1.055	0	0
variance					341	13.04	0.008	0	0
se					4	0.81	0.020	0	0

	yellow=1 rock=2 halibut=3	mud=1 sand=2	high=3 lo=2 cont=1	in days	mm	mg		growth rate length	growth rate weight
tank no	species	substrate	oil level	time	length	weight	condition	GrowthL	GrowthW
16	2	2	1	0	49	1.14	0.966	0	0
16	2	2	1	0	56	1.63	0.929	0	0
16	2	2	1	0	56	2.05	1.164	0	0
16	2	2	1	0	48	1.06	0.959	0	0
16	2	2	1	0	51	1.45	1.094	0	0
16	2	2	1	0	54	1.56	0.993	0	0
16	2	2	1	0	101	10.58	1.027	0	0
16	2	2	1	0	68	3.13	0.994	0	0
16	2	2	1	0	101	11.91	1.156	0	0
16	2	2	1	0	73	3.27	0.841	0	0
11	2	2	1	0	60	2.15	0.994	0	0
11	2	2	1	0	60	2.44	1.131	0	0
11	2	2	1	0	48	1.05	0.949	0	0
11	2	2	1	0	60	2.27	1.050	0	0
11	2	2	1	0	70	3.11	0.906	0	0
11	2	2	1	0	61	2.43	1.068	0	0
11	2	2	1	0	101	11.24	1.091	0	0
11	2	2	1	0	66	2.80	0.975	0	0
11	2	2	1	0	102	11.11	1.047	0	0
11	2	2	1	0	64	2.90	1.105	0	0
mean					67	3.96	1.022	0	0
variance					350	14.33	0.007	0	0
se					4	0.85	0.019	0	0
1	3	2	3	0	55	1.64	0.986	0	0
1	3	2	3	0	80	6.10	1.192	0	0
1	3	2	3	0	68	3.14	0.998	0	0
1	3	2	3	0	76	4.63	1.055	0	0
1	3	2	3	0	57	1.82	0.984	0	0
1	3	2	3	0	84	6.16	1.039	0	0
1	3	2	3	0	49	1.88	1.595	0	0
1	3	2	3	0	79	5.08	1.031	0	0
1	3	2	3	0	65	2.59	0.945	0	0
1	3	2	3	0	51	1.42	1.068	0	0
3	3	2	3	0	61	2.24	0.986	0	0
3	3	2	3	0	68	2.83	0.899	0	0
3	3	2	3	0	47	1.02	0.986	0	0
3	3	2	3	0	81	6.10	1.147	0	0
3	3	2	3	0	66	2.81	0.978	0	0
3	3	2	3	0	81	6.04	1.136	0	0
3	3	2	3	0	82	5.63	1.021	0	0
3	3	2	3	0	81	5.59	1.052	0	0
3	3	2	3	0	74	4.57	1.128	0	0
3	3	2	3	0	77	5.12	1.122	0	0
mean					69	3.82	1.067	0	0
variance					149	3.38	0.021	0	0
se					3	0.41	0.032	0	0

	yellow=1 rock=2 halibut=3	mud=1 sand=2	high=3 lo=2 cont=1	in days	mm	mg		growth rate length	growth rate weight
tank no	species	substrate	oil level	time	length	weight	condition	GrowthL	GrowthW
2	3	2	2	0	66	3.31	1.151	0	0
2	3	2	2	0	76	4.45	1.014	0	0
2	3	2	2	0	65	2.86	1.042	0	0
2	3	2	2	0	70	3.76	1.097	0	0
2	3	2	2	0	89	7.23	1.025	0	0
2	3	2	2	0	73	4.06	1.044	0	0
2	3	2	2	0	71	3.58	1.000	0	0
2	3	2	2	0	60	2.12	0.982	0	0
2	3	2	2	0	80	5.55	1.083	0	0
2	3	2	2	0	79	5.52	1.120	0	0
4	3	2	2	0	79	4.90	0.994	0	0
4	3	2	2	0	71	4.00	1.117	0	0
4	3	2	2	0	53	1.31	0.881	0	0
4	3	2	2	0	79	5.32	1.079	0	0
4	3	2	2	0	84	6.94	1.170	0	0
4	3	2	2	0	59	2.16	1.050	0	0
4	3	2	2	0	60	2.28	1.056	0	0
4	3	2	2	0	75	4.58	1.086	0	0
4	3	2	2	0	65	3.01	1.096	0	0
4	3	2	2	0	74	3.89	0.959	0	0
mean					71	4.04	1.052	0	0
variance					86	2.47	0.005	0	0
se					2	0.35	0.015	0	0
7	3	2	1	0	81	5.95	1.120	0	0
7	3	2	1	0	73	4.30	1.106	0	0
7	3	2	1	0	61	2.43	1.071	0	0
7	3	2	1	0	70	3.47	1.012	0	0
7	3	2	1	0	64	2.85	1.088	0	0
7	3	2	1	0	66	2.96	1.030	0	0
7	3	2	1	0	80	5.40	1.054	0	0
7	3	2	1	0	80	5.43	1.060	0	0
7	3	2	1	0	66	3.38	1.175	0	0
7	3	2	1	0	78	5.26	1.107	0	0
8	3	2	1	0	73	4.38	1.125	0	0
8	3	2	1	0	61	2.30	1.014	0	0
8	3	2	1	0	64	2.48	0.948	0	0
8	3	2	1	0	82	6.63	1.202	0	0
8	3	2	1	0	78	4.72	0.994	0	0
8	3	2	1	0	74	4.08	1.007	0	0
8	3	2	1	0	70	3.69	1.077	0	0
8	3	2	1	0	68	2.91	0.925	0	0
8	3	2	1	0	81	5.78	1.087	0	0
8	3	2	1	0	71	3.50	0.979	0	0
mean					72	4.09	1.059	0	0
variance					49	1.70	0.005	0	0
se					2	0.29	0.016	0	0

	yellow=1 rock=2 halibut=3	mud=1 sand=2	high=3 lo=2 cont=1	in days	mm	mg		growth rate length	growth rate weight
tank no	species	substrate	oil level	time	length	weight	condition	GrowthL	GrowthW
5	1	1	3	30	59	2.01	0.978	0.033	0.000
5	1	1	3	30	56	1.89	1.078	0.033	0.023
5	1	1	3	30	55	2.14	1.286	0.000	0.226
5	1	1	3	30	70	2.90	0.845	0.067	0.163
5	1	1	3	30	95	8.64	1.008	0.033	0.097
5	1	1	3	30	68	3.39	1.077	0.133	0.264
5	1	1	3	30	104	11.00	0.978	0.033	0.132
5	1	1	3	30	57	1.89	1.021	0.033	0.206
5	1	1	3	30	91	7.67	1.017	0.133	0.210
5	1	1	3	30	111	13.28	0.971	0.000	0.092
19	1	1	3	30	64	2.38	0.909	0.033	0.000
19	1	1	3	30	58	2.00	1.024	0.067	0.074
19	1	1	3	30	70	3.76	1.097	0.133	0.549
19	1	1	3	30	62	2.88	1.206	0.033	0.380
19	1	1	3	30	108	14.53	1.153	0.133	0.276
19	1	1	3	30	61	2.31	1.018	0.033	0.058
19	1	1	3	30	103	12.39	1.134	0.133	0.497
19	1	1	3	30	62	2.91	1.220	0.033	0.334
19	1	1	3	30	77	3.90	0.854	0.100	0.000
19	1	1	3	30	67	3.25	1.080	0.033	0.145
mean					75	5.26	1.048	0.062	0.186
variance					375	18.44	0.013	0.002	0.026
se					4	0.96	0.026	0.011	0.036

14	1	1	2	30	59	2.37	1.152	0.033	0.347
14	1	1	2	30		DEAD			
14	1	1	2	30	55	1.61	0.968	0.000	0.000
14	1	1	2	30	61	2.59	1.140	0.067	0.519
14	1	1	2	30	101	9.98	0.969	0.067	0.067
14	1	1	2	30		DEAD			
14	1	1	2	30	115	16.04	1.054	0.133	0.857
14	1	1	2	30	71	3.62	1.011	0.333	1.188
14	1	1	2	30	82	5.71	1.035	0.100	0.194
14	1	1	2	30	65	3.07	1.119	0.000	0.000
22	1	1	2	30	61	2.41	1.063	0.067	0.134
22	1	1	2	30	56	1.84	1.050	0.067	0.257
22	1	1	2	30	67	2.90	0.965	0.067	0.001
22	1	1	2	30	68	3.07	0.976	0.167	0.415
22	1	1	2	30	90	7.23	0.992	0.133	0.226
22	1	1	2	30	73	2.98	0.766	0.167	0.000
22	1	1	2	30	116	16.01	1.026	0.200	0.290
22	1	1	2	30	64	2.54	0.969	0.167	0.561
22	1	1	2	30	95	8.73	1.018	0.067	0.000
22	1	1	2	30	85	5.85	0.953	0.133	0.242
mean					77	5.48	1.013	0.109	0.294
variance					378	20.51	0.008	0.007	0.105
se					4	1.01	0.019	0.018	0.072

	yellow=1 rock=2 halibut=3	mud=1 sand=2	high=3 lo=2 cont=1	in days	mm	mg		growth rate length	growth rate weight
tank no	species	substrate	oil level	time	length	weight	condition	GrowthL	GrowthW
9	1	1	1	30	57	1.73	0.932	0.000	0.408
9	1	1	1	30	63	2.60	1.039	0.000	0.127
9	1	1	1	30	59	2.05	0.996	0.133	0.567
9	1	1	1	30	67	2.93	0.973	0.200	0.836
9	1	1	1	30	78	4.74	0.999	0.233	0.638
9	1	1	1	30	76	4.44	1.012	0.067	0.508
9	1	1	1	30	104	11.86	1.054	0.100	0.235
9	1	1	1	30	69	3.37	1.027	0.000	0.202
9	1	1	1	30	110	12.45	0.935	0.200	0.520
9	1	1	1	30	73	4.09	1.052	0.167	0.756
20	1	1	1	30	61	2.52	1.108	0.167	0.843
20	1	1	1	30	57	2.25	1.215	0.000	0.000
20	1	1	1	30	56	1.38	0.788	0.233	0.601
20	1	1	1	30	66	2.23	0.776	0.167	0.520
20	1	1	1	30	80	5.70	1.113	0.133	0.489
20	1	1	1	30	73	4.07	1.046	0.333	0.951
20	1	1	1	30	109	12.84	0.992	0.267	0.467
20	1	1	1	30	100	11.45	1.145	0.133	0.356
20	1	1	1	30	74	5.31	1.311	0.100	0.833
20	1	1	1	30	68	3.21	1.021	0.200	0.484
mean					75	5.06	1.027	0.142	0.517
variance					301	14.63	0.015	0.009	0.064
se					4	0.86	0.028	0.021	0.057

10	2	1	3	30	51	1.30	0.978	0.067	0.000
10	2	1	3	30	58	2.11	1.082	0.033	0.035
10	2	1	3	30	58	2.23	1.141	0.000	0.015
10	2	1	3	30	64	2.39	0.913	0.100	0.000
10	2	1	3	30	64	2.94	1.122	0.000	0.165
10	2	1	3	30	103	11.00	1.007	0.067	0.268
10	2	1	3	30	95	8.91	1.039	0.133	0.233
10	2	1	3	30	58	2.18	1.115	0.000	0.000
10	2	1	3	30	72	4.07	1.090	0.033	0.199
10	2	1	3	30	72	4.13	1.106	0.067	0.410
21	2	1	3	30	60	2.14	0.992	0.033	0.286
21	2	1	3	30	59	1.91	0.930	0.033	0.072
21	2	1	3	30	49	1.13	0.963	0.033	0.000
21	2	1	3	30	61	1.86	0.819	0.100	0.000
21	2	1	3	30	74	4.14	1.020	0.067	0.000
21	2	1	3	30	93	7.68	0.954	0.200	0.419
21	2	1	3	30	82	5.79	1.050	0.067	0.102
21	2	1	3	30	67	3.04	1.010	0.033	0.011
21	2	1	3	30	111	14.15	1.034	0.033	0.129
21	2	1	3	30	74	4.39	1.083	0.100	0.467
mean					71	4.37	1.022	0.060	0.141
variance					300	12.25	0.007	0.002	0.025
se					4	0.78	0.018	0.011	0.035

	yellow=1	mud=1	high=3	in days	mm	mg		growth	growth
	rock=2	sand=2	lo=2					rate	rate
	halibut=3		cont=1					length	weight
tank no	species	substrate	oil level	time	length	weight	condition	GrowthL	GrowthW
12	2	1	2	30	51	1.57	1.181	0.000	0.409
12	2	1	2	30	61	2.45	1.079	0.200	1.218
12	2	1	2	30	55	1.78	1.067	0.000	0.117
12	2	1	2	30	52	1.31	0.934	0.033	0.000
12	2	1	2	30	67	3.16	1.052	0.267	0.877
12	2	1	2	30	63	2.65	1.060	0.000	0.217
12	2	1	2	30	68	3.40	1.081	0.000	0.007
12	2	1	2	30	79	5.77	1.170	0.100	0.472
12	2	1	2	30	116	17.05	1.092	0.200	0.400
12	2	1	2	30	85	6.11	0.995	0.100	0.000
17	2	1	2	30	60	2.46	1.137	0.133	1.164
17	2	1	2	30	65	2.70	0.984	0.133	0.759
17	2	1	2	30	54	1.63	1.035	0.100	0.604
17	2	1	2	30	61	2.12	0.932	0.100	0.565
17	2	1	2	30	100	10.00	1.000	0.000	0.000
17	2	1	2	30	110	12.90	0.969	0.133	0.000
17	2	1	2	30	63	2.67	1.067	0.133	0.831
17	2	1	2	30	58	2.06	1.055	0.067	0.236
17	2	1	2	30	103	12.24	1.120	0.233	0.859
17	2	1	2	30	63	2.75	1.100	0.100	0.490
mean					72	4.84	1.056	0.102	0.461
variance					406	20.56	0.005	0.007	0.155
se					5	1.01	0.016	0.018	0.088
15	2	1	1	30	53	1.59	1.067	0.000	0.366
15	2	1	1	30	54	1.58	1.006	0.100	0.570
15	2	1	1	30	48	1.09	0.981	0.067	0.579
15	2	1	1	30	50	1.23	0.982	0.133	0.971
15	2	1	1	30	100	10.04	1.004	0.100	0.000
15	2	1	1	30	71	4.40	1.230	0.233	1.336
15	2	1	1	30	109	11.02	0.851	0.000	0.000
15	2	1	1	30	70	3.74	1.090	0.200	0.872
15	2	1	1	30	67	2.92	0.970	0.233	0.807
15	2	1	1	30	61	2.50	1.102	0.100	0.853
24	2	1	1	30	55	1.89	1.136	0.133	0.000
24	2	1	1	30	75	4.99	1.182	0.100	0.604
24	2	1	1	30	49	1.07	0.912	0.100	0.388
24	2	1	1	30	53	1.47	0.986	0.067	0.620
24	2	1	1	30	69	3.19	0.971	0.100	0.261
24	2	1	1	30	64	2.77	1.057	0.133	0.819
24	2	1	1	30	98	9.70	1.031	0.067	0.203
24	2	1	1	30	79	5.67	1.150	0.300	1.704
24	2	1	1	30	96	9.38	1.061	0.133	0.291
24	2	1	1	30	67	3.54	1.177	0.200	1.151
mean					69	4.19	1.047	0.125	0.620
variance					342	10.71	0.009	0.006	0.209
se					4	0.73	0.022	0.017	0.102

	yellow=1 rock=2 halibut=3	mud=1 sand=2	high=3 lo=2 cont=1	in days	mm	mg		growth rate length	growth rate weight
tank no	species	substrate	oil level	time	length	weight	condition	GrowthL	GrowthW
13	2	2	3	30	58	2.01	1.030	0.100	0.642
13	2	2	3	30	63	2.24	0.894	0.067	0.000
13	2	2	3	30	43	0.84	1.059	0.000	0.000
13	2	2	3	30	60	2.34	1.083	0.167	1.055
13	2	2	3	30	69	3.57	1.085	0.000	0.398
13	2	2	3	30	64	2.99	1.139	0.033	0.322
13	2	2	3	30	96	9.03	1.020	0.000	0.000
13	2	2	3	30	67	3.30	1.098	0.067	0.229
13	2	2	3	30	81	5.71	1.074	0.167	0.407
13	2	2	3	30	69	3.53	1.076	0.033	0.102
23	2	2	3	30	59	2.00	0.972	0.133	0.773
23	2	2	3	30	56	2.14	1.216	0.033	0.090
23	2	2	3	30	70	3.22	0.938	0.167	0.531
23	2	2	3	30	57	2.05	1.104	0.067	0.826
23	2	2	3	30	71	3.97	1.109	0.267	0.813
23	2	2	3	30	69	2.92	0.887	0.133	0.242
23	2	2	3	30	76	3.51	0.799	0.267	0.277
23	2	2	3	30	66	2.19	0.760	0.100	0.000
23	2	2	3	30	71	3.38	0.943	0.100	0.279
23	2	2	3	30	72	4.33	1.161	0.200	0.959
mean					67	3.26	1.022	0.105	0.397
variance					116	2.95	0.014	0.007	0.117
se					2	0.38	0.027	0.018	0.076

6	2	2	2	30		DEAD			
6	2	2	2	30	60	1.97	0.912	0.067	0.204
6	2	2	2	30	48	0.82	0.741	0.067	0.000
6	2	2	2	30	70	3.14	0.914	0.100	0.549
6	2	2	2	30	68	3.29	1.045	0.100	0.182
6	2	2	2	30	94	8.16	0.983	0.033	0.174
6	2	2	2	30	72	4.16	1.115	0.067	0.557
6	2	2	2	30	106	12.00	1.008	0.167	0.498
6	2	2	2	30	72	4.36	1.168	0.100	0.380
6	2	2	2	30	84	7.18	1.211	0.100	0.462
18	2	2	2	30	45	0.85	0.928	0.000	0.000
18	2	2	2	30	63	2.71	1.084	0.133	0.574
18	2	2	2	30	50	1.18	0.942	0.033	0.000
18	2	2	2	30	59	2.32	1.131	0.133	0.776
18	2	2	2	30	115	16.55	1.088	0.167	0.671
18	2	2	2	30	74	4.58	1.130	0.200	0.736
18	2	2	2	30	69	3.45	1.051	0.133	0.648
18	2	2	2	30	66	3.05	1.059	0.067	0.521
18	2	2	2	30	105	12.55	1.084	0.167	0.333
18	2	2	2	30	77	4.46	0.976	0.000	0.040
mean					74	5.09	1.030	0.096	0.384
variance					386	18.84	0.012	0.003	0.069
se					4	0.97	0.025	0.013	0.059

	yellow=1 rock=2 halibut=3	mud=1 sand=2	high=3 lo=2 cont=1	in days	mm	mg		growth rate length	growth rate weight
tank no	species	substrate	oil level	time	length	weight	condition	GrowthL	GrowthW
16	2	2	1	30	52	1.30	0.925	0.100	0.450
16	2	2	1	30	60	2.06	0.952	0.133	0.771
16	2	2	1	30	58	2.92	1.495	0.067	1.184
16	2	2	1	30	50	1.11	0.890	0.067	0.156
16	2	2	1	30	57	2.08	1.123	0.200	1.200
16	2	2	1	30	55	1.56	0.936	0.033	0.000
16	2	2	1	30	106	12.01	1.008	0.167	0.423
16	2	2	1	30	71	4.04	1.127	0.100	0.851
16	2	2	1	30	105	12.06	1.042	0.133	0.043
16	2	2	1	30	70	3.84	1.120	0.000	0.535
11	2	2	1	30	63	3.00	1.201	0.100	1.116
11	2	2	1	30	65	3.24	1.179	0.167	0.939
11	2	2	1	30	51	1.35	1.019	0.100	0.843
11	2	2	1	30	65	2.78	1.012	0.167	0.679
11	2	2	1	30	74	5.32	1.313	0.133	1.791
11	2	2	1	30	65	2.82	1.027	0.133	0.503
11	2	2	1	30	106	13.56	1.139	0.167	0.626
11	2	2	1	30	70	4.80	1.400	0.133	1.795
11	2	2	1	30	104	12.60	1.120	0.067	0.420
11	2	2	1	30	67	3.65	1.214	0.100	0.771
mean					71	4.80	1.112	0.113	0.755
variance					360	17.15	0.025	0.003	0.242
se					4	0.93	0.035	0.011	0.110

1	3	2	3	30	56	1.70	0.970	0.033	0.124
1	3	2	3	30	80	6.10	1.191	0.000	0.000
1	3	2	3	30	70	3.25	0.948	0.067	0.116
1	3	2	3	30	76	4.65	1.059	0.000	0.014
1	3	2	3	30	57	1.80	0.973	0.000	0.000
1	3	2	3	30	85	6.31	1.028	0.033	0.082
1	3	2	3	30	50	1.96	1.567	0.033	0.143
1	3	2	3	30	81	5.32	1.001	0.067	0.153
1	3	2	3	30	65	2.60	0.947	0.000	0.008
1	3	2	3	30	51	1.50	1.131	0.000	0.190
3	3	2	3	30	61	2.21	0.972	0.000	0.000
3	3	2	3	30	70	2.96	0.862	0.067	0.149
3	3	2	3	30	48	1.10	0.993	0.033	0.233
3	3	2	3	30	82	6.29	1.141	0.033	0.106
3	3	2	3	30	66	2.31	0.803	0.000	0.000
3	3	2	3	30	81	6.32	1.188	0.000	0.150
3	3	2	3	30	83	6.09	1.064	0.033	0.259
3	3	2	3	30	81	5.50	1.035	0.000	0.000
3	3	2	3	30	75	4.79	1.135	0.033	0.155
3	3	2	3	30	78	5.32	1.120	0.033	0.123
mean					70	3.90	1.056	0.023	0.100
variance					150	3.68	0.025	0.001	0.007
se					3	0.43	0.035	0.005	0.019

	yellow=1 rock=2 halibut=3	mud=1 sand=2	high=3 lo=2 cont=1	in days	mm	mg		growth rate length	growth rate weight
tank no	species	substrate	oil level	time	length	weight	condition	GrowthL	GrowthW
2	3	2	2	30	70	3.60	1.050	0.133	0.282
2	3	2	2	30	81	4.44	0.836	0.167	0.000
2	3	2	2	30	70	3.67	1.069	0.167	0.826
2	3	2	2	30	75	4.31	1.021	0.167	0.449
2	3	2	2	30	93	9.00	1.119	0.133	0.733
2	3	2	2	30	78	4.69	0.989	0.167	0.482
2	3	2	2	30	75	4.38	1.039	0.133	0.676
2	3	2	2	30	66	2.59	0.901	0.200	0.664
2	3	2	2	30	85	6.67	1.085	0.167	0.613
2	3	2	2	30	84	6.40	1.080	0.167	0.491
4	3	2	2	30	84	5.42	0.915	0.167	0.336
4	3	2	2	30	76	4.82	1.097	0.167	0.621
4	3	2	2	30	59	1.39	0.675	0.200	0.183
4	3	2	2	30	85	6.01	0.978	0.200	0.405
4	3	2	2	30	89	7.78	1.103	0.167	0.381
4	3	2	2	30	64	2.48	0.946	0.167	0.467
4	3	2	2	30	65	2.70	0.983	0.167	0.561
4	3	2	2	30	80	5.21	1.017	0.167	0.427
4	3	2	2	30	69	3.60	1.096	0.133	0.597
4	3	2	2	30	77	4.51	0.988	0.100	0.496
mean					76	4.68	0.999	0.162	0.484
variance					83	3.44	0.012	0.001	0.037
se					2	0.41	0.024	0.006	0.043

7	3	2	1	30	88	8.75	1.284	0.233	1.285
7	3	2	1	30	79	7.11	1.442	0.200	1.675
7	3	2	1	30	67	3.93	1.307	0.200	1.601
7	3	2	1	30	76	5.50	1.253	0.200	1.534
7	3	2	1	30	71	4.78	1.334	0.233	1.718
7	3	2	1	30	72	4.94	1.324	0.200	1.705
7	3	2	1	30	85	8.26	1.345	0.167	1.419
7	3	2	1	30	86	8.17	1.284	0.200	1.363
7	3	2	1	30	72	4.00	1.071	0.200	0.561
7	3	2	1	30	84	7.88	1.330	0.200	1.352
8	3	2	1	30	80	6.67	1.302	0.233	1.403
8	3	2	1	30	68	4.00	1.271	0.233	1.841
8	3	2	1	30	70	4.11	1.197	0.200	1.675
8	3	2	1	30	86	10.01	1.573	0.133	1.373
8	3	2	1	30	85	7.23	1.177	0.233	1.421
8	3	2	1	30	79	6.68	1.354	0.167	1.643
8	3	2	1	30	77	6.32	1.384	0.233	1.790
8	3	2	1	30	73	4.80	1.234	0.167	1.669
8	3	2	1	30	87	8.70	1.322	0.200	1.365
8	3	2	1	30	78	6.01	1.266	0.233	1.798
mean					78	6.39	1.303	0.203	1.510
variance					47	3.43	0.010	0.001	0.080
se					2	0.41	0.023	0.006	0.063

	yellow=1	mud=1	high=3	in days	mm	mg		growth	growth
	rock=2	sand=2	lo=2					rate	rate
	halibut=3		cont=1					length	weight
tank no	species	substrate	oil level	time	length	weight	condition	GrowthL	GrowthW
5	1	1	3	60	59	1.99	0.967	0.000	0.000
5	1	1	3	60	54	1.64	1.043	0.000	0.000
5	1	1	3	60	57	2.28	1.231	0.000	0.211
5	1	1	3	60	72	3.11	0.834	0.067	0.236
5	1	1	3	60	97	8.89	0.974	0.067	0.094
5	1	1	3	60	71	3.90	1.090	0.100	0.470
5	1	1	3	60	106	11.57	0.971	0.067	0.168
5	1	1	3	60	58	2.05	1.051	0.033	0.271
5	1	1	3	60	96	8.85	1.000	0.167	0.477
5	1	1	3	60	114	14.00	0.945	0.100	0.176
19	1	1	3	60	63	2.26	0.902	0.000	0.000
19	1	1	3	60	59	2.04	0.994	0.033	0.071
19	1	1	3	60	74	4.95	1.221	0.133	0.910
19	1	1	3	60	68	3.45	1.098	0.200	0.610
19	1	1	3	60	113	16.15	1.119	0.167	0.352
19	1	1	3	60	63	2.41	0.963	0.067	0.138
19	1	1	3	60	107	12.88	1.051	0.133	0.128
19	1	1	3	60	67	3.40	1.131	0.167	0.525
19	1	1	3	60	82	3.90	0.707	0.167	0.000
19	1	1	3	60	69	3.61	1.099	0.067	0.350
mean					77	5.67	1.020	0.087	0.259
variance					412	21.26	0.015	0.004	0.058
se					5	1.03	0.028	0.015	0.054

14	1	1	2	60	62	2.75	1.156	0.100	0.506
14	1	1	2	60	DEAD				
14	1	1	2	60	59	2.20	1.071	0.133	1.041
14	1	1	2	60	64	3.02	1.154	0.100	0.520
14	1	1	2	60	106	12.00	1.008	0.167	0.613
14	1	1	2	60	DEAD				
14	1	1	2	60	116	16.16	1.035	0.033	0.027
14	1	1	2	60	72	3.28	0.879	0.033	0.000
14	1	1	2	60	85	6.90	1.124	0.100	0.632
14	1	1	2	60	74	3.98	0.983	0.300	0.864
22	1	1	2	60	64	2.56	0.975	0.100	0.191
22	1	1	2	60	57	1.91	1.029	0.033	0.110
22	1	1	2	60	65	2.85	1.036	0.000	0.000
22	1	1	2	60	72	3.61	0.967	0.133	0.539
22	1	1	2	60	93	7.80	0.970	0.100	0.253
22	1	1	2	60	75	4.20	0.996	0.067	1.144
22	1	1	2	60	120	16.07	0.930	0.133	0.012
22	1	1	2	60	67	2.90	0.964	0.100	0.443
22	1	1	2	60	96	11.70	1.323	0.033	0.977
22	1	1	2	60	84	6.88	1.160	0.000	0.539
mean					80	6.15	1.042	0.093	0.467
variance					378	22.36	0.011	0.005	0.139
se					4	1.06	0.024	0.016	0.083

	yellow=1 rock=2 halibut=3	mud=1 sand=2	high=3 lo=2 cont=1	in days	mm	mg		growth rate length	growth rate weight
tank no	species	substrate	oil level	time	length	weight	condition	GrowthL	GrowthW
9	1	1	1	60	69	1.95	0.594	0.400	0.407
9	1	1	1	60	73	3.60	0.926	0.333	1.092
9	1	1	1	60	67	3.01	1.000	0.267	1.285
9	1	1	1	60	75	4.42	1.049	0.267	1.379
9	1	1	1	60	86	6.82	1.072	0.267	1.211
9	1	1	1	60	84	5.74	0.968	0.267	0.852
9	1	1	1	60	112	16.62	1.183	0.267	1.126
9	1	1	1	60	84	3.92	0.661	0.500	0.498
9	1	1	1	60	115	15.54	1.022	0.167	0.740
9	1	1	1	60	84	6.15	1.038	0.367	1.359
20	1	1	1	60	69	2.99	0.911	0.267	0.579
20	1	1	1	60	70	2.78	0.810	0.433	0.701
20	1	1	1	60	64	3.14	1.198	0.267	2.733
20	1	1	1	60	72	3.63	0.971	0.200	1.618
20	1	1	1	60	87	6.74	1.024	0.233	0.562
20	1	1	1	60	72	3.83	1.025	0.000	0.000
20	1	1	1	60	115	14.32	0.942	0.200	0.364
20	1	1	1	60	106	12.04	1.011	0.200	0.168
20	1	1	1	60	78	6.82	1.437	0.133	0.833
20	1	1	1	60	72	3.92	1.050	0.133	0.663
mean					83	6.40	0.995	0.258	0.909
variance					272	20.46	0.032	0.013	0.370
se					4	1.01	0.040	0.025	0.136
10	2	1	3	60	52	1.30	0.925	0.033	0.008
10	2	1	3	60	59	2.20	1.071	0.033	0.138
10	2	1	3	60	58	2.14	1.095	0.000	0.000
10	2	1	3	60	64	2.74	1.045	0.000	0.449
10	2	1	3	60	68	3.76	1.196	0.133	0.818
10	2	1	3	60	105	12.27	1.060	0.067	0.364
10	2	1	3	60	96	8.71	0.984	0.033	0.000
10	2	1	3	60	68	3.67	1.168	0.333	1.745
10	2	1	3	60	76	4.66	1.061	0.133	0.450
10	2	1	3	60	78	5.73	1.207	0.200	1.093
21	2	1	3	60	60	2.03	0.938	0.000	0.000
21	2	1	3	60	60	1.99	0.919	0.033	0.128
21	2	1	3	60	DEAD				
21	2	1	3	60	59	1.61	0.784	0.000	0.000
21	2	1	3	60	75	4.52	1.072	0.033	0.297
21	2	1	3	60	96	8.45	0.955	0.100	0.320
21	2	1	3	60	80	5.70	1.113	0.000	0.000
21	2	1	3	60	67	2.60	0.864	0.000	0.000
21	2	1	3	60	115	14.89	0.979	0.133	0.171
21	2	1	3	60	74	3.98	0.983	0.000	0.000
mean					74	4.89	1.022	0.067	0.315
variance					304	13.99	0.013	0.008	0.212
se					4	0.84	0.025	0.020	0.103

	yellow=1 rock=2 halibut=3	mud=1 sand=2	high=3 lo=2 cont=1	in days	mm	mg		growth rate length	growth rate weight
tank no	species	substrate	oil level	time	length	weight	condition	GrowthL	GrowthW
12	2	1	2	60	59	2.19	1.066	0.267	1.114
12	2	1	2	60	65	2.85	1.038	0.133	0.504
12	2	1	2	60	55	1.75	1.052	0.000	0.000
12	2	1	2	60	53	1.30	0.875	0.033	0.000
12	2	1	2	60	71	3.92	1.094	0.133	0.711
12	2	1	2	60	65	3.00	1.093	0.067	0.415
12	2	1	2	60	68	3.37	1.072	0.000	0.000
12	2	1	2	60	82	7.65	1.387	0.100	0.940
12	2	1	2	60	122	19.22	1.058	0.200	0.399
12	2	1	2	60	87	7.29	1.107	0.067	0.587
17	2	1	2	60	66	3.14	1.091	0.200	0.816
17	2	1	2	60	71	3.19	0.892	0.200	0.558
17	2	1	2	60	57	1.97	1.063	0.100	0.628
17	2	1	2	60	63	2.32	0.927	0.067	0.304
17	2	1	2	60	101	9.42	0.914	0.033	0.000
17	2	1	2	60	111	13.01	0.951	0.033	0.028
17	2	1	2	60	67	3.21	1.067	0.133	0.613
17	2	1	2	60	61	2.54	1.121	0.100	0.705
17	2	1	2	60	108	13.61	1.081	0.167	0.354
17	2	1	2	60	66	3.00	1.043	0.100	0.289
mean					75	5.40	1.049	0.107	0.448
variance					409	23.68	0.012	0.005	0.110
se					5	1.09	0.025	0.016	0.074

15	2	1	1	60	58	2.13	1.091	0.167	0.977
15	2	1	1	60	59	1.95	0.949	0.167	0.693
15	2	1	1	60	49	1.18	1.002	0.033	0.277
15	2	1	1	60	57	1.81	0.977	0.233	1.296
15	2	1	1	60	100	11.61	1.161	0.000	0.486
15	2	1	1	60	79	5.52	1.120	0.267	0.753
15	2	1	1	60	110	12.91	0.970	0.033	0.528
15	2	1	1	60	77	4.62	1.013	0.233	0.710
15	2	1	1	60	70	3.72	1.085	0.100	0.813
15	2	1	1	60	66	3.21	1.117	0.167	0.831
24	2	1	1	60	60	2.30	1.066	0.167	0.659
24	2	1	1	60	79	5.75	1.167	0.133	0.477
24	2	1	1	60	54	1.51	0.959	0.167	1.139
24	2	1	1	60	56	1.69	0.962	0.100	0.469
24	2	1	1	60	79	5.81	1.178	0.333	1.999
24	2	1	1	60	69	2.56	0.778	0.167	0.000
24	2	1	1	60	100	10.32	1.032	0.067	0.206
24	2	1	1	60	73	4.01	1.031	0.000	0.000
24	2	1	1	60	100	10.01	1.001	0.133	0.216
24	2	1	1	60	70	3.64	1.060	0.100	0.090
mean					73	4.81	1.036	0.138	0.631
variance					307	13.00	0.009	0.008	0.234
se					4	0.81	0.021	0.020	0.108

	yellow=1 rock=2 halibut=3	mud=1 sand=2	high=3 lo=2 cont=1	in days	mm	mg		growth rate length	growth rate weight
tank no	species	substrate	oil level	time	length	weight	condition	GrowthL	GrowthW
13	2	2	3	60	61	2.44	1.077	0.100	0.652
13	2	2	3	60	64	2.25	0.858	0.033	0.021
13	2	2	3	60	44	0.77	0.904	0.033	0.000
13	2	2	3	60	65	2.97	1.082	0.167	0.797
13	2	2	3	60	72	3.80	1.018	0.100	0.213
13	2	2	3	60	72	2.96	0.794	0.267	0.000
13	2	2	3	60	101	9.51	0.923	0.167	0.172
13	2	2	3	60	71	3.41	0.953	0.133	0.109
13	2	2	3	60	86	6.74	1.060	0.167	0.556
13	2	2	3	60	72	4.09	1.097	0.100	0.489
23	2	2	3	60	60	2.18	1.009	0.033	0.294
23	2	2	3	60	58	2.14	1.096	0.067	0.006
23	2	2	3	60	71	3.61	1.009	0.033	0.383
23	2	2	3	60	59	1.89	0.922	0.067	0.000
23	2	2	3	60	76	4.24	0.967	0.167	0.222
23	2	2	3	60	72	3.07	0.821	0.100	0.168
23	2	2	3	60	74	4.23	1.044	0.000	0.622
23	2	2	3	60	70	3.79	1.105	0.133	1.834
23	2	2	3	60	72	3.55	0.951	0.033	0.168
23	2	2	3	60	80	5.31	1.037	0.267	0.677
mean					70	3.65	0.986	0.108	0.369
variance					135	3.58	0.009	0.006	0.185
se					3	0.42	0.021	0.017	0.096

6	2	2	2	60	DEAD				
6	2	2	2	60	63	2.27	0.907	0.100	0.468
6	2	2	2	60	48	0.88	0.793	0.000	0.228
6	2	2	2	60	77	3.87	0.848	0.233	0.702
6	2	2	2	60	74	4.12	1.016	0.200	0.750
6	2	2	2	60	100	9.79	0.979	0.200	0.606
6	2	2	2	60	81	5.11	0.962	0.300	0.686
6	2	2	2	60	110	13.70	1.030	0.133	0.442
6	2	2	2	60	78	5.00	1.054	0.200	0.457
6	2	2	2	60	90	8.29	1.138	0.200	0.480
18	2	2	2	60	45	0.91	0.993	0.000	0.225
18	2	2	2	60	67	3.11	1.033	0.133	0.455
18	2	2	2	60	51	1.23	0.927	0.033	0.147
18	2	2	2	60	61	2.00	0.881	0.067	0.000
18	2	2	2	60	122	20.50	1.129	0.233	0.714
18	2	2	2	60	82	6.23	1.130	0.267	1.027
18	2	2	2	60	73	3.92	1.007	0.133	0.420
18	2	2	2	60	71	3.80	1.062	0.167	0.739
18	2	2	2	60	110	13.52	1.015	0.167	0.247
18	2	2	2	60	79	4.98	1.010	0.067	0.368
mean					78	5.96	0.995	0.149	0.482
variance					449	26.62	0.009	0.008	0.064
se					5	1.15	0.021	0.019	0.056

	yellow=1 rock=2 halibut=3	mud=1 sand=2	high=3 lo=2 cont=1	in days	mm	mg		growth rate length	growth rate weight
tank no	species	substrate	oil level	time	length	weight	condition	GrowthL	GrowthW
16	2	2	1	60	60	1.48	0.687	0.267	0.441
16	2	2	1	60	66	2.92	1.014	0.200	1.162
16	2	2	1	60	60	2.99	1.385	0.067	0.084
16	2	2	1	60	57	1.52	0.821	0.233	1.042
16	2	2	1	60	63	2.69	1.076	0.200	0.857
16	2	2	1	60	63	2.57	1.029	0.267	1.674
16	2	2	1	60	109	13.41	1.035	0.100	0.368
16	2	2	1	60	78	6.01	1.266	0.233	1.328
16	2	2	1	60	108	14.00	1.112	0.100	0.497
16	2	2	1	60	78	5.18	1.092	0.267	0.998
11	2	2	1	60	68	4.30	1.368	0.167	1.198
11	2	2	1	60	67	4.21	1.400	0.067	0.875
11	2	2	1	60	54	1.55	0.983	0.100	0.451
11	2	2	1	60	71	4.45	1.243	0.200	1.568
11	2	2	1	60	81	6.50	1.224	0.233	0.669
11	2	2	1	60	71	4.12	1.151	0.200	1.263
11	2	2	1	60	109	14.51	1.121	0.100	0.226
11	2	2	1	60	76	5.11	1.165	0.200	0.208
11	2	2	1	60	107	14.00	1.143	0.100	0.350
11	2	2	1	60	76	4.36	0.993	0.300	0.590
mean					76	5.79	1.115	0.180	0.792
variance					326	19.60	0.032	0.006	0.223
se					4	0.99	0.040	0.017	0.105

1	3	2	3	60	58	1.80	0.920	0.067	0.175
1	3	2	3	60	82	6.69	1.214	0.067	0.309
1	3	2	3	60	72	3.49	0.934	0.067	0.234
1	3	2	3	60	81	5.10	0.960	0.167	0.311
1	3	2	3	60	57	2.04	1.099	0.000	0.405
1	3	2	3	60	87	6.77	1.028	0.067	0.235
1	3	2	3	60	51	2.07	1.564	0.033	0.190
1	3	2	3	60	83	5.60	0.980	0.067	0.172
1	3	2	3	60	68	2.89	0.918	0.100	0.350
1	3	2	3	60	52	1.57	1.114	0.033	0.144
3	3	2	3	60	64	2.50	0.953	0.100	0.416
3	3	2	3	60	73	3.12	0.803	0.100	0.183
3	3	2	3	60	51	1.14	0.859	0.100	0.125
3	3	2	3	60	85	6.67	1.086	0.100	0.194
3	3	2	3	60	69	3.09	0.941	0.100	0.972
3	3	2	3	60	81	6.66	1.252	0.000	0.175
3	3	2	3	60	85	6.22	1.013	0.067	0.074
3	3	2	3	60	84	6.21	1.048	0.100	0.404
3	3	2	3	60	77	5.01	1.098	0.067	0.153
3	3	2	3	60	79	5.68	1.151	0.033	0.218
mean					72	4.22	1.047	0.072	0.272
variance					156	4.08	0.028	0.002	0.037
se					3	0.45	0.037	0.009	0.043

	yellow=1 rock=2 halibut=3	mud=1 sand=2	high=3 lo=2 cont=1	in days	mm	mg		growth rate length	growth rate weight
tank no	species	substrate	oil level	time	length	weight	condition	GrowthL	GrowthW
2	3	2	2	60	75	4.41	1.046	0.167	0.678
2	3	2	2	60	86	5.24	0.823	0.167	0.547
2	3	2	2	60	76	4.37	0.995	0.200	0.582
2	3	2	2	60	81	4.80	0.903	0.200	0.361
2	3	2	2	60	98	10.78	1.145	0.167	0.600
2	3	2	2	60	84	5.41	0.912	0.200	0.471
2	3	2	2	60	81	6.01	1.130	0.200	1.051
2	3	2	2	60	72	3.06	0.820	0.200	0.555
2	3	2	2	60	90	7.69	1.055	0.167	0.475
2	3	2	2	60	89	7.29	1.035	0.167	0.436
4	3	2	2	60	90	5.95	0.816	0.200	0.309
4	3	2	2	60	81	5.71	1.074	0.167	0.568
4	3	2	2	60	65	1.69	0.616	0.200	0.667
4	3	2	2	60	90	6.57	0.902	0.167	0.300
4	3	2	2	60	94	8.59	1.034	0.167	0.332
4	3	2	2	60	69	2.80	0.853	0.167	0.406
4	3	2	2	60	70	3.16	0.921	0.167	0.524
4	3	2	2	60	87	6.11	0.928	0.233	0.534
4	3	2	2	60	74	4.19	1.034	0.167	0.504
4	3	2	2	60	81	5.12	0.963	0.133	0.419
mean					82	5.45	0.950	0.180	0.516
variance					81	4.50	0.016	0.001	0.028
se					2	0.47	0.028	0.005	0.037
7	3	2	1	60	96	11.96	1.352	0.267	1.042
7	3	2	1	60	86	9.22	1.449	0.233	0.866
7	3	2	1	60	75	5.91	1.401	0.267	1.360
7	3	2	1	60	83	8.01	1.401	0.233	1.253
7	3	2	1	60	81	7.00	1.317	0.333	1.275
7	3	2	1	60	78	7.12	1.500	0.200	1.217
7	3	2	1	60	90	11.00	1.509	0.167	0.954
7	3	2	1	60	91	10.67	1.415	0.167	0.889
7	3	2	1	60	79	6.87	1.393	0.233	1.804
7	3	2	1	60	91	10.44	1.386	0.233	0.938
8	3	2	1	60	87	8.78	1.333	0.233	0.917
8	3	2	1	60	76	5.93	1.352	0.267	1.317
8	3	2	1	60	76	6.00	1.366	0.200	1.263
8	3	2	1	60	91	13.56	1.799	0.167	1.013
8	3	2	1	60	92	10.00	1.284	0.233	1.081
8	3	2	1	60	85	9.20	1.499	0.200	1.069
8	3	2	1	60	85	9.01	1.466	0.267	1.182
8	3	2	1	60	80	6.66	1.301	0.233	1.092
8	3	2	1	60	94	11.56	1.391	0.233	0.945
8	3	2	1	60	86	8.75	1.376	0.267	1.253
mean					85	8.88	1.414	0.232	1.136
variance					41	4.86	0.012	0.002	0.049
se					1	0.49	0.025	0.009	0.049

	yellow=1 rock=2 halibut=3	mud=1 sand=2	high=3 lo=2 cont=1	in days	mm	mg		growth rate length	growth rate weight
tank no	species	substrate	oil level	time	length	weight	condition	GrowthL	GrowthW
5	1	1	3	90	60	1.97	0.913	0.033	0.000
5	1	1	3	90	53	1.32	0.889	0.000	0.000
5	1	1	3	90	63	2.42	0.967	0.200	0.196
5	1	1	3	90	74	3.36	0.828	0.067	0.251
5	1	1	3	90	100	9.14	0.914	0.100	0.094
5	1	1	3	90	76	4.59	1.045	0.167	0.542
5	1	1	3	90	108	12.39	0.983	0.067	0.229
5	1	1	3	90	60	2.24	1.037	0.067	0.294
5	1	1	3	90	100	9.73	0.973	0.133	0.317
5	1	1	3	90	119	15.51	0.920	0.167	0.340
19	1	1	3	90	63	2.27	0.909	0.000	0.025
19	1	1	3	90	61	2.13	0.940	0.067	0.147
19	1	1	3	90	77	5.24	1.148	0.100	0.193
19	1	1	3	90	71	4.23	1.183	0.100	0.680
19	1	1	3	90	118	18.78	1.143	0.167	0.504
19	1	1	3	90	64	2.50	0.953	0.033	0.121
19	1	1	3	90	107	13.43	1.096	0.000	0.140
19	1	1	3	90	70	3.91	1.140	0.100	0.463
19	1	1	3	90	80	3.88	0.757	0.000	0.000
19	1	1	3	90	71	4.11	1.147	0.067	0.431
mean					80	6.16	0.994	0.082	0.248
variance					438	26.55	0.014	0.004	0.039
se					5	1.15	0.027	0.014	0.044
14	1	1	2	90	68	3.57	1.135	0.200	0.863
14	1	1	2	90	DEAD				
14	1	1	2	90	62	2.62	1.098	0.100	0.579
14	1	1	2	90	67	3.20	1.064	0.100	0.188
14	1	1	2	90	111	13.70	1.002	0.167	0.442
14	1	1	2	90	DEAD				
14	1	1	2	90	120	17.44	1.009	0.133	0.254
14	1	1	2	90	77	5.05	1.106	0.167	1.437
14	1	1	2	90	88	8.08	1.186	0.100	0.526
14	1	1	2	90	76	4.88	1.112	0.067	0.679
22	1	1	2	90	66	2.82	0.982	0.067	0.334
22	1	1	2	90	58	2.16	1.109	0.033	0.422
22	1	1	2	90	66	3.17	1.101	0.033	0.355
22	1	1	2	90	76	4.59	1.045	0.133	0.800
22	1	1	2	90	97	9.91	1.086	0.133	0.797
22	1	1	2	90	79	5.28	1.070	0.133	0.760
22	1	1	2	90	122	19.76	1.088	0.067	0.688
22	1	1	2	90	68	3.28	1.044	0.033	0.412
22	1	1	2	90	97	14.71	1.612	0.033	0.762
22	1	1	2	90	90	8.36	1.147	0.200	0.651
mean					83	7.37	1.111	0.106	0.608
variance					390	30.41	0.018	0.003	0.084
se					4	1.23	0.030	0.013	0.065

	yellow=1 rock=2 *halibut*=3	mud=1 sand=2	high=3 lo=2 cont=1	in days	mm	mg		growth rate length	growth rate weight
tank no	species	substrate	oil level	time	length	weight	condition	GrowthL	GrowthW
9	1	1	1	90	73	3.66	0.940	0.133	2.094
9	1	1	1	90	77	4.94	1.082	0.133	1.050
9	1	1	1	90	71	3.83	1.069	0.133	0.803
9	1	1	1	90	81	5.94	1.118	0.200	0.983
9	1	1	1	90	93	9.34	1.162	0.233	1.051
9	1	1	1	90	94	8.13	0.978	0.333	1.160
9	1	1	1	90	117	20.07	1.253	0.167	0.629
9	1	1	1	90	93	7.14	0.888	0.300	2.000
9	1	1	1	90	120	20.64	1.194	0.167	0.946
9	1	1	1	90	92	8.75	1.123	0.267	1.172
20	1	1	1	90	72	4.08	1.092	0.100	1.031
20	1	1	1	90	76	3.73	0.849	0.200	0.983
20	1	1	1	90	71	3.14	0.877	0.233	0.000
20	1	1	1	90	77	4.96	1.085	0.167	1.042
20	1	1	1	90	93	9.52	1.184	0.200	1.150
20	1	1	1	90	78	4.91	1.034	0.200	0.831
20	1	1	1	90	118	20.77	1.264	0.100	1.239
20	1	1	1	90	112	20.40	1.452	0.200	1.756
20	1	1	1	90	91	8.80	1.168	0.433	0.850
20	1	1	1	90	78	5.09	1.072	0.200	0.870
mean					89	8.89	1.094	0.205	1.082
variance					271	39.31	0.021	0.007	0.212
se					4	1.40	0.033	0.018	0.103

10	2	1	3	90	53	1.29	0.866	0.033	0.000
10	2	1	3	90	61	2.23	0.984	0.067	0.050
10	2	1	3	90	58	2.17	1.112	0.000	0.053
10	2	1	3	90	71	4.18	1.168	0.233	1.411
10	2	1	3	90	73	4.58	1.176	0.167	0.654
10	2	1	3	90	106	11.05	0.928	0.033	0.000
10	2	1	3	90	97	8.70	0.953	0.033	0.000
10	2	1	3	90	68	3.67	1.167	0.000	0.000
10	2	1	3	90	77	4.60	1.008	0.033	0.000
10	2	1	3	90	85	5.97	0.972	0.233	0.137
21	2	1	3	90	62	2.82	1.182	0.067	1.098
21	2	1	3	90	62	2.28	0.955	0.067	0.456
21	2	1	3	90	DEAD				
21	2	1	3	90	59	1.67	0.814	0.000	0.126
21	2	1	3	90	76	4.19	0.954	0.033	0.000
21	2	1	3	90	98	9.71	1.032	0.067	0.464
21	2	1	3	90	80	5.82	1.136	0.000	0.068
21	2	1	3	90	70	2.58	0.752	0.100	0.000
21	2	1	3	90	118	14.91	0.907	0.100	0.004
21	2	1	3	90	74	4.47	1.103	0.000	0.386
mean					76	5.10	1.009	0.067	0.258
variance					310	13.02	0.016	0.005	0.165
se					4	0.81	0.029	0.016	0.091

	yellow=1 rock=2 halibut=3	mud=1 sand=2	high=3 lo=2 cont=1	in days	mm	mg		growth rate length	growth rate weight
tank no	species	substrate	oil level	time	length	weight	condition	GrowthL	GrowthW
12	2	1	2	90	67	3.39	1.128	0.267	1.461
12	2	1	2	90	70	3.40	0.991	0.167	0.588
12	2	1	2	90	56	1.71	0.976	0.033	0.000
12	2	1	2	90	55	1.62	0.971	0.067	0.718
12	2	1	2	90	75	4.14	0.980	0.133	0.182
12	2	1	2	90	69	3.86	1.175	0.133	0.839
12	2	1	2	90	68	3.38	1.074	0.000	0.006
12	2	1	2	90	85	7.10	1.157	0.100	0.000
12	2	1	2	90	128	20.07	0.957	0.200	0.145
12	2	1	2	90	93	9.32	1.159	0.200	0.821
17	2	1	2	90	72	3.56	0.955	0.200	0.425
17	2	1	2	90	75	3.83	0.909	0.133	0.608
17	2	1	2	90	61	2.22	0.979	0.133	0.405
17	2	1	2	90	64	2.45	0.933	0.033	0.179
17	2	1	2	90	103	8.73	0.799	0.067	0.000
17	2	1	2	90	112	13.20	0.940	0.033	0.050
17	2	1	2	90	71	3.86	1.079	0.133	0.618
17	2	1	2	90	65	2.89	1.051	0.133	0.420
17	2	1	2	90	112	15.71	1.118	0.133	0.478
17	2	1	2	90	73	3.60	0.925	0.233	0.609
mean					79	5.90	1.013	0.127	0.428
variance					414	25.65	0.010	0.005	0.141
se					5	1.13	0.022	0.016	0.084
15	2	1	1	90	62	2.81	1.180	0.133	0.927
15	2	1	1	90	65	2.95	1.075	0.200	1.383
15	2	1	1	90	55	1.71	1.028	0.200	1.239
15	2	1	1	90	64	2.51	0.958	0.233	1.091
15	2	1	1	90	110	14.31	1.075	0.333	0.696
15	2	1	1	90	86	7.33	1.153	0.233	0.946
15	2	1	1	90	111	13.73	1.004	0.033	0.205
15	2	1	1	90	80	6.20	1.211	0.100	0.979
15	2	1	1	90	76	5.21	1.186	0.200	1.119
15	2	1	1	90	71	4.08	1.139	0.167	0.796
24	2	1	1	90	67	3.32	1.104	0.233	1.218
24	2	1	1	90	82	6.71	1.217	0.100	0.514
24	2	1	1	90	59	2.32	1.130	0.167	1.433
24	2	1	1	90	69	3.17	0.965	0.433	2.097
24	2	1	1	90	84	6.52	1.100	0.167	0.384
24	2	1	1	90	69	3.38	1.027	0.000	0.928
24	2	1	1	90	112	15.01	1.068	0.400	1.248
24	2	1	1	90	80	5.29	1.033	0.233	0.922
24	2	1	1	90	104	11.79	1.048	0.133	0.546
24	2	1	1	90	76	5.64	1.286	0.200	1.466
mean					79	6.20	1.099	0.195	1.007
variance					310	17.68	0.008	0.011	0.187
se					4	0.94	0.020	0.024	0.097

	yellow=1 rock=2 halibut=3	mud=1 sand=2	high=3 lo=2 cont=1	in days	mm	mg		growth rate length	growth rate weight
tank no	species	substrate	oil level	time	length	weight	condition	GrowthL	GrowthW
13	2	2	3	90	65	2.66	0.967	0.133	0.277
13	2	2	3	90	66	2.97	1.033	0.067	0.925
13	2	2	3	90	44	0.81	0.956	0.000	0.185
13	2	2	3	90	71	3.87	1.081	0.200	0.878
13	2	2	3	90	76	4.08	0.928	0.133	0.233
13	2	2	3	90	69	3.42	1.041	0.000	0.476
13	2	2	3	90	103	10.87	0.995	0.067	0.447
13	2	2	3	90	74	4.22	1.041	0.100	0.706
13	2	2	3	90	86	7.00	1.101	0.000	0.127
13	2	2	3	90	76	4.70	1.070	0.133	0.459
23	2	2	3	90	63	2.49	0.995	0.100	0.442
23	2	2	3	90	58	2.01	1.028	0.000	0.000
23	2	2	3	90	75	4.18	0.990	0.133	0.485
23	2	2	3	90	59	1.88	0.915	0.000	0.000
23	2	2	3	90	76	4.58	1.043	0.000	0.254
23	2	2	3	90	73	3.52	0.906	0.033	0.464
23	2	2	3	90	79	4.69	0.951	0.167	0.346
23	2	2	3	90	73	3.88	0.998	0.100	0.080
23	2	2	3	90	72	3.69	0.989	0.000	0.132
23	2	2	3	90	80	5.62	1.098	0.000	0.191
					72	4.06	1.006	0.068	0.355
mean					140	4.45	0.003	0.005	0.069
variance					3	0.47	0.013	0.015	0.059
se									

6	2	2	2	90	DEAD				
6	2	2	2	90	74	2.51	0.619	0.367	0.339
6	2	2	2	90	50	1.66	1.331	0.067	2.135
6	2	2	2	90	84	6.44	1.087	0.233	1.699
6	2	2	2	90	81	5.05	0.950	0.233	0.682
6	2	2	2	90	108	12.37	0.982	0.267	0.779
6	2	2	2	90	90	7.25	0.995	0.300	1.165
6	2	2	2	90	116	15.82	1.013	0.200	0.478
6	2	2	2	90	85	6.46	1.051	0.233	0.852
6	2	2	2	90	96	9.45	1.068	0.200	0.434
18	2	2	2	90	53	1.29	0.864	0.267	1.171
18	2	2	2	90	70	3.60	1.050	0.100	0.495
18	2	2	2	90	53	1.41	0.947	0.067	0.455
18	2	2	2	90	69	3.39	1.032	0.267	1.759
18	2	2	2	90	131	20.07	0.893	0.300	0.000
18	2	2	2	90	94	9.46	1.139	0.400	1.393
18	2	2	2	90	80	5.13	1.001	0.233	0.896
18	2	2	2	90	79	5.01	1.015	0.267	0.917
18	2	2	2	90	117	15.04	0.939	0.233	0.355
18	2	2	2	90	90	7.62	1.045	0.367	1.418
mean					85	7.32	1.001	0.242	0.917
variance					491	27.86	0.019	0.008	0.322
se					5	1.18	0.030	0.020	0.127

	yellow=1	mud=1	high=3	in days	mm	mg		growth rate length	growth rate weight
	rock=2	sand=2	lo=2						
	halibut=3		cont=1						
tank no	species	substrate	oil level	time	length	weight	condition	GrowthL	GrowthW
16	2	2	1	90	67	2.95	0.981	0.233	2.290
16	2	2	1	90	74	4.65	1.149	0.267	1.560
16	2	2	1	90	74	4.65	1.149	0.467	1.474
16	2	2	1	90	62	2.39	1.003	0.167	1.509
16	2	2	1	90	71	3.88	1.083	0.267	1.217
16	2	2	1	90	71	3.71	1.037	0.267	1.220
16	2	2	1	90	115	14.78	0.972	0.200	0.325
16	2	2	1	90	88	8.12	1.191	0.333	1.001
16	2	2	1	90	114	5.80	0.391	0.200	0.000
16	2	2	1	90	91	8.73	1.158	0.433	1.737
11	2	2	1	90	78	5.73	1.207	0.333	0.956
11	2	2	1	90	77	5.25	1.150	0.333	0.736
11	2	2	1	90	61	2.29	1.009	0.233	1.307
11	2	2	1	90	80	6.06	1.184	0.300	1.029
11	2	2	1	90	88	8.07	1.184	0.233	0.720
11	2	2	1	90	81	5.75	1.083	0.333	1.114
11	2	2	1	90	115	15.71	1.033	0.200	0.264
11	2	2	1	90	84	7.28	1.229	0.267	1.179
11	2	2	1	90	114	15.90	1.073	0.233	0.425
11	2	2	1	90	83	6.22	1.089	0.233	1.189
mean					84	6.90	1.068	0.277	1.063
variance					303	16.92	0.032	0.006	0.299
se					4	0.92	0.040	0.017	0.122

1	3	2	3	90	DEAD				
1	3	2	3	90	DEAD				
1	3	2	3	90	DEAD				
1	3	2	3	90	DEAD				
1	3	2	3	90	DEAD				
1	3	2	3	90	DEAD				
1	3	2	3	90	DEAD				
1	3	2	3	90	83	5.90	1.032	0.000	0.174
1	3	2	3	90	DEAD				
1	3	2	3	90	DEAD				
3	3	2	3	90	DEAD				
3	3	2	3	90	DEAD				
3	3	2	3	90	DEAD				
3	3	2	3	90	86	7.12	1.120	0.033	0.219
3	3	2	3	90	DEAD				
3	3	2	3	90	DEAD				
3	3	2	3	90	DEAD				
3	3	2	3	90	DEAD				
3	3	2	3	90	DEAD				
3	3	2	3	90	DEAD				
mean					85	6.51	1.076	0.017	0.197
variance					5	0.75	0.004	0.001	0.001
se					0	0.19	0.014	0.005	0.007

	yellow=1 rock=2 halibut=3	mud=1 sand=2	high=3 lo=2 cont=1	in days	mm	mg		growth rate length	growth rate weight
tank no	species	substrate	oil level	time	length	weight	condition	GrowthL	GrowthW
2	3	2	2	90	81	5.28	0.994	0.200	0.600
2	3	2	2	90	93	6.07	0.755	0.233	0.493
2	3	2	2	90	84	5.32	0.897	0.267	0.658
2	3	2	2	90	90	5.39	0.740	0.300	0.390
2	3	2	2	90	103	12.78	1.169	0.167	0.568
2	3	2	2	90	90	6.12	0.839	0.200	0.413
2	3	2	2	90	87	6.02	0.914	0.200	0.006
2	3	2	2	90	78	3.57	0.752	0.200	0.514
2	3	2	2	90	95	8.79	1.025	0.167	0.445
2	3	2	2	90	98	8.24	0.875	0.300	0.406
4	3	2	2	90	96	6.69	0.756	0.200	0.390
4	3	2	2	90	87	6.96	1.057	0.200	0.661
4	3	2	2	90	71	2.44	0.681	0.200	1.217
4	3	2	2	90	98	7.63	0.810	0.267	0.495
4	3	2	2	90	99	9.46	0.975	0.167	0.320
4	3	2	2	90	77	3.09	0.677	0.267	0.327
4	3	2	2	90	76	4.24	0.966	0.200	0.982
4	3	2	2	90	93	7.70	0.958	0.200	0.772
4	3	2	2	90	79	4.94	1.002	0.167	0.549
4	3	2	2	90	83	6.94	1.214	0.067	1.018
mean					88	6.38	0.903	0.208	0.561
variance					81	5.69	0.023	0.003	0.075
se					2	0.53	0.034	0.012	0.061
7	3	2	1	90	105	15.55	1.343	0.300	0.873
7	3	2	1	90	94	13.72	1.652	0.267	1.326
7	3	2	1	90	84	8.32	1.403	0.300	1.139
7	3	2	1	90	90	10.64	1.459	0.233	0.946
7	3	2	1	90	89	9.38	1.330	0.267	0.974
7	3	2	1	90	85	8.90	1.449	0.233	0.746
7	3	2	1	90	98	14.68	1.559	0.267	0.961
7	3	2	1	90	99	13.77	1.420	0.267	0.852
7	3	2	1	90	88	9.95	1.460	0.300	1.235
7	3	2	1	90	99	13.32	1.373	0.267	0.811
8	3	2	1	90	95	11.09	1.294	0.267	0.780
8	3	2	1	90	85	7.87	1.282	0.300	0.942
8	3	2	1	90	83	7.92	1.386	0.233	0.928
8	3	2	1	90	98	17.31	1.839	0.233	0.814
8	3	2	1	90	99	12.83	1.322	0.233	0.831
8	3	2	1	90	92	11.54	1.482	0.233	0.754
8	3	2	1	90	93	11.09	1.379	0.267	0.695
8	3	2	1	90	89	8.57	1.215	0.300	0.838
8	3	2	1	90	101	14.64	1.421	0.233	0.790
8	3	2	1	90	98	11.52	1.224	0.400	0.916
mean					93	11.63	1.415	0.270	0.908
variance					41	7.60	0.021	0.002	0.027
se					1	0.62	0.032	0.009	0.036

Appendix Three. Raw Data. Biomarkers of Fish Health (Chapter Four)

	yellow=1	mud=1	high=3	in days		
	rock=2	sand=2	lo=2			
	halibut=3		cont=1			
tank no.	species	substrate	oil level	time	erosion	macrophage
5	1	1	3	90	66	7
5	1	1	3	90	10	9
5	1	1	3	90	15	10
5	1	1	3	90	0	12
5	1	1	3	90	2	10
19	1	1	3	90	10	11
19	1	1	3	90	26	9
19	1	1	3	90	33	8
19	1	1	3	90	30	11
19	1	1	3	90	27	10
mean					22	9.7
variance					376	2.2
se					6	0.5
14	1	1	2	90	5	17
14	1	1	2	90	0	17
14	1	1	2	90	23	15
14	1	1	2	90	0	19
14	1	1	2	90	10	17
22	1	1	2	90	0	18
22	1	1	2	90	0	20
22	1	1	2	90	7	17
22	1	1	2	90	0	17
22	1	1	2	90	5	19
mean					5	17.6
variance					53	2.0
se					2	0.5
9	1	1	1	90	0	18
9	1	1	1	90	0	18
9	1	1	1	90	0	14
9	1	1	1	90	0	15
9	1	1	1	90	0	17
20	1	1	1	90	0	15
20	1	1	1	90	0	17
20	1	1	1	90	0	15
20	1	1	1	90	0	17
20	1	1	1	90	0	17
mean					0	16.3
variance					0	2.0
se					0	0.4

tank no.	yellow=1 rock=2 halibut=3	mud=1 sand=2	high=3 lo=2 cont=1	in days time	erosion	macrophage
10	2	1	3	90	44	9
10	2	1	3	90	14	10
10	2	1	3	90	25	8
10	2	1	3	90	5	9
10	2	1	3	90	33	10
21	2	1	3	90	36	10
21	2	1	3	90	22	11
21	2	1	3	90	15	10
21	2	1	3	90	5	10
21	2	1	3	90	23	11
mean					22	9.8
variance					167	0.8
se					4	0.3
12	2	1	2	90	0	17
12	2	1	2	90	10	16
12	2	1	2	90	0	15
12	2	1	2	90	5	18
12	2	1	2	90	10	20
17	2	1	2	90	0	15
17	2	1	2	90	0	16
17	2	1	2	90	12	17
17	2	1	2	90	10	17
17	2	1	2	90	5	18
mean					5	16.9
variance					25	2.3
se					2	0.5
15	2	1	1	90	0	20
15	2	1	1	90	0	21
15	2	1	1	90	0	17
15	2	1	1	90	0	18
15	2	1	1	90	5	18
24	2	1	1	90	5	17
24	2	1	1	90	0	18
24	2	1	1	90	0	19
24	2	1	1	90	10	20
24	2	1	1	90	0	20
mean					2	18.8
variance					12	2.0
se					1	0.4

tank no.	yellow=1 rock=2 halibut=3	mud=1 sand=2	high=3 lo=2 cont=1	in days time	erosion	macrophage
13	2	2	3	90	100	11
13	2	2	3	90	30	13
13	2	2	3	90	100	9
13	2	2	3	90	60	12
13	2	2	3	90	73	11
23	2	2	3	90	53	11
23	2	2	3	90	59	13
23	2	2	3	90	73	10
23	2	2	3	90	75	12
23	2	2	3	90	69	12
mean					69	11.4
variance					439	1.6
se					7	0.4
6	2	2	2	90	0	19
6	2	2	2	90	0	18
6	2	2	2	90	10	16
6	2	2	2	90	20	16
6	2	2	2	90	23	18
18	2	2	2	90	0	19
18	2	2	2	90	20	16
18	2	2	2	90	18	17
18	2	2	2	90	10	18
18	2	2	2	90	12	16
mean					11	17.3
variance					80	1.6
se					3	0.4
16	2	2	1	90	0	20
16	2	2	1	90	0	16
16	2	2	1	90	0	18
16	2	2	1	90	0	16
16	2	2	1	90	0	19
11	2	2	1	90	0	20
11	2	2	1	90	0	18
11	2	2	1	90	0	17
11	2	2	1	90	0	18
11	2	2	1	90	0	17
mean					0	17.9
variance					0	2.1
se					0	0.5

tank no.	yellow=1 rock=2 halibut=3	mud=1 sand=2	high=3 lo=2 cont=1	in days time	erosion	macrophage
1	3	2	3	90	100	6
1	3	2	3	90	100	7
mean					100	7
variance					0	1
se					0	0
2	3	2	2	90	66	18
2	3	2	2	90	54	17
2	3	2	2	90	73	20
2	3	2	2	90	64	15
2	3	2	2	90	95	16
4	3	2	2	90	65	18
4	3	2	2	90	66	16
4	3	2	2	90	100	16
4	3	2	2	90	43	19
4	3	2	2	90	0	19
mean					63	17.4
variance					774	2.7
se					9	0.5
7	3	2	1	90	0	19
7	3	2	1	90	0	19
7	3	2	1	90	0	20
7	3	2	1	90	0	21
7	3	2	1	90	0	21
8	3	2	1	90	0	17
8	3	2	1	90	0	19
8	3	2	1	90	0	19
8	3	2	1	90	0	18
8	3	2	1	90	0	20
mean					0	19.3
variance					0	1.6
se					0	0.4