

LETHAL AND SUBLETHAL EFFECTS ON SELECTED  
ALASKAN MARINE SPECIES AFTER ACUTE AND LONG-TERM  
EXPOSURE TO OIL AND OIL COMPONENTS

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# Annual Report

(For the Period April 1, 1977 to March 31, 1978)

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## Summary and Implications of Research

Progress on FY 77 studies for research unit #72 was generally good except for a few studies requiring considerable chemical expertise. Loss of three chemists (**GS-11, GS-9, GS-7**) from the program mid-way through the fiscal year followed by a delay of 2 months before replacement with one permanent chemist (**GS-9**) and a temporary chemist (**GS-5**) required rescheduling of some projects. Chemical support is now nearly back to normal with the exception that the **GS-11** chemist has not been replaced, reducing our capacity for chemical R&D and manuscript preparation. Contract support from the NOAA National Analytical Facility, including co-authorship of some chemically oriented studies has helped to relieve this problem of chemical support.

Progress on FY 78 studies is going well and is generally on schedule. Progress on studies requiring chemical **R&D** is satisfactory with no major **problems** encountered.

A number of manuscripts are in preparation but a **significant** back-log of manuscripts exists. Progress on manuscripts is returning to normal, assisted by the fact that several junior scientists within our research unit have developed professionally to the level of competent writers. An up-grading of professional competence in oil research is common throughout the research unit, which is paradoxical in light of the recent indications of reduced funding for effects studies and the continued need for such studies.

A substantial base of oil effects data has been accumulated. Implications are that

- (1) Oil effects and responses of organisms to oil are complex, species dependent, and variably modified by environmental factors (temperature, salinity **etc**).
- (2) Effects studies are producing a number of observations useful in evaluating the impact of oil in the real environment.

(3) There is a need to test laboratory findings in the **field**.

(4) Several research findings indicate the need for further effects research, especially with long-term chronic exposures.

(5) Extremely low concentrations of hydrocarbons reduce survival of marine organisms.

(6) Immediate or delayed death is not necessarily a prerequisite to impact on a life stage; behavioral changes of larvae (**nonswimming** response) may be just as effective in eliminating the individual (predators) as outright death from oil.

(7) Much of the research data generated in this program has had immediate application and use by regulatory **agencies**, e.g. Alaska Governor's Office, legislative committees, and Federal and State Agencies.

## Introduction

### General Nature and Scope of Study

The research is addressed to the general question, "What are the effects of petroleum hydrocarbons on arctic and subarctic **biota**"? It involves physiological and bioassay tests of applied research on species indigenous to the Gulf of Alaska, Bering Sea, and Beaufort Sea. The major emphasis of research has shifted from strictly descriptive acute toxicity determinations to mechanistic studies and sublethal tests that will eventually **allow** prediction of oil impact on the **biota**.

Our studies can be broken down into two basic themes. (1) Toxicity challenge experiments, where we attempt to identify sensitive species, life stages, factors that affect toxicity, or components that are most responsible for toxicity, and (2) sublethal physiological response, where we attempt to identify, measure, and characterize physiological responses that are indicative of oil stress. Eventually these sublethal studies will provide information useful in identifying exposed animals in the field, an evaluation of how stressed the exposed animals are, and possibly the mode or mechanism of toxic **action**. We often conduct uptake-depuration studies in parallel to the above tests, to correlate tissue concentrations with effects, which will aid in the interpretation of results.

OCSEAP funding on effects studies at Auke Bay began in the last 2 months of FY 75, continued at significant funding levels through FY 76, 77, and will continue through at least FY 78. This report describes progress associated with **OCSEAP** funding only, and draws from published or drafted manuscripts and unwritten but completed studies up to April 1978.

### Specific Program Objectives

General program objectives have remained relatively constant throughout the life of this research unit, although emphasis has changed as information on

various aspects of oil impact has accumulated. A **list** of current and recent past objectives follows. Objectives which have significant data accumulation are indicated with an asterisk \*.

1. Toxicity challenge experiments

**A\***. Determine the comparative sensitivity of Alaskan marine organisms to oil and oil components.

**B\***. Compare static and flow-through tests--determine the validity and reliability of static tests for comparing animal sensitivities to oil.

**C\***. Determine whether Alaskan species are more sensitive to oil than animals from warmer climates.

D. Determine the effect of temperature on toxicity and sensitivity.

**E\***. Determine the sensitivity of comparative life stages of fish to oil.

**F\***. Determine the sensitivity of comparative life stages of crustaceans to oil.

**G\***. Identify the major toxic compounds or fractions in the **water-soluble fraction (WSF)** of oil.

H. Determine whether the major toxic components have similar modes of action, metabolic rates, uptake-depuration rates and if they are synergistic or antagonistic to each other.

2. Sublethal effects experiments

A. Determine behavioral responses of organisms to oil and the effect on survival.

**B\***. Determine the effects of aromatic hydrocarbons on **byssal** thread formation in mussels.

**C\***. Determine the effects of aromatic hydrocarbons on oxygen consumption and breathing rates in pink salmon fry.

D. Determine the pathway of elimination from fish of aromatic hydrocarbons

**E\***. Determine the effect of aromatic hydrocarbons exposures on crab heart rates and oxygen consumption.

F. Determine the effect of aromatic hydrocarbons on growth of fish and invertebrates.

**Relevance** to Problems of **Petroleum** Development

The above objectives when answered will allow an evaluation of the relative contribution of each important oil component to the toxicity of oil WSP. This information will allow some prediction of effects of oil contamination on the **biota** by relating chemical analyses of the water (amount of each important oil component) to the toxicity of each component. In addition, the other objectives will evaluate comparative sensitivities of Alaskan organisms, effects of temperature on toxicity, and effects on sublethal physiological parameters.

Uptake-depuration information relating tissue burdens with particular exposure regimes, with observed sublethal effects, and with measured stress factors is of particular value in understanding the impact of hydrocarbons in the environment. Coincident with this is the need for information on the duration that a particular physiological perturbation exists in an animal following exposure. In other words, can we identify animals in the field which have been exposed to oil for short durations or chronic levels of oil? Do they recover rapidly? Information collected is expected to have considerable value in detecting, predicting, and monitoring oil impacts in the environment when coupled with hydrocarbon baseline information, information on concentrations achieved in the field following spills, and the persistence of oil in arctic environments.

The research completed on a number of spills indicating that oil from spills in temperate and Arctic regions is much more persistent than oil spilled in warmer waters shows the value of research on the effects of temperature on oil toxicity, sublethal effects, metabolism, etc. Our data indicate that

temperature and salinity affects the sensitivity of marine organisms to oil and these effects are species dependent, therefore the need for more information on temperature and salinity effects is apparent.

#### Current State of Knowledge

Prior **to** this research, information on acute and chronic toxicity to Alaskan organisms was limited to certain commercial species. Beyond acute toxicity determinations little was known about sublethal effects or the relative toxicity of important oil components. A base of information has now begun to accumulate on acute toxicity, sublethal effects, behavioral responses, and the effects of various factors on these parameters, but it is only a small part of the information needed to predict and evaluate the major impacts of hydrocarbons in the marine environment. Essentially little is yet known about the effects of temperature, salinity, and pressure (depth) on the ability of arctic organisms to metabolize, eliminate or recover from petroleum exposure.

A recent (December **1977**) review of RU #72 prepared for **OCSEAP** discusses the current status of knowledge and research needs relevant to research objectives of this project. The list of completed and in-progress publications at the end of this report forms part of the information base for that discussion.

A considerable amount of information has been generated in this effects study and in other effects studies throughout the scientific community. There is a need to review the current status of knowledge, take a close look at information gaps, and the direction of future research. **In** our opinion there is **still** a great need for effects research information, especially the correlation of effects observed in the laboratory with effects noted in field exposures, and the determination of exposure regimes and tissue burdens in field and laboratory exposures.

## Progress on FY-77 <sup>'</sup>Studies

### Situation

The progress in FY 77 was generally good, except in a few studies requiring chemical expertise. The death in late FY 77 of a competent chemist spearheading two projects was a severe blow to the entire program. The transfer of one chemist and the resignation of a second chemist for personal reasons, created additional voids during the same time period. A replacement chemist was finally hired after a **delay** of 2 months and by mid-summer we had added a temporary chemist to carry us through the fiscal year.

This series of events, especially the death of a colleague and a friend, created an emotional atmosphere which undoubtable affected our performance and resulted in the loss of two positions, one permanent and one part-time permanent from the program.

### Accomplishment (Study Title, Objectives, Methods, Status, Results, and Significance)

1. Determination of the acute toxicity of the water-soluble fraction WSF of crude oil:

Objective 1A: Determine the acute **toxicity** to species not tested previously.

Methods: Acute static 96 h **bioassays** were conducted before being phased out and flow-through tests substituted. Animals were retained in clean water for observation of delayed mortality.

Status: The manuscript "Toxicity of Cook Inlet Crude Oil and No. 2 Fuel Oil to Several Alaskan Marine Fishes and Invertebrates" was presented at the AIBS meeting in Washington, **D.C.** 1976, but included no **GC** measurements. Some of these tests had GC measurements, but were not included, while many of the earlier tests with sensitive and commercial species were conducted prior to our ability to analyze **WSF's** by GC. Consequently, we have conducted a few more static

**bioassays** with crude and No. 2 fuel oil to several new species that represent groups we have not tested and several old species that we had tested before, but lacked GC analysis. Except for a study comparing static and flow-through tests with sensitive and tolerant species, this is the last study containing static tests and is intended to be a final study comparing sensitivities based on GC and IR measurements of over 30 subarctic species from several phyla and environments. With only 2 species remaining to be tested (availability is limited by season), the drafting of a final manuscript is in progress now. A summary of **bioassays** completed by species follows in Table 1.

Results: We found fish and shrimp to be the more sensitive animals tested with oil, with some exceptions, like starry flounder. Most subtidal invertebrates such as shrimp, crabs, and sea urchins were also moderately sensitive. Intertidal animals were quite tolerant to 96 h static exposures, probably because they are adapted to environmental stress and can "close-up" during brief static exposures to oil solutions which are declining in concentrations over time. The sensitivities to 96 h static exposures, with declining doses, vary considerably between various animal groups, much more so than sensitivities during 96 h **flow-through** exposures (see next objective #1B).

Significance of Results: These results simulate brief exposure to **oil** from a point source and give environmental managers information on the types of animals that are most sensitive to short-term, acute oil exposures.

Objective 1B: Compare sensitivities of tolerant and sensitive species to toluene and **naphthalene** when exposed in static and flow-through tests.

Methods: Pink salmon, the black sea cucumber *Cucumaria* **vegae**, the shore crab *Hemigrapsus*, *Eualus* shrimp, and a **subtidal** snail *Colus* sp. were tested with **naphthalene** and **toluene** using both static and continuous-flow bioassay methods. In the static **bioassays**, the concentrations decline with time, while in flow-through tests, the concentrations remain stable.

Table 1. Status of static crude oil and No. 2 fuel oil bioassays  
 Species tested using 96 h static bioassays with Cook Inlet Crude oil  
 (CI) and No. 2 fuel oil (FO).

Species	Completed by FY 77		Completed or <u>scheduled</u> <sup>1/</sup> in FY 78	
<b>Fish</b>				
1. <u>Oncorhynchus gorboscha</u>	CI	FO		
2. <u>Salvelinus malma</u>		FO	(CI)	
3. <u>Theragra chalcogramma</u>	CI	FO		
4. <u>Clupea pallasii</u>	CI	FO		
5. <u>Platichthys stellatus</u>	CI			(FO)
6. <u>Myoxocephalus polyacanthocephalus</u>	CI	FO		
<b>Crustacea</b>				
7. <u>Orchomene pinguis</u>	CI	FO		
8. <u>Acanthomysis pseudomacropsis</u>	CI	FO		
<b>Crustacea-shrimp</b>				
9. <u>Pandalus borealis</u>			CI	FO
10. <u>Eualus suckleyi</u>	CI			FO
11. <u>Crago alaskensis</u>	CI	FO		
<b>Crustacea-crabs</b>				
12. <u>Pagurus sp.</u>			CI	FO
13. <u>Hemigrapsus nudus</u>	CI	FO		
14. <u>Paralithodes camtschatica</u>			CI	FO
<b>Echinoderms</b>				
15. <u>Strongylocentrotus drobachiensis</u>			CI	FO
16. <u>Leptasterias hexactis</u>			CI	FO
17. <u>Eupentacta quinquesemita</u>	CI	FO		
18. <u>Cucumaria vega</u>	CI	FO		
<b>Mollusks - Limpets and Chitons</b>				
19. <u>Collisella scutum</u>	CI	FO		
20. <u>Notoacmaea pelta</u>	CI	FO		
21. <u>Katharina tunicata</u>	CI			FO
22. <u>Tonicella lineata</u>	CI			FO
23. <u>Mopalia ciliata</u>	CI			FO
<b>Mollusks-clams</b>				
24. <u>Chlamys hericus</u>	CI			FO
25. <u>Mytilus edulis</u>		FO	CI	
26. <u>Protothaca staminea</u>	CI	FO		
<b>Mollusks-snails</b>				
27. <u>Margarita pupillus</u>	CI			FO
28. <u>Littorina sitkana</u>	CI	FO		
29. <u>Nucella lima</u>	CI	FO		
30. <u>Colus halli</u>		FO	CI	
31. <u>Buccinum plectrum</u>	CI			FO

<sup>1/</sup> Those in parentheses are scheduled and will be completed by June 1978.

Status: All tests are complete. The manuscript "Comparative sensitivities of five marine organisms exposed to **toluene** and **naphthalene** by static and flow-through tests" is in preparation.

Results: The differences between static **and** flow-through tests for a given species have been insignificant with the sensitive fish and shrimp, and dramatically different with tolerant species. The differences between animal response to the static and flow-through exposures is probably caused by the rate of toxicity. The "so called" sensitive species take up hydrocarbons quickly, effects are noted within a few hours, and deaths appear in 12 h or so. The tolerant species do not show signs of stress (except the slowing down and lack of movement), and deaths are not noted for at **least** 24-48 h. These **animals** have the ability to "hold up" and wait for the static concentrations to decline to sublethal concentrations.

Significance: These tests tell environmental managers that some animals can be tolerant to short-term exposure to **WSF's** of oil, and can have better survival rates if the concentrations decline within 24-48 h. If the concentrations **continue** at relatively high concentrations, all animals appear vulnerable.

Objective **1C:** Determine acute toxicity of WSF and aromatic hydrocarbons to (a) larvae of new species, previously untested, and (b) crustacean larvae; before, during, and after molting.

Methods: Tests will be static. Larvae of previously untested species (such as mussels, barnacles, snails, and sea urchins) will be tested with WSF, **toluene**, and **naphthalene**. Massive cultures of daily released crustacean **larvae** will be reared on **phytoplankton** until tested.

Status: (a) Tests with several species were cancelled because of hiring freezes that caused administrative delays in hiring key personnel.

(b) Toxicity tests with **coonstripe** shrimp 1 arvae before, during, and after molting from **Stage I to II** were completed with both **toluene** and **naphthalene**. A manuscript that compares toxicity data and uptake data (see objective 4A) is in progress. "Acute toxicity and uptake-depuration of **toluene** and **naphthalene** by **coonstripe** shrimp larvae exposed before, during, and after molting from **Stage I to II**".

Results: Molting animals were more sensitive than **nonmolting** animals when exposed to **toluene**, but little difference was observed for **naphthalene**.

Significance of Results: Molting larvae seem to be the most sensitive stage ever tested to oil. Mortality to **toluene** was found at ppb levels which could occur in the environment after an oil spill.

Objective 1D: Determine sensitivity changes of several **salmonid** species when transferred to seawater at time of normal seaward migration.

Method: Salmon smelts were tested (static bioassay) in fresh water and after acclimation to salt water.

Status: Data acquisition and analyses have been completed. The data on out-migrant smelt tests in seawater has been included in the manuscript "Sensitivity of Alaskan Freshwater and **Anadromous** Fishes to **Prudhoe** Bay Crude Oil and Benzene". The manuscript will be submitted to a journal in April 1978.

Results: The sensitivity of three species of **salmonid** smelts in seawater (after rapid acclimation-3 days) was about twice the sensitivity of **out-**migrant smelts tested in freshwater, to **toluene**, **naphthalene**, and WSF of **Prudhoe** Bay crude oil. Even though the out-migrant smelts are "pre-adapted" for the normal migration to seawater, the initial introduction is stressful, resulting in a lowered tolerance to oil.

Significance of Results: Salmon are most sensitive to oil pollution at the time of smelt migration from fresh- to seawater. Environmental managers

will want to minimize the risk of **oil** pollutants in estuaries that first receive out-migrant smelts.

2. Determine which components of oil account for toxicity:

Objective 2A: Assess the toxicity role of phenols and **heterocycles** by determining quantities available in oil and **WSF's**, determining the acute toxicity of the major compounds found in the **WSF** to two species and determining the persistent compounds in the tissues of exposed organisms.

Method: Standard 96 h static assays were used. Oil and WSF samples were analyzed by **GC-MS**. Labeled compounds were used in uptake-depuration tests.

Status: All bioassay and uptake tests have been completed. Detailed analyses by National Analytical Laboratory at Seattle are completed, The manuscript "Occurrence of toxicity, accumulation, and depuration of phenol and **cresol** in salmon and shrimp" is in preparation.

Results: Phenols and **cresols** were found in low concentrations in oil and oil **WSF**. **Cresol** was more toxic than phenol (**TLM's** were in the low ppm range). Salmon were more sensitive to both compounds than shrimp. Phenol and **cresol** accumulate in both species and the retention was longer in shrimp than salmon.

Significance: The relatively low toxicity, low accumulation, and low concentration (low availability) of the compounds in oil WSF indicates that phenol and **cresol** are not major contributors to the toxicity of **un-aged** oil WSF.

Objective 26: Determine toxicity of natural WSF and a synthetic WSF to three species with flow-through tests to determine whether the synthetic WSF accounts for all the toxicity.

Method: A synthetic **WSF will** be created by combining the most important aromatic oil components (as determined by **GC**) in the same ratio that they are found in the natural WSF. Flow-through **bioassays** will compare the

toxicity of the synthetic and natural **WSF's**. Compounds will be added or deleted as needed to account for toxicity.

Status: This study was delayed by the death of Loren **Cheatham**, chemist, and resignation of chemist Sue Way, who operated the GC. The new chemist, Steve Lindsay, has received specific **GC** training for WSF hydrocarbons; and **GC** analyses have been calibrated with standards from NOAA analytical facility at Seattle. Some **R&D** was started in FY 77, but **R&D** for WSF generation was not completed until early FY 78 and this project is currently **in** progress in FY 78.

Objective 2C: Determine the magnitude of synergistic action of mono- and **dinuclear** aromatic hydrocarbons by conducting time-dependent toxicity recovery curves with mono- and **dinuclear** aromatics to three species with flow-through tests.

Methods: Tests will be with individual compounds and combined mixtures. Standard flow-through assays with stable concentrations will be used. This will be a beginning effort to assess the relative importance of mono- and **dinuclear** aromatics.

Status: This study was also delayed by the loss of two chemists. The project was **re-evaluated** and included as FY 78 goal and is currently in progress (see section 4).

3. Effects of oil on survival of animals in the field:

Objective 3A: Determine the effects of short-term exposures to WSF'S on the survival of tagged scallops that are returned to the natural environment.

Methods: Scallops will be tagged, exposed, and returned to pens in Auke Bay, with exposed and nonexposed starfish (**Pycnopodia helianthodes**) also being introduced into the pens. Their survival will be monitored for up to 3 months. Tests with pure aromatic fractions will also be used.

Status: Preliminary tests with scallops exposed to sublethal doses of oil and returned to pens in the natural environment indicated that oil exposure reduced survival. Control and exposed scallops were preyed upon by the sunflower star Pycnopodia helianthodes which entered the pens. A follow-up study with scallops and Pycnopodia was completed; however, reduced feeding by both control and oil exposed starfish made the results of the experiment inconclusive. Handling of starfish is believed to have disrupted their incentive to feed, As we have found previously, field studies are unpredictable and often require large samples, difficult logistics, and much R&D due to the many uncontrollable variables.

4. Determine the tissue burden of several species exposed to oil or labeled aromatics, and their ability to rid themselves of hydrocarbons.

Objective 4A: Determine the accumulation in larvae of WSF's spiked with labeled isotopes.

Method: Larvae were exposed for varying lengths of time to radiolabeled compounds and analyzed by liquid scintillation counting. The form of the isotope (parent compound vs metabolite) were determined by the method of Roubal (1976). No attempt was made to identify metabolites.

Status: Data acquisition completed. Coonstripe shrimp larvae (stages I, II, and I molting to II) were exposed to labeled toluene and naphthalene. Analyses of live vs dead larvae (killed with O<sub>2</sub> deprivation) and of molted skins and bodies of stage 11, were completed. Roubal samples for metabolites were taken. The manuscript "Accumulation and deputation of <sup>14</sup>C toluene and naphthalene in stage I, stage II, and molting coonstripe shrimp larvae" is being prepared.

Results: Larvae accumulated oil components very rapidly (equilibrium levels in 20-60 minutes), probably because of the high surface area to volume

ratio. We showed that labeled hydrocarbons were taken up, rather than adsorbed on the carapace surface, by finding essentially **all** the isotope in Stage II larvae that had been exposed only as Stage I, prior to molting (and no isotope was found in the cast exoskeleton). Deputation of **naphthalene** was slow (96 h), but **toluene** deputation was even slower (most remaining after 96 h). Live larvae accumulated more rapidly than dead larvae.

Significance: Shrimp larvae accumulate oil components very rapidly. Even a brief exposure will cause the maximum amount of accumulation. The observation of rapid toxicity and rapid uptake have been used by oil discharge permit reviewers to justify the implementation of lower discharges during the spring in **Cook Inlet**.

The slow deputation, especially **toluene**, indicates low or nonexistent metabolic potential. Shrimp larvae would transfer accumulated residues to their predators (next **trophic** level).

Objective 4B: Determine the aromatic hydrocarbon uptake deputation pattern in **salmonid** smelts when exposed in seawater and in fresh water.

Methods: Effects of salinity on uptake, metabolism and deputation were investigated by exposing pink and chum salmon out-migrant fry (fresh water and salt water simultaneously) to the toxicants for 24 h and sampling for **isotopic** content and percent metabolize (by the method of **Roubal**) in whole animals during exposure and during the clean water deputation phase. Isotope counting was by liquid scintillation.

Status: The uptake, metabolism, and deputation of radio-labeled **toluene**, **naphthalene**, and methyl **naphthalene** were determined with pink salmon and chum salmon fry acclimated to fresh water and to seawater. The comparison of uptake in fresh and seawater is included in a manuscript currently in progress "uptake and deputation of  $^{14}\text{C}$  **toluene** and **naphthalene** by different

life stages of salmon".

Results: Salinity did not affect accumulation or deputation of labeled compounds in whole animals. The increased sensitivity of salmon in seawater vs fresh water cannot be explained by different uptake **patterns** between fresh and seawater exposures. A small follow-up study to look at other parameters to explain this difference is currently in progress in FY 78.

Objective 4C: Determine the pathway and rate of elimination of labeled mono- and **dinuclear** aromatics in fish; identify the labeled compounds as "parent" or "metabolite".

Methods: **Salmon** were fed  $^{14}\text{C}$  **naphthalene** or **toluene**. The fish were placed in partitioned chambers which separated the gill area from the anal area, and with the ureters catheterized. Samples of water from the gill and anal chambers were analyzed periodically during the 24 h experiments. Samples of urine and tissue were analyzed **radiometrically** at the end of 24 h. Metabolize percentage was determined for each sample.

Status: Data collection was completed. The manuscript "Excretion of labeled **toluene** and **naphthalene** by gills vs gut of Dolly Varden trout" is in review.

Results: **Toluene** was eliminated more rapidly than naphthalene, primarily from the gill as parent hydrocarbon. There was less metabolism of toluene than naphthalene. Very little **naphthalene** was excreted in 24 h. Small percentages of the total amount ingested appeared in liver, gall bladder, intestine, but was almost entirely metabolize.

Significance: These results explain the mechanisms of oil hydrocarbon elimination, and the differences observed in the retention of mono- and **dinuclear** aromatic hydrocarbons.

Objective 4D: Determine the uptake, metabolism, distribution and elimination

of naphthalene in intertidal Hemigrapsus crabs.

Methods: Adult crabs were exposed to <sup>14</sup>C **naphthalene** and the blood, hepatopancreas, muscle, gut, and nerve ganglion sampled periodically during exposure and a deputation period. The **Roubal** method was used to determine metabolize level. Crabs were exposed to naphthalene in their bath then divided into three groups for deputation **in** water, in air, or normal tidal cycle.

Status: The study was completed. Results from this study are to be combined with another test with **toluene** scheduled for this spring 1978.

Results: Hepatopancreous accumulated the most, followed by nerve ganglion, green gland and muscle. The most metabolism occurred in the green gland. Residues are deputed at a slower rate than in fish. Deputation was slowed considerably during air exposures.

Significance: Understanding the pathways and metabolic rates of aromatics in invertebrates 'brings us closer to the point of predicting the impact of oil on this species. This is some of the first work ever on this type of intertidal invertebrate.

5. Effects of oil and oil components on **byssal** thread formation in mussels.

Objective 5A: Determine the rate of **byssal** thread extrusion of mussels exposed to WSF, **toluene** and **naphthalene**.

Method: **Mussels** were exposed to oil, **toluene**, and naphthalene for 48 h under static conditions. The rate of **byssal** thread extrusion was monitored during and after exposure.

Status: The study was completed and the manuscript "Effects of crude oil WSF, **toluene**, and naphthalene on **byssal** thread extrusion rates of mussels" is in preparation.

Results: The **byssal** thread extrusion rate was reduced by exposure to

oil components at sublethal levels. The reduction was linearly related to oil concentration for each of the **toxicants**.

Significance: Reduction of **byssal** thread extrusion could have major implications in the field. The success of juvenile mussel attachment could be lowered, and adults could have reduced survival due to their inability to replace damaged **byssal** threads and subsequent loss from the substrate.

6. Temperature effects on oil toxicity and metabolism.

Objective 6A: Determine the effect of temperature on fish and shrimp sensitivity to toluene and naphthalene, and the effect on uptake-depuration.

Method: Uptake tests and **bioassays** were flow-through. Samples were taken during the uptake tests to determine percent **of metabolite (Roubal method)**. During the uptake studies, viscera, muscle and whole body residues were determined.

Status: Flow-through assays and uptake studies were completed at 4°, 8°, and 12°C with **toluene** and naphthalene exposures to pink salmon juveniles and **Eualus** shrimp. The manuscript "Effects of temperature on accumulation and depuration of aromatic oil components in pink salmon and **Eualus** shrimp" is in preparation.

Results: No effects of **temperature** were found on oil component accumulation and depuration. Flow-through tests supported previous results of the earlier static temperature study in that toluene was more toxic to fish at lower temperatures and naphthalene was more toxic to shrimp at high temperatures. These results were expected because both species are relatively sensitive, with rapid uptake, and effects are observed quickly after beginning exposure; indicating that most of the "action" is in the first few hours, when there is little difference between static and flow-through exposures. Effects of temperature on uptake would probably be noted on

"slow" species, such as **sessile molluscs**.

Significance: Effects of temperature on toxicity vary with species. Certain animals are more sensitive at cold Alaskan temperatures indicating the need for more study. Toxicity mechanisms differ between fish and shrimp.

## Progress on FY 78 Studies

## Situation

In FY 78, our studies are generally going well, and are on schedule. The preparation of manuscripts is increasing, probably about normal now, although the finished-reviewed products are some time away.

Accomplishments (study, title, objectives, methods, and status)

## 1. Toxic components and synergism of toxic components:

Objective 1A: Compare the toxicity of water-soluble fractions (**WSF's**) of crude oil with synthetically produced **WSF's**.

Methods: Exposures are flow-through, analyses by GC, and test animals are pink salmon fry and shrimp (**Eualus**). Synthetic mix contains the most important aromatics in the same amounts as oil WSF.

Status: Experimental design is complete. The apparatus has been constructed, and is being tested, and tests on shrimp are scheduled to begin in spring 1978. GC analytical equipment has been upgraded (automatic sample changer and integrator) and is functional.

2. Synergistic effects of **toluene** and **naphthalene**:

Objective 2A: Determine if **toluene** and naphthalene have synergistic toxicities to pink salmon fry, snails (**Colus<sub>SP</sub>**), **Mytilus**, and **Eualus** shrimp under flow-through conditions.

Methods: Flow-through **bioassays will** be used with both compounds individually and simultaneously.

Status: The assays testing **toluene** and **naphthalene** with **Eualus** shrimp were completed in December 1977 and data analyses are in progress. Tests with the snails, **Colus jordani** and the mussel, **Mytilus edulis** have been completed (March 1978). Final tests with pink salmon fry are scheduled for spring 1978, when they are available.

Objective 2B: Determine if **toluene** and naphthalene have synergistic effects on uptake and/or deputation in pink salmon fry, snails (Colus sp) and Eualus shrimp.

Methods: Uptake studies (2-4 days) will be run to determine the equilibrium levels of each **toxicant** alone, then together. Static solutions are changed as needed to keep the concentration stable (above 90% of initial). Liquid scintillation counting is used for residue analyses, and some samples are analyzed for percent metabolize (Roubal et al. 1974).

Status: Synergistic uptake studies with Eualus shrimp were completed, and will be followed by tests in April with snails, and in May with pink salmon fry.

### 3. Larval Studies

Objective 3A: Determine the sensitivity of eggs and larvae from several noncommercial species, e.g. barnacles, mussels, snails, and sea urchins.

Methods: Static exposures will be used for these microscopic larvae, and will include tests with **WSF's, toluene, and naphthalene.**

Status: Larval **bioassays** are scheduled for spring and summer 1978 when wild test organisms will be available. Eggs have been collected from several species and are being incubated.

Objective 3B: Determine what concentrations impair swimming ability of larvae.

Methods: Several species will be tested with **WSF's, toluene, and naphthalene.** Inability to swim will be interpreted as equivalent to death in the natural environment,

Status: This study is scheduled for spring and summer 1978 when larvae become available.

#### 4. Accumulation Studies

Objective 4A: Determine the uptake and retention of hydrocarbons into gonads, new eggs, and old eggs carried by Eualus shrimp.

Methods: Exposures will be **WSF's** and isotopes, and analyses by GC and liquid scintillation. Isotope exposures are 24 h at each stage. Shrimp carrying new eggs are exposed continuously for 10 days to oil WSF. Shrimp gonad, muscle, and **hepatopancreas** will be sampled and **analysed** by liquid scintillation or GC.

Status: All studies are finished. GC analyses of tissues is in progress. Data work-up of isotope exposures is in progress.

Objective 4B: Determine the uptake of isotopes into tissues of fresh water and seawater adapted **salmonid** smelts.

Methods: Fresh water and salt water adapted smelts will be simultaneously exposed to <sup>14</sup>C **aromatics** for 24 h. Brain, **liver, muscle, gill and intestine** will be analyzed by liquid scintillation.

Status: This study is scheduled for summer 1978. Dr. R. Thomas, Chico State University will be co-investigator for this project,

#### 5. Effects of salinity on oil toxicity

Objective 5A: Determine the osmotic and ionic composition of blood in fresh water and seawater adapted smelts exposed to **toluene** and **naphthalene**.

Methods: Salmon smelt will be exposed under flow-through conditions to **toluene** and naphthalene while their blood osmotic pressure and ionic composition is monitored. Dr. W. Stickle, Louisiana State University, **will** be co-investigator for this experiment.

Status: Osmotic studies are scheduled for May-June 1978 when wild smelts normally migrate from fresh water to salt water.

## 6. Long-term Exposures

Objective 6A: Determine the effects of flow-through **toluene** and **naphthalene** exposures on growth and survival of pink salmon fry exposed at different temperatures.

Methods: Tests will be 40 days long, with samples of fish taken at 10-day intervals to assess effects on growth, fat content and caloric content. Tests will be replicated at two temperatures to determine the influence of temperature on toxicity in long-term exposures.

Status: Design of the apparatus is complete with experiments scheduled for spring and summer 1978.

Objective 66: Determine the sensitivity of several intertidal species to **toluene** and **naphthalene** exposures, with and without intermittent exposures to air.

Method: Flow-through **bioassays** will be used with animals in water and animals exposed on a tidal cycle (air and water).

Status: Bioassay with **Hemigrapsus nudus** to **toluene** and **naphthalene** are scheduled for spring 1978.

## 7. Dispersant testing

Literature review and R&D on methods of analysis and exposure will be probed. This will be a trial exercise in preparation for expanded testing in FY 79. **We** will conduct a literature survey and identify the dispersants of interest, and conduct some preliminary tests with dispersants on fish and shrimp in the summer of FY 78.

Status: Literature review has started. New information **was** learned at the recent ASTM Conference on **dispersants**. Five **dispersants** are now approved by EPA for uses that include "minimizing environmental damage". New

dispersants are relatively non-toxic, effective even at ratios of **1/50**. Exposures scheduled for summer 1978.

Recent preliminary information on FY 79 indicate a substantial reduction in funds, **eliminating** the continued effort in dispersants. If this is true, perhaps the remaining **dispersant** effort should be **cancelled** and reprogrammed into the current studies and the manuscript backlog.

8, Writing-up of previous results:

Manuscripts describing FY 77 research projects will be completed.

Status: Robert Thomas and Stan Rice presented a paper "The significance of exposure temperatures on the sensitivity and respiration of pink salmon fry exposed to **toluene** and **naphthalene**" at the symposium on Pollution and Physiology of Marine Organisms, at Georgetown, South Carolina in November 1977. The manuscript is being reviewed for publication.

The manuscript "Effects of temperature on median tolerance limits of pink salmon fry and shrimp exposed to **toluene, naphthalene**, and Cook Inlet crude oil" was revised and resubmitted for publication.

Several manuscripts have been worked on, but progress has been less than hoped for. A reduction from \$500k in FY 77 to \$300k in FY **78** has eliminated several personnel from the staff who contributed in the writing of manuscripts. Some manuscripts are being co-authored by co-investigators working for other agencies in other parts of the country. As for three manuscripts, the senior author is deceased, however, completion by junior authors is in progress.

Problems Encountered

No major problems have been encountered in research activities. Functionally, however, the present funding level is below optimum for the research unit.

MILESTONE CHART

- ▽ Technique development RU #: 72
- ◻ Experiment in progress
- Data collected
- △ Data analysis
- Proposed - Open
- Actual - Darkened

PI: Karinen, Rice, and Kern

Major Milestones: Reporting, data management and other significant contractual requirements: periods of field work; workshops; etc.

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MAJOR MILESTONES	77												8				
	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D		
A. Toxic components & synergism (flow-thru)																	
1. Comparison of natural & synthetic WSF																	
a. pink salmon fry			▽			▼										◻	
b. <u>Eualus</u> shrimp			▽			▼											
2. 'Synergism of toluene, naphthalene																	
a. Toxicity bioassay			▼														
(1) pink salmon fry																◻	
(2) <u>Eualus</u> shrimp			▼			■											
b. Uptake/depuration																	
(1) fry																◻	
(2) shrimp						■											
B. 'Larvae																	
1. Sensitivity of noncommercial species to static exposure of WSF, toluene & naphthalene.																◻	
2. Failure to swim after WSF, toluene, naphthalene																◻	
a crustacean																	

### MILESTONE CHART

▽ Technique development  
 □ Experiment in progress RU #: 72  
 ○ Data collected  
 △ Data analysis  
 Proposed - Open  
 Actual - Darkened

PI: Karinen, Rice, and Kern"

Major Milestones; Reporting, data management and other significant contractual requirements; periods of field work; workshops; etc.

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MAJOR MILESTONES	1977			1978												
	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	
b. non-crustacean																
3. Uptake and retention of labeled WSF into new and old shrimp eggs			■			●	△		□			□				
C. Sensitivity increase to smelts in sea H <sub>2</sub> O																
1. Isotope uptake into tissues									□			□				
2. "Osmotic and ionic composition of blood									□			□				
D. Long-term exposure (flow-thru)																
1. Effect of toluene, naphthalene (40d exposure) on pink salmon fry, f(T) " " "																
2. Survival of tolerant and sensitive species to " toluene, naphthalene, WSF			▽			▽	■		□			□				
E. Effect of intermittent air exposure on sensitivity of intertidal species to																
1. Sensitivity																
2. Uptake/depuration of labeled compounds																
F. Dispersant testing																
1. Literature review																
2. R&D on methods and exposure																

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## Estimate of Funds Expended for FY 78

	<u>Annual Plan</u>	<u>costs to Feb. 28, 1978</u>
Salary Costs (base pay+ benefits &COLA)	\$150.3	<b>\$150.3<sup>1/</sup></b>
Travel	9.7	7.8
Contracts	18.5	8.3
Equipment and Supplies	42.3	30.1
Other Direct & Indirect Costs	<u>79.2</u>	<u><b>79.2<sup>1/</sup></b></u>
Total	\$300.0K	\$275.7K

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<sup>1/</sup> Salary costs projected through September 30.

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- \***Rice, S.D., D.L. Cheatham, and D. Brown.** In preparation. The toxicity, uptake, and availability of phenol, substituted **phenols, naphthols,** and heterocycles compounds from crude oil **WSF**.

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