# Program Work Statements

# Environmental Assessment of the Alaskan Continental Shelf

## Volume 4 – Effects of Contaminants

U. S. DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration

U. S. DEPARTMENT OF INTERIOR
Bureau of Land Management

CONTENTS - EFFECTS OF CONTAMINANTS

WORK STATEMENT #62

I. TITLE: The Physiological Effects of Acute and Chronic Exposure to Hydrocarbons and of Petroleum on the Near-shore Fishes of the Bering Sea.

II. PRINCIPAL INVESTIGATOR

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III. GEOGRAPHIC AREA AND INCLUSIVE DATES

Bering Sea (Area: shallow waters near the shore around St. Lawrence Isld., Nome and Nunivak Isld.)

Dates: June 1, 1975 September 30, 1976. The area will be visited for only a month during the summer and winter to collect fish specimens.

IV. COST SUMMARY

FY 1975--$8,980. FY 1976--$38,691.

V. PROPOSED RESEARCH

A. Background and Objectives--We plan to investigate how certain hydrocarbons and heavy metals will affect the physiology and survival of the near-shore fish fauna of the Bering Sea. Two or three species, Myoxocephalus verrucosus, M. scorpius and Eleginus gracilis will be studied. These fishes are year around residents of the Bering sea and have been chosen for study because of their importance in the food chain of the area. These species are fed upon by many of the birds and mammals of the area and are also eaten by the eskimos living in the small villages of this area. It is important to study these species because being food fishes they could be a pathway by which toxic hydrocarbons are introduced into humans. It is well known that petroleum hydrocarbons are conserved as they pass through the various trophic levels of food chains (1).

State of knowledge of effects of hydrocarbons on cold-water fishes--In general there is not a great deal known about the effects of hydrocarbons on the physiology of fishes in temperate regions even where intensive fisheries research has been

carried out for several decades. Virtually nothing is known about the effects of hydrocarbons on polar fishes and it is only recently that the physiology of these cold-water species has begun to be studied(2)

Studies of temperate fishes indicate that when possible fishes will avoid petroleum spills (3). Sedentary organisms cannot avoid spills and thus become contaminated and in turn pass the pollutants on to fishes which feed upon them. A number of studies have been done where fishes have been exposed to various petroleum hydrocarbon fractions and the results indicate that the low boiling hydrocarbons such as the aromatic and water soluble petroleum hydrocarbon acids are the most toxic. For example hydrocarbon acids such as hexhydrobenzoic acid and napthenic acid which occur in some crude oils in concentrations as high as 4.5% kill the minnow, Phoxinus phoxinus in a few hours at concentrations of 92-118 ppm (4). Chronic exposure to a concentration of only 5 ppm will eventually result in death. Benzene is toxic to fish at concentrations between 10-40 ppm. Toluene and xylene are less toxic with concentrations of 50-60 ppm being lethal (4)

The toxicity of longer chain hydrocarbons to fishes is considerably less. Residual fuel oil and diesel fuel produce $LC_{50}$s at 2417 and 167 ppm respectively in 48 hours (4) Values around 2900 ppm have been reported for kerosene using sunfish (5).

Long term effects of sub-lethal concentrations of these petroleum hydrocarbons have not been established (6). It is clear that they will be difficult to detect because they may result in decreased growth or tumor initiation which may have obscure causes.

Some of the short chain hydrocarbons are known to produce a reversible narcotic effect and it is thought that it is because of their solubility in the lipid phase of the plasma membrane (7). It is known that certain polar fishes have higher lipid contents in their tissues (8) and thus it is possible that hydrocarbons may be more readily dissolved in their cell membranes.

It is expected that by September 30, 1976 that we will have data which will indicate whether or not the Bering Sea fishes show similar tolerances to the hydrocarbons described above. We will also have data to show to what extent these pollutants disrupt the physiological processes of these fishes such as their seasonal oxygen consumption levels and ability to acclimate to freezing conditions.

B. Methods--Little information about the fishes described above exists. Only a few studies have been done which were concerned only with their taxonomy. Thus, studies of their physiology and responses to pollutants will be a new area of research.

We plan to examine specimens of the sculpin, M. verrucosus, M. scorpius and the cod E. gracilis during the summer when the waters are relatively warm and during the winter when they are ice covered. First we plan to determine the doses of water soluble hydrocarbon fractions such as the napthalenes which will produce death in 48 hours. This will be done in both summer and winter fishes. As relatively large

numbers of specimens are needed for such acute toxicity studies, it is proposed to arrange to do this part of the study at the NMFS laboratory at Auke Bay, Alaska. We will also inquire about laboratory space and aquarium space at the Arctic Institute of Biology Laboratory at Point Barrow Alaska. The behavioral and physiological responses to these high level exposures will be noted.

It appears that we will be able to collaborate with Dr. Stan Rice at the National Marine Fisheries Laboratory at Auke Bay. We plan to catch about 75-100 small saffron cod and ship them to this laboratory where they will be exposed to the water soluble fraction of Prudhoe Bay crude oil. This water soluble fraction is rich in napthalenes which are known to be extremely toxic to organisms. The concentration of napthalenes in the water both before and after the death of the fish will be determined by extracting the water with hexane and measuring the absorbance of 221 mu on a spectrophotometer. This is the technique which Dr. Rice has used for his studies of toxicity and will be used here because of the need for standarization. We will also determine whether the napthalenes are entering the circulatory system by extracting the blood serum and assaying for napthalenes.

Once the short term lethal doses have been determined we will initiate some long term studies where doses will be sublethal. We plan to return shipped saffron cod and sculpin to our laboratory at Scripps where they will be subjected to low concerntrations of pure napthalenes. With these fishes we will determine the effects on the whole organism oxygen consumption, tissue oxygen consumption and the effect on their ability to acclimate to freezing seawater of the winter season. Their ability to acclimate will be used as a model system for determining the effect of this pollutant because of its importance for survival. These fishes elaborate glycoproteins and proteins in the liver which are secreted into the body fluids and protect them from freezing. Since these antifreeze proteins and glycoproteins are elaborated in the liver, accumulation and detoxification of napthalenes in the liver may damage this protein synthetic system and lead to death from freezing in the winter.

To monitor the effect on the synthesis of the antifreeze glycoproteins and proteins, periodic blood samples will be taken and extracted with hexane and the level of napthalene determined to find out whether it is getting into the circulatory system and liver. Dr. Rice will measure the concentration of serum napthalene at Auke Bay. Freezing and melting points of the blood serum will be determined and used as an indicator of the appearance of the antifreeze compounds in the blood of polar fishes. It is also possible that the napthalenes may affect the function of the antifreeze in the blood, thus the effect of napthalenes on the "antifreeze" activity of the antifreeze glycoproteins and proteins will be determined.

The oxygen consumption measurements will be done in a closed system using a clark oxygen electrode and the tissue oxygen consumption will be determined with a Gilson Respirometer on muscle, gill, and liver tissue slices according to the method of Lin et al.(3). These measurements should give us some idea of the extent to which these pollutants are affecting the gross physiology of these fishes.

If long term exposure to napthalenes inhibits the synthesis of antifreeze proteins then we plan to examine the morphology of the liver since we suspect they are synthesized there. Thus, we will examine the ultrastructure of the liver cells

to determine whether they have undergone significant changes.

VI. INFORMATION PRODUCTS

Upon completion of this work we should have some data on the tolerances of a few Bering Sea fishes to some of the more toxic components of petroleum hydrocarbons. Probably only a few of the more obvious effects will be known from the experiments involving exposure to low concentrations of these pollutants. We will also have baseline measurements for whole organism and tissue oxygen consumption.

VII. DATA OR SAMPLE EXCHANGE INTERFACES

As explained under methods, we will be cooperating with Dr. Stan Rice of NMFS-Auke Bay. In order for this study to be as meaningful as possible, we should know what the aromatic and hydrocarbon acid content of the Alaskan Crude oil is. We would like to have this information by July of 1975 or as soon after that as possible. Data on the heavy metal content of this crude oil would also be helpful. This information should become available through literature surveys by NWFC/NMFS.

VIII. SAMPLE ARCHIVAL REQUIREMENTS

During this study we will have collected a number of fishes which may be of use to other programs involved in this survey. We will provide extra specimens if the need arises. Also I think it would be worthwhile for someone to carry out base line measurements on the heavy metal content of these specimens. We will be willing to set aside some of the specimens for this purpose.

IX. SCHEDULE

We propose to begin the acute toxicity experiments this summer (August - September) providing we can make arrangements to catch the fishes and arrangements at an Alaskan laboratory for space. If ship time is made available this summer of next summer and the ship has laboratory space some of these acute experiments could be done aboard ship. Perhaps arrangements could be made with the programs involving sampling of the fish populations of the Bering Sea. If this sort of arrangement could be worked out it would greatly simplify some of the transportation requirements.

During this summer both sculpin and cod will be shipped to the Scripps laboratory where respiratory metabolism and chronic toxicity experiments will be initiated on the summer fishes. These experiments will be done July, August and September.

Examination of the physiology and chronic toxicity studies of the winter fishes will be done during March of 1976. These fishes will be collected through the ice and transported to the NMFS Laboratory at Auke Bay for the acute toxicity experiments. For the base line and chronic exposure studies they will be shipped to the Scripps Laboratory.

X. EQUIPMENT REQUIREMENTS

One piece of laboratory equipment is required for this study -- a Gilson Respirometer for measuring oxygen consumption of the various tissues. This item will be needed early in the summer of 1975.

XI. LOGISTIC REQUIREMENTS

During the winter we are able to collect the fishes through the ice with little problem. Transporting them to a laboratory will present some problem especially with large numbers of fish. Arrangements have been made to work at the NMFS laboratory at Auke Bay for the short term toxicity study.

To facilitate collection of the summer fishes we would like to request ship time which we anticipate will be provided by NOAA. Five days are required during each summer period (sometime between July and August) for trawling fish with a small otter trawl. A small oceanographic winch and wire will be required for towing the trawl. Holding tanks for the fishes will also be required.

XII. COST

(See attached budget sheets)

## REFERENCES


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**U.S. DEPARTMENT OF COMMERCE**
**National Oceanic and Atmospheric Administration**
NATIONAL MARINE FISHERIES SERVICE

NATIONAL MARINE FISHERIES SERVICE, NWFC
MARINE MAMMAL DIVISION
NAVAL SUPPORT ACTIVITY, BLDG. 192
SEATTLE, WA 98115

(Research Unit #71)

# WORK STATEMENT

C-2 -- Determine the acute and chronic effects of crude oil, its component fractions, and other petroleum-associated chemicals on physiological and behavioral mechanisms of selected arctic and subarctic organisms.

C-3 -- Determine the effects of crude oil on the thermoregulatory and other functions of marine birds and mammals.

A-2 -- Determine the seasonal density distribution, critical habitats, migratory routes, and breeding locales for marine mammals. Identify critical species, particularly in regard to possible effects of oil and gas development.

| | | |
|---|---|---|
| I RESEARCH UNIT 71 | | Physiological Impact of Oil on Pinnipeds |
| II PRINCIPAL INVESTIGATORS | | Roger L. Gentry and W. Bruce McAlister, NMFS, Seattle |
| III ST. GEORGE ISLAND, BERING SEA | | 1 May 1975 to 30 September 1976 |
| IV COST SUMMARY | -0-<br>$75,000 | FY 1975 - through 30 June 1975<br>FY 1976 - 1 July 1975 - 30 Sept. 1976 |

V PROPOSED RESEARCH

A. Background and Objectives

1. Primary Tasks in this project: C-2 and C-3.

2. Secondary Tasks in this project: A-2.

3. Present state of knowledge: Two papers have been written about the effects of oil pollution on phocid seals (Brownell and LeBoeuf, 1971; Smith and Geraci, 1974), and Kenyon (1971) has reviewed past incidents of oil pollution involving sea otters and fur seals. These papers do not form an adequate basis for predicting the impact of oil spills on individuals or, by extrapolation, on pinniped populations, especially on fur bearers.

4. Information required: We anticipate that oil will have an impact on pinnipeds through (1) increasing their maintenance costs through increased heat loss in air and in water as a result of pelage contamination. The magnitude of this increase depends on whether the affected animal uses fur as a thermoregulatory mechanism and therefore will vary among species or even between neonates and adults of the same species. External oil may also (2) impair their diving and feeding abilities. Kenyon (1974) noted that malnutrition was common in contaminated fur seals. Any such impariment would accentuate increased maintenance costs in (1). Finally we would anticipate (3) direct metabolic effects of ingested oil. In this research we propose to obtain data relating to subjects (1) and (2) above for the northern fur seal (Callorhinus ursinus). Studies on the metabolic fate of ingested oil may be included in future studies, and preliminary evaluation may be attempted by cooperating investigation if funds and circumstances allow. Specifically we will obtain data on the following topics which relate to subjects (1) and (2) above. The details of each item appear under "Sampling Scheme."

    1. Susceptibility of fur seals to fouling by oil on land and in water.

    2. Heat loss to air from animals with oil on the fur compared to animals without oil on fur.

        a. measure heart and respiration rates as indicators of metabolic rate

        b. measure heat conductance of pelts to quantify the insulative properties of fur

    3. Heat loss during immersion of oiled vs non-oiled animals.

        a. measure differences in thermal neutral zone

        b. measure energetics of immersion by standard $O_2$ analysis and attempt direct calorimetry

    4. Effects of oil on feeding behavior (of lactating females only).

        a. number and depth of dives made during a feeding excursion

        b. duration of trips to sea and duration of stays on land

    5. Heat loss of washed fur seals.

        a. measure heat loss to air and water of seals washed free of oil with detergents.

6. Metabolic fate of ingested oil - preliminary studies only in FY 75.

The results of this research will apply to other marine mammals that use fur for thermoregulation such as southern fur seals (all species), the young of ice breeding seals, and the sea otter. The applicability of results to adult hair bearing marine mammals (such as sea lions and all phocids) is uncertain.

5. Meeting requirements by 1976: By October 1976, 85% of items 1 through 5 above will be accomplished for one species only, the northern fur seal Callorhinus ursinus. Research on other species will be proposed after evaluation of fur seal results, and upon receipt of a permit under the MMPA.

6. Related Research: No similar research is presently being carried out to our knowledge. Mr. C. Ohata is conducting thermoregulatory and metabolic studies on fur seals in captivity at the University of Alaska, Fairbanks. His study uses different methods and has different goals, but contacts will be made with him to discuss current problems. Dr. Ronald has studied effects of oil on harp seals at Guelph University. Drs. T. Smith and J. Geraci are conducting research on effects of ingested oil on phocid seals in Canada. An increase of present funding may be requested in the event that Smith and Geraci are willing to extend their research to fur seals.

B. Methods

1. Utilization of previous data: Some methods and materials developed in past research are applicable to the present effort; little past data is of help. Methods are:

a. Thermal Conductance. Data obtained by McEwan et al (1974) on muskrats will be reviewed and used if applicable. No conductance studies have been done on fur seals to date, but methods to be used will resemble those of Kooyman et al (1974) on penguin pelts and live birds.

b. Diving. Kooyman (1968) developed hardware and techniques for measuring diving in Weddell seals. These instruments will be modified for use on fur seals.

c. Thermoregulation. Much research has been done on thermoregulation in phocids (McGinnis, 1968) using biotelemetry. Instruments similar to his are available commercially and will be modified to telemeter heart and respiratory rates on fur seals.

d. Feeding cycles. Gentry (1974) developed methods for marking and observing fur seals. The data he obtained on feeding cycles of normal (non-oiled) fur seals will be used as a control for comparison with feeding cycles observed in oiled females.

2. Sampling scheme.

a. Susceptibility of seals to fouling by oil -- up to six female fur seals will be placed in a water tank containing small, measured amounts of oil, and another six will be placed in a dry cage with an oiled floor. The extent of oil-covered fur resulting from timed exposure will be measured in both groups as will any tendency to spread the oil during grooming. Care will be taken not to expose any of the wild fur seal population to oil pollution.

b. Heat loss in air -- The twelve oiled animals above, plus twelve non-oiled females, along with six oiled and six non-oiled pups, will constitute the experimental and control groups on the breeding sites. Metabolic rates will be taken prior to release of some experimental and control animals, and a correlation between this rate and heart and respiratory rates will be sought. If heart and respiratory rates can be used as an index of metabolic rates then a telemetry pack will be made to monitor these rates in both groups when they are released to the breeding grounds. Differences in these index rates between the two groups under similar environmental conditions will reveal the extent of heat loss to air resulting from oil on the pelage. As a further test, the thermal conductance of two fur seal pelts will be measured to define the insulative properties of fur, using the technique of Kooyman et al (1974).

c. Heat loss during immersion -- the thermal neutral zone and the energetics of immersion will be measured on at least six of the experimentals and six controls if possible. Metabolic rates will be measured in a test chamber using a paramagnetic oxygen analyzer (Kooyman et al 1974) to measure $O_2$ content of expired air when the animals are partly immersed in waters of several different (2°-20°C) temperatures. Calorimetry as a means of measuring heat loss to water (and thereby derive metabolic rate) will be evaluated during these studies.

d. Feeding cycles -- Movements to sea and back of the experimental and control females will show the effects of oil pollution on feeding and diving behavior. After being marked (and subjected to oil if

an experimental), equipped with dive recorders and released, seals will be observed on the breeding sites during all daylight hours for the remainder of the breeding season. Females return to land about every eight days to suckle their young. The duration of alternate stays at sea and visits to land will be compared in the two groups. Females in both groups will be recaptured during returns to land, dive recorders will be collected and replaced, and data on the number duration and depth of dives made during the feeding excursion will be obtained from the recovered dive recorders. Based on past data, we do not anticipate that repeated handling will alter the duration of the feeding cycle.

e. Heat loss of washed fur seals -- At the termination of observations all oiled seals will be recaptured, washed free of oil using several different compounds, and held until their pelage, behavior, and heart and respiratory rates appear normal. During this period metabolic rates will be measured to compare with oiled and non-oiled animals. All captives will be fed frozen herring and squid, supplemented with vitamins using methods developed in 1974. None of the experimental or control animals will be intentionally killed. Any oiled animals showing responses that jeopardize its survival, or the survival of its young, will be immediately washed and removed from the experiment.

## VI INFORMATION PRODUCTS

A. Physiological data. We have no existing format for coding or storing the physiological data. Some of it, such as heart and respiratory rates and various temperatures, can be put in digital form relatively easily. But the diving recorders produce a continuous curve that can be described by a series of coordinate readings only after considerable hand manipulation. The proposed budget does not provide for reduction of these data to computer form. Summary copies of processed physiological data will be included in the quarterly reports.

## VII DATA OR SAMPLE EXCHANGE INTERFACES

A. No input from other investigators will be necessary for the performance of the proposed research. The hardware and methods needed in this study have been developed by personnel in the project, or by the proposed contract physiologist, and require only modifications for the present application. The only capabilities we lack involve biotelemetry instruments which are available commercially.

B. Time Frame for Interface DNA.

C. Products required by other researchers: None to our knowledge.

VIII SAMPLE ARCHIVAL REQUIREMENTS. DNA

IX SCHEDULE

A. May-August 1975 (St. George Island)
   1. Modification of existing depth recorders to produce fur seal recorder prototype.
   2. Further quantification of female feeding cycles in the field.

B. September 1975 (St. George Island)
   1. Experiment with methods of attaching depth recorders to seals at St. George Island.
   2. Make initial tests of the depth recorder on released females.
   3. Obtain raw pelts for conductance studies to be done in laboratory of contract physiologist.
   4. Transport four yearling animals to same laboratory.

C. October, 1975 to June, 1976 (in laboratory of contract physiologist).
   1. Construction of 24 depth recorders and harnesses
   2. Construction of telemetry packs (monitoring heart and respiratory rates) and testing on captive seals.
   3. Construction of chamber for experiments on pelt conductance.
   4. Construction of wet environment chamber (for metabolic rates).
   5. Preliminary experiments on energetics of immersed fur seals.

D. June-August 1976 (St. George Island)
   1. Capture animals, create experimental & control groups, release and conduct all studies listed under V, B,2 above.

E. August-October 1976 (St. George Island)
   1. Recapture and wash all experimental animals, measure metabolic rates.
   2. Data analysis and preparation of final report.

F. Interfaces. The main interface to be made within project is to couple the behavioral observations with physiology. Behavioral staff and the physiologist will work together closely at St. George Island, and will share the same observation blind. No interfaces between this and other projects are anticipated.

X EQUIPMENT REQUIREMENTS

A. Dive recorders. See "Schedule" under IX above.

B. Heat Flow Transducers. Conductance of pelts is to be measured in the laboratory of the contract physiologist. A Beckman-Whitely T 200-3 transducer used to make these measurements is available through Dr. H. T. Hammel, Scripps Institution of Oceanography.

C. Biotelemetry equipment. To be purchased commercially and shipped to St. George Island via M. V. Pribilof in March 1976.

D. Environmental bath - this chamber with bubble top and air flow and air collection aparatus is to be constructed and shipped to St. George Island aboard the M. V. Pribilof in March 1976. Dr. H. T. Hammel, Scripps, will act as advisor on construction of this chamber.

XI LOGISTIC REQUIREMENTS

A. No vessels or aircraft other than those normally employed in transportation of personnel and material to St. George Island will be required.

B. Lodging. NMFS maintains several houses on St. George Island for the use of research personnel.

C. Special facilities. The unused skin processing plant on St. George Island has been modified as a holding area for captive fur seals. It contains five water pools and a 25 x 70 ft. outdoor caged enclosure. This facility, with some modification, will be adequate for the experimental portion described in this research plan. Modifications are to begin in May 1975.

D. Laboratory of the Physiologist. It is anticipated that Dr. G. L. Kooyman will contract for the physiological research. His laboratory, Physiological Research Laboratory at Scripps, is acknowledged to be the best in the nation for keeping captive marine mammals, and for measurements such as those proposed. This project must transport four live yearling fur seals to San Diego in the event the contract is let to Dr. Kooyman. Kooyman and Gentry have conducted measurements similar to those proposed, both together and separately, in the past.

## XIII LITERATURE CITED

Brownell, R. L. and B. J. LeBoeuf. 1971. In Biological and Oceanographical Survey of the Santa Barbara Channel Oil Spill, 1969-70 (ed. D. Straughan), 287(Allan Hancock Foundation, Univ. Southern Calif.)

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Smith, T. G., and J. R. Geraci. 1974. The effect of contact and ingestion of crude oil on ringed seals of the Beaufort Sea. Interim Report of Beaufort Sea Project Study A5, December 1974 (unpublished).

Work Statement to Bureau of Land Management/Environmental Research Laboratories for Alaska Outer Continental Shelf Oil Development Impact Study

Task:

C-2--Determine the acute and chronic effects of crude oil, its component fractions, and other petroleum-associated chemicals on physiological and behavioral mechanisms of selected arctic and subarctic organisms.

C-4--Conduct laboratory and field studies to determine recovery rates of selected organisms and ecosystems from perturbations resulting from either contamination or other disturbances associated with petroleum development.

I. RESEARCH UNIT 72A--Acute and Chronic Toxicity-euphausids, mysids, sandlances, and other organisms.

RESEARCH UNIT 72B--Uptake and Depuration-sublethal metabolic response of various Alaskan organisms.

RESEARCH UNIT 331--Sublethal Effects-early egg development in crabs.

RESEARCH UNIT 334--Sublethal Effects--salmon fry respiration.

II. PRINCIPAL INVESTIGATORS--Stanley D. Rice and John F. Karinen, National Marine Fisheries Service, Auke Bay Laboratory, Auke Bay, Alaska (NWFC-Seattle).

III. AREA--Non-specific (physiological effects).

IV. COST SUMMARY--27.8K FY 1975-through June 30, 1975
200.0K FY 1976-July 1, 1975-September 30, 1976

V PROPOSED RESEARCH:

A. Background and Objectives

This proposal is addressed to the general question: "What are the effects of hydrocarbons and associated contaminants on arctic and subarctic biota?" It will involve physiology and bioassay tests of applied research on species indigenous to the three study areas (Gulf of Alaska, Bering and Beaufort Seas) described in the BLM Draft Study Plan.

Studies will be of two types; (1) measurements of acute and chronic toxicity of crude oil to selected arctic and subarctic organisms, (see Task C-2) and (2) measurement of hydrocarbon uptake, depuration, and metabolism by various tissues and species for the purpose of quantitating recovery ability by organisms (See Task C-4).

Although considerable oil toxicity data and some sublethal effects data are available for some Alaskan commercial species, the present information base is insufficient to evaluate oil impacts on the ecosystem of Alaska. Little is known about the toxicity of oil to noncommercial Alaskan species, or the effect of extreme low temperatures on oil toxicity. Essentially nothing is known about the quantitative ability of arctic and subarctic organisms to avoid, metabolize, eliminate, or recover from hydrocarbon exposure.

The amounts and kinds of information needed to meet the task objectives depends upon the similarity or dissimilarity of responses of the various species to oil exposure. Uniform responses by genera or family will minimize information requirements for evaluating the environmental impacts of oil exposure.

The amount of information on oil toxicity needed to evaluate the differences and complexities of species responses is unknown at this time. Information needs for predictive capabilities can only be evaluated after a series of bioassays and sublethal effects observation have been completed on several species at various temperatures.

We propose to generate the following toxicity data:

1. Determine oil toxicity to previously untested organisms (Euphausids, Mysids, sandlances, and others).

2. Determine oil toxicity to above organisms and previously tested organisms at several developmental stages where practical.

3. Determine oil toxicity to above organisms at various temperatures (-1° to 10° C.).

4. Determine chronic oil toxicity to eggs of shrimp, crab and herring.

To evaluate sublethal effects and recovery potential of various organisms,

we propose to generate the following sublethal effects measurements.

1. Determine hydrocarbon uptake and depuration on several previously untested species (herring eggs, crab eggs, Euphausids, Mysids, etc.)

2. Determine hydrocarbon uptake and depuration of several species at various temperatures.

3. Determine the doses of oil that cause sustained changes in metabolic rates of pink salmon fry, crabs, scallops, and other invertebrate species.

4. Determine the effect of oil on metabolic rates of various tissues of crab, salmon, and scallops after in vitro and in vivo oil exposures.

5. Determine the doses of oil that will inhibit scallop growth and modify behavior.

6. Determine the dose of oil and conditions that causes autotomy in molted crabs.

7. Determine histopathological changes in various target tissues (gut, gills, chemosensory organs, liver, etc.) of pink salmon fry and some invertebrates when exposed to lethal and sublethal oil doses.

8. Determine the effects of short term oil exposure on early development of crab eggs.

It is our intention to complete all the above experiments outlined above by September 30, 1976. By September 1976, enough data should be generated to evaluate the need for tests with additional species or variables.

Studies by Anderson et al. of Texas A.M with Gulf of Mexico species and temperatures are similar to ours in scope and methods. Quantitative and qualitative similarities and differences will be continually compared with their data.

Studies by Malins et al. (NMFS/NWFC) will be coordinated with ours to prevent unnecessary overlap. Coordination is anticipated in metabolism, detoxification rates and mechanisms, and histolopathological changes. Studies in similar areas are expected to complement each other and provide insight into adaptive abilities of various species. Analytical methods for monitoring oil concentration will be evaluated and coordinated between the laboratories. A committee for coordinating research activities with Dr. Malins group has been formed.

Studies by Caldwell, at Oregon State University parallel and supplement our crab studies. Cooperation will be sought to coordinate research efforts and monitoring of exposure levels.

Considerable coordination of research efforts are anticipated with Intertidal Baseline studies at Auke Bay conducted by Merrell, Zimmerman, and Myren; and with Feder of the University of Alaska. Bioassays with predominant intertidal species will be done.

B. Methods

1. Oil exposures will be to the water soluble fraction of Cook Inlet

and Prudhoe Bay crude oils. Slow mixing will be at low temperatures for 20 hrs. and patterned after Anderson's et al. techniques.

2. Oil concentrations in water will be monitored by I.R. spectrophotometry (Gruenfeld, 1973) and by U.V. (Anderson, et al. 1973).

3. Oil concentrations in tissues will be monitored by G.C. (contracted to Warner of Battelle, Columbus) and by U.V. spectrophotometry (Neff and Anderson).

4. Acute bioassays will be 4-day standard tests and analyzed statistically by probit analysis.

5. Dosing, handling, etc. are methods developed at Auke Bay for individual species.

6. Metabolism measurements of salmon fry will be by the method of Spoor et al. (1971) for quantitating opercular movements in free swimming fish.

7. Metabolism of tissue slices will be quantitated in a Gilson respirometer. Oxygen consumption of invertebrates will be measured by quantitating inflow and outflow water by an oxygen electrode.

8. Effects of oil on crab autotomy will be refined experiments patterned after Karinen and Rice (1973).

## VI. INFORMATION PRODUCTS

All data will be available in a summary form by September 30, 1976. Information will be published in scientific journals as units of data accumulate. Some of these products will be in manuscript form by September 30, 1976. Content is described in the following list of expected publications.

1. Comparison of acute toxicity data by species and life stages.

2. Comparison of oil concentrations that are acutely and chronically toxic to small shrimp and eggs of shrimp, crab, and herring.

3. The effect of temperature on oil toxicity.

4. Comparison of hydrocarbon uptake and depuration rates by species.

5. Comparison of hydrocarbon uptake and depuration rates by temperature.

6. Comparison of the effect of oil on metabolism of several species and tissues.

7. Comparison of oil concentrations that are acutely toxic to scallops with those concentrations that affect metabolism, growth, and behavior.

8. Autotomy responses in molted crabs after sublethal oil exposures and at varying time periods following the molt.

VII. DATA OR SAMPLE EXCHANGE INTERFACES

N/A

VIII. SAMPLE ARCHIVAL REQUIREMENTS

The OASIS system for updating our information requirements on specific subjects related to effects of hydrocarbons will be needed as various subtasks are begun.

IX. SCHEDULING OF EXPERIMENTS

1. Acute toxicity tests--untested species and life stages start--September 15, 1975--end September 15, 1976. Specific dates depend on animal availability.

2. Acute toxicity--different temperatures, Salmon fry-August 1975, Scallops-September 1975, Shrimp-October 1975, and 2 other species-1976.

3. Chronic toxicity to shrimp, shrimp eggs, crab eggs, and herring eggs. Preliminary--spring and summer 1975. Final--spring 1976.

4. Uptake and depuration determinations for untested species start September 15, 1975, end September 15, 1976. Depends on animal availability--U.V. analysis will be completed within 30 days after exposure and data from contracted G.C. analysis will be expected 60 days after samples sent.

5. Uptake and depuration determination at various temperatures. Salmon fry--August 1975, Scallops--September 1975, Shrimp--October 1975, and 2 other species--1976.

6. Determine effect of oil on metabolism.

Salmon fry - start June 1975 end September 1, 1975

Crabs - Fall 1975
Other Species - 1976

7. Determine effect of oil on tissue metabolism.

Summer 1976

8. Determine effect of oil on scallop growth and behavior.

Summer 1975 and 1976

9. Determine effect of oil on crab autotomy response.

Feburary 1, 1976 to May 15, 1976

10. Samples for histopathology using routine histology, enzyme histochemistry, microscopy, and scanning electron microscopy will be taken from selected exposures and tissues during routine oil exposures in acute and chronic tests. Samples will be analyzed inhouse and/or in cooperation with similar studies at the NWFC under Donald Malins.

X EQUIPMENT REQUIREMENTS.

N/A

XI LOGISTIC REQUIREMENTS.

Laboratory facilities will be needed at the Auke Bay Fisheries Laboratory from July 1, 1975, to September 30, 1976. Collection of test organisms will require occasional use of the Murre II and samll boats of the laboratory. Collection of animals will be made in cooperation with other units of the laboratory whenever possible.

May 13, 1975



(Research Unit #73)

WORK STATEMENT

I. TITLE: Research Unit 73. Sublethal effects as reflected by morphological, chemical, physiological, and behavioral indices.

II. PRINCIPAL INVESTIGATORS: Drs. Donald C. Malins, Harold O. Hodgins, and Mr. Douglas D. Weber, National Marine Fisheries Service, Northwest Fisheries Center, 2725 Montlake Boulevard East, Seattle, Washington 98112, (206)442-7737 and (206)442-7740.

III. GEOGRAPHIC AREA AND INCLUSIVE DATES: Beaufort Sea, Bering Sea, and Gulf of Alaska. April 1, 1975 to September 30, 1976.

IV. COST SUMMARY:

| FY 1975 | FY 1976 |
|---|---|
| through June 30, 1975 | July 1, 1975 - Sept. 30, 1976 |
| -0- | 150.0K |

V. PROPOSED RESEARCH:

A. Background and objectives

Research Unit 73 addresses itself to Task Element C2 "Determine the acute and chronic effects of crude oil, its component fractions, and other petroleum-associated chemicals on physiological and behavioral mechanisms of selected arctic and subarctic organisms."

From the combined literature available and our observations, it is increasingly apparent that marine organisms exposed to petroleum hydrocarbons and trace metals undergo considerable stress. Histologically, there is evidence for degeneration of sensory receptors (1), depletion of surface mucus cells and liver glycogen reserves (2), and melanization and disruption

of respiratory epithelium followed by necrosis and eventual sluffing of necrotic tissues (3,4). Behaviorally, sublethal levels of petroleum hydrocarbons interfere with settling and attachment of juvenile invertebrates (5), cause detachment of invertebrate adults (6), and modify or inhibit responses necessary for food-gathering, migration, and reproduction of a variety of species (7,8). Petroleum components also cause teratogenic effects in developing larvae (9), morphological anomalies in adults (10), and a reduction in feeding rates along with concomitant growth lag (11).

Many of the above physiological disruptions are typical of generalized stress conditions (e.g., following exposure to pathogens or thermal variation); however, for trace metals and petrochemicals there is an added complication resulting from biological accumulation which may amplify the contaminant effect, even though the environmental level is negligible. In tuna, for example, lead is found only in small amounts ($<1$ ppb) in muscle and other tissues, but accumulates in the epidermis to as much as 2 ppm (12).

The objectives of studies proposed by Research Unit 73 are to identify and evaluate the following with respect to chronic exposure to petroleum hydrocarbons and trace metals:

(1) Structural and ultrastructural changes in internal tissues, epithelial tissues, and their secretions.

(2) Perturbations in chemosensory systems of fish and shellfish and related behavioral modifications.

(3) Life history stages of crustacea and molluscs most sensitive to uptake and disruption of normal physiological responses.

(4) Uptake and resultant alterations in chemical and physicochemical properties of mucus.

The products of this unit which can be expected by September 30, 1976 are the following:

(1) Effects of total water soluble oil components, and selected aromatic hydrocarbon fractions on (a) structure and ultrastructure of flatfish and salmonid skin and gill epithelium, and crustacean chemoreceptors; (b) sperm and egg viability, hatching time, metamorphosis, settling success, and uptake in selected larval and adult invertebrates; (c) chemosensory-related behavior modification in crustacea, and neurophysiological changes in olfactory responses of salmonids.

(2) Effects from ingestion of whole crude oil and selected fractions thereof on: (a) structure and ultrastructure of internal tissues of salmonids; (b) feeding rate, growth, and uptake in selected juvenile and adult invertebrates.

(3) Effects from surface coating of fresh and weathered whole crude oil on: (a) sperm and egg viability, hatching time, and metamorphosis of selected invertebrates; (b) structural and ultrastructural changes of epithelial tissues of selected molluscs and crustacea.

(4) Effects of trace metals (e.g., lead, cadmium, and vanadium): (a) uptake and accumulation of metals into body and gill mucus of salmon and flatfish; (b) changes in chemical and physiocochemical properties of mucus; (c) structural, ultrastructural, and histochemical changes of skin and gill epithelium; and (d) chemosensory impairment in crustacea and salmonids.

Studies proposed in Research Unit 73 are planned to be carried out at NWFC, Seattle. Other research units concerned with sublethal physiological effects of chronic exposure on fishes are located at the NWFC Auke Bay Laboratory, Juneau. A committee has been formed within the NWFC to coordinate activities of the Seattle and Juneau laboratories. The members of this coordinating committee will maintain contact with other research units (e.g., R.U. 183, Corvallis, Oregon) to insure that related research is complementary and not duplicative.

B. Methods

Under Task C, full utilization will be made of available existing and related publications, reports, and techniques. To facilitate these efforts, one of the principal investigators for Research Unit 73 is also the principal investigator for Research Unit 75, Contaminant Effects - Literature Review.

In respect to animal species, emphasis will be on physiological principles as demonstrated by studies on representative marine species from a broad range of taxonomic groups. All organisms considered for investigation are indigenous to the subarctic biota, and are locally available:

Phylum Chordata

Class Osteichthyes

Order Salmoniformes

genus Oncorhynchus

Order Pleuronectiformes

genus Parophrys

Phylum Arthropoda

Class Crustacea

Order Decapoda

genus Cancer

genus Pandalus

Phylum Mollusca

Class Lamellibranchiata

Order Filibranchiata

genus Mytilus

Order Eulamellibranchiata

genus Ostrea

A multidisciplinary approach is proposed in this research unit; thus, methods of data analysis will be variable and dependent on the discipline involved. Initial stages of this research through September 30, 1976, will be laboratory-oriented with methods of exposure to varying concentrations of pollutants and to environmental variables (e.g., temperature ($2^{\circ}$ to $10^{\circ}$C), dissolved oxygen, photoperiod, salinity, and pH) parallel in all comparative experiments. Concentrations of hydrocarbons in water samples will be determined via techniques such as liquid and gas chromatography, and photofluorometry. Basically, the methodology to be employed is as follows:

(1) Analysis of structural, ultrastructural, and histochemical changes will be conducted with light microscopy, and transmission and scanning electron microscopy. Target organs have been identified and methods of tissue fixation have been perfected for species of salmonids, flatfish (pleuronectids), and crustacean sensory receptors in previous preliminary experiments carried out by NMFS.

(2) In assessing behavioral modifications of crustacea after exposure to hydrocarbons and trace metals, biologically meaningful stimuli (i.e., taurine and sex pheromones) will be employed to test for overt sensory responses. Possible changes in olfactory responses of salmon will be evaluated using electrophysiological techniques.

(3) Sensitivity evaluations of life history stages of crustacea and molluscs to contaminants will employ behavioral, histochemical, and histological methods to detect developmental anomalies. Radioactive tracers will be used in determining uptake, tissue localization, and concentration of contaminants in larval and adult invertebrates.

(4) Salmon and flatfish will be exposed to trace metals under flow-through bioassay conditions. The degree of uptake by mucus will be measured with radioactive tracers at different intervals of time consistent with chronic exposure. Alterations in the chemical and physical structure of mucus will be

evaluated by electron spin resonance spectroscopy, other special techniques, and rheometry. Histological and histochemical evaluations will be conducted to identify and evaluate possible incidences of cellular alterations or aberations.

VI. INFORMATION PRODUCTS:

Specific information expected to be derived from this unit by September 30, 1976, was presented in Section V-A. It is anticipated that the results will substantially contribute to new knowledge important to an area where little information currently exists. The result will also provide a data base to determine the most productive route for future research on physiological effects of the impact of OCS activity on marine species.

VII. DATA OR SAMPLE EXCHANGE INTERFACES:

Personnel of Unit 73 will share expertise and equipment as feasible to supplement requirements of other Research Units. We will also attempt to obtain and analyze for our studies samples of organisms used by other investigators in their defined and controlled experiments. This will be done where either they have surpluses or where analyses of the same samples by two research groups are compatible with both obtaining useful data. In this way maximum information will be obtained from diverse experiments.

VIII. SAMPLE ARCHIVAL REQUIREMENTS;

Data will be submitted as published manuscripts

IX. SCHEDULE:

Not applicable

X. EQUIPMENT REQUIREMENTS:

Major laboratory equipment requirements are slated for purchase during FY 1975 and are given in Section XII (COST).

XI. LOGISTICS REQUIREMENTS

Not applicable

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(Research Unit #74)

WORK STATEMENT

I. TITLE: Research Unit 74. Identification of major processes in biotransformations of petroleum hydrocarbons and trace metals

II. PRINCIPAL INVESTIGATORS: Drs. Donald C. Malins, William L. Reichert, and William T. Roubal, National Marine Fisheries Service, Northwest Fisheries Center, 2725 Montlake Boulevard East, Seattle, Washington 98112 ((206)442-7737)

III. GEOGRAPHIC AREA AND INCLUSIVE DATES: Gulf of Alaska, Bering Sea, and Beaufort Sea. April 1, 1975 to September 30, 1976

IV. COST SUMMARY

| FY 1975 through June 30, 1975 | FY 1976 July 1, 1975-Sept. 30, 1976 |
|---|---|
| -0- | 61,000 |

V. PROPOSED RESEARCH

A. Background and Objectives

Research Unit 74 addresses itself to Task Element C2 "Determine the acute and chronic effects of crude oil, its component fractions, and other petroleum-associated chemicals on physiological and behavioral mechanisms of selected arctic and subarctic organisms." The hydrocarbons and trace metals occur to a significant degree as co-soluble components with petroleum oils in the marine environment. For clarity, we will treat the contaminants separately in discussing background and objectives.

Hydrocarbons: A recent review published by the Ford Foundation (2) included the following comments: "There are reasons to believe that the effects of spilled oil in polar regions might be serious and long lasting, including: (1) cold temperatures do not permit rapid evaporation of aromatics in oil, thus allowing more of these toxic hydrocarbons to enter solution in sea water even though the solubility of these compounds is lower at low temperatures; (2) the rate of bacterial degradation and other processes of weathering are comparatively slower at very cold temperatures; and (3) the marine biota of polar regions are generally long-lived, have low reproductive potentials and do not have wide ranging dispersal stages."

Most of the data on accumulation of hydrocarbons is from short periods of exposure and relatively high levels. Further, much of the information has been related to whole animals, an approach which sidesteps the possible accumulation of hydrocarbons in specific tissues and the likelihood of detrimental effects occurring at such loci (1-12). The whole-organism data are of slight value in relating possible accumulations to physiological damage under environmental conditions. If tissues associated with high accumulations and physiological impairment are delineated for marine animals from arctic waters, such tissues would have high priority for sampling and analysis during monitoring

operations. The results of this approach would have considerable value over monitoring carried out in the absence of such relevant information.

Consistent with the principles stated above, the objectives of the proposed research are to determine accumulations of hydrocarbons and identify potential effects from chronic exposures to oil on arctic and subarctic biota. The research during the contract period will: (a) Determine the degree to which different compounds accumulate in biota from continuous exposure to nonlethal doses; (b) Determine effects of environmental conditions, such as temperature and exposure levels, on accumulation of petroleum hydrocarbons; (c) Identify whether specific sites exist in fish where accumulations result in damage to normal physiology; (d) Determine the metabolic stability (biological half-life of selected aromatic hydrocarbons in the test organisms.

Trace Metals: A wide spectrum of data exists suggesting that marine organisms are susceptible to detrimental physiological changes when exposed to trace metals (1-8) which accumulate in tissue sites, such as the kidney, liver, and gills. Lead, cadmium, and vanadium have been chosen for investigation because: (a) They are known to have toxic effects on marine organisms under conditions of chronic exposure; (b) Environmental levels would be expected to increase substantially from drilling operations;

and (c) Accumulations and physiological effects at the lower temperatures characteristic for arctic conditions have not been delineated.

Objectives for the initial studies are: (a) Measure the uptake and accumulation of lead, cadmium, and vanadium in salmon and flatfish employing both radioactive and non-radioactive trace metals; (b) Delineate the relative distributions of the trace metals in key tissues (e.g., liver, kidney, and gills) in salmon and flatfish; identify interactions with cellular components via techniques, such as autoradiography.

The above objectives will be completed and reports will be written during the 15-month contract period (see VI. Information Products).

The work under this Research Unit will be closely coordinated with R.U. 73 and the Research Units at the Auke Bay Fisheries Laboratory. A NMFC committee has been appointed to ensure that the Research Units studying the effects of petroleum hydrocarbons and heavy metals are complementary. Individual investigators will also coordinate their work with other laboratories investigating the effects of petroleum hydrocarbons and heavy metals, such as those funded by EPA and API. Mechanisms have been initiated, for example, to maintain contacts with Northwest laboratories having related projects, such as Battelle Northwest, Washington State Department of Ecology, and the University of Washington.

B. Methods:

Work described on both petroleum hydrocarbons and trace metals will be an extension of ongoing work within the Environmental Conservation Division. Techniques used will be based on published works and on data arising from ongoing programs within the Division.

Petroleum Hydrocarbons: Salmon, flatfish, and spotted shrimp and larvae will be exposed (2 to 10°C) to petroleum oil, fractions thereof, and standard aromatic reference compounds under either static, flow-through, or suitable water-exchange systems. Concentrations of hydrocarbons in tissues and water samples will be determined via techniques, such as head-space analysis for volatile compounds, liquid chromatography ($^{13}$), photofluorometry, thin-layer chromatography, and gas chromatography.

The degree of accumulation of radioactively labeled hydrocarbons (e.g., $^{14}$C-naphthalene) and petroleum oil fractions will be evaluated in the test species. The accumulation of hydrocarbons at key tissue sites will be assessed, together with an evaluation of the nature of metabolic products and incidences of damage as reflected at the cellular and subcellular level. The work will be carried out in close cooperation with complementary activities conducted at the Auke Bay Fisheries Laboratory and will be modified as indicated by the proposed literature review

(Research Unit 75). The work closely intermeshes with the investigations at NWFC titled "Sublethal effects as reflected by morphological, chemical, physiological, and behavioral indeces" (Research Unit 73), which will be carried out concurrently with the present activities.

Trace Metals: Salmon and flatfish will be exposed to various concentrations of lead, cadmium, and vanadium under typical chronic bioassay (flow-through) conditions. Using both radioactive and non-radioactive trace metals under simulated arctic temperature conditions (2 to 10°C), (a) measurements will be obtained of the uptake and accumulation in key tissue sites (e.g., liver, kidney, and gills), (b) identification of interactions of these metals at the cellular level will be evaluated using autoradiography and other suitable techniques, and (c) possible alterations at the cellular and subcellular level will be delineated. The findings will be presented in report form at the end of the contract period and ultimately submitted for publication and/or oral presentation at scientific meetings. This work will be carried out in close cooperation with the studies on metal interactions with epithelial mucus (Research Unit 73) and will be consistent with data arising from the literature review (Research Unit 75).

VI. INFORMATION PRODUCTS

The data and information from this work will be submitted in scheduled progress reports and publications will be submitted to appropriate journals.

The work will provide important preliminary data on the uptake, accumulation, and biotransformation of both petroleum hydrocarbons and trace metals in specific key tissue sites of selected species found in the arctic and subarctic regions. Moreover, the results will contribute to a data base where virtually no detailed information presently exists. The data will show whether hydrocarbons and trace metals are deposited and accumulated in key sites, such as kidney, liver, brain, and fat deposits. The chemical nature of altered hydrocarbons, notably aromatics, will be determined. The proposed work will lead to a rational basis for sampling of organisms and for establishing a basis for the selection of tissue sites for monitoring uptake by organisms during and after oil operations.

VII through XI.

To achieve optimal information, personnel of Research Unit 74 will maintain contact with personnel on related programs, not only on Alaskan projects but also on other projects and programs on the biological effects of petroleum oil. Interface with the physiological effects project at Auke Bay will be both informal between individual researchers and more formal through the MMFC coordination committee.

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(Research Unit #75)

WORK STATEMENT

I. TITLE: Research Unit 75. Assessment of available literature on effects of oil pollution on biota in arctic and subarctic waters.

II. PRINCIPAL INVESTIGATOR: Dr. Donald C. Malins, Director, Environmental Conservation Division, National Marine Fisheries Service, Northwest Fisheries Center, 2725 Montlake Boulevard East, Seattle, Washington 98112 ((206)442-7737).

III. DATES: July 1, 1975 to September 30, 1976

IV. COST: $32.3 K

V. OBJECTIVES AND METHODS

A. Objectives

Review the available literature as described in Task Element C1: (a) On toxicity of crude oils and crude oil components (including heavy metals), and (b) On the composition and toxicity of formation waters, various drilling muds, and their components. Compare these toxicities on the basis of species, life stage, temperature at exposure, water source, oil source, geographic source of organisms, and presence of heavy metals.

B. Methods

Existing information and literature delineating effects on marine biota from crude oils and their components or from activities related to offshore drilling will be compiled and evaluated, particularly in relation to operations and

pollution in arctic or subarctic waters. The bibliographies will be compiled from existing reviews, as well as computerized bibliographies from OASIS and additional hand literature searches by staff. The individual literature references will be evaluated, particularly in relation to the application of results to arctic conditions and to offshore drilling operations. Published data on toxicities and pathological manifestations will be compared on the basis of species, life stage, temperature, water source, oil source, geographic source of organisms, and presence of heavy metals.

VI. INFORMATION PRODUCTS:

A report and probably a publication evaluating current knowledge and delineating areas of ignorance on the effects of oil pollution and offshore drilling on the biota and ecological systems in arctic and subarctic waters will be prepared.

Personnel: Selected staff members of NWFC with no charge for salaries

June 30, 1975

ALASKA MARINE ENVIRONMENTAL ASSESSMENT PROGRAM
WORK STATEMENT (Research Unit #123)

I. Title: Acute Toxicity - Pacific Herring Roe in the Gulf of Alaska

II. Principal Investigator:
Dr. Ronald L. Smith
University of Alaska
Fairbanks, Alaska
(907) 479-7542
SS#: 546-56-3254

III. Geographic Area and Inclusive Dates:

Gulf of Alaska - July 1, 1975 - September 30, 1976

IV: Cost Summary:

| FY 1975 through June 30, 1975 | FY 1976 July 1, 1975 - Sept 30, 1976 |
|---|---|
| | $62,005 |

V. Proposed Research:

A. Background and objectives

This proposal addresses itself to Task C-2.

A significant literature has developed on the biology of herring and their spawning. The effects of various environmental parameters on larval development and hatching success have been dealt with by a number of authors including McMynn and Hoar, 1953; Blaxter and Hempel, 1966; Taylor, 1971; Jones, 1972. Other information relative to herring life history can be found in papers by Taylor, 1964, Holliday and Jones, 1965; Stevenson and Outramm, 1953; Svetividov, 1949 and others. I have not yet been able to explore the literature for pertinent references on pollutant effects on herring roe. Information about herring spawning, including times and locations in Gulf of Alaska waters, has been gathered over the past 15 years by personnel of the Alaska Department of Fish and Game (Pirtle, pers. comm.).

The information required to meet the objectives of Task C- 2 is, of course, much more extensive than will be provided by this project alone. We are hoping to use hatching success as a measure of the

effect of various exposure times to oil pollutants on the physiological and developmental processes of a single species of fish. These data should be easily attainable. Additionally, we plan to express the effects of these pollutants on the normalcy of larvae and on pollutant uptake by developing embryos. Since spawning will occur some time in April or May, 1976, analysis of data cannot possibly begin before that time. It should be possible, however, to complete analyses by September, 1976. Report preparation should be completed by the September 30 deadline.

In looking over the other proposals submitted, it appears that similar work on toxicity effects in herring roe will be conducted by Stanley D. Rice and John Karinen, NMFS, Seattle, as part of their studies on other marine organisms. Also, hydrocarbon uptake and depuration will be studied by Rice and Karinen, NMFS, Juneau, employing herring eggs among other organisms. The details of experimental variables we will utilize are dependent on the results of current studies by Rice.

B. Methods

Sampling of herring roe will take place as soon as possible after spawning. Egg-laden algal fronds or eelgrass blades will be collected, placed in containers with gauze soaked in seawater. These containers will be transported to Seward or Auke Bay by the most rapid means possible.

These egg-covered fronds will be transferred to a series of 15 aerated aquaria, three to serve as controls (no oil contaminants)

and 12 to serve as experimental material. We plan to expose roe to water equilibrated with Prudhoe Bay crude oil. The exposure times will include 24 hours, 48 hours, one week, and continuous. Each tank will contain several dozen eggs, enough, hopefully, to insure statistical validity. Mortality and abnormality results will be compared statistically. At hatching time individual larvae will be examined for morphological abnormalities, and wet weight. The larvae (and unhatched eggs) will be frozen and retained for chemical analysis in Fairbanks. Chemical analysis will include the following procedures (see attached flow sheet). Volatile nonpolar hydrocarbons will be evaluated by gas chromatography and mass spectroscopy. Concentration of contaminants will be accomplished by the head space sampling technique of Hertz et al. (1974). These volatile components are particularly interesting due to their relatively high solubilities in water and to their noted toxicity in biological systems. After analysis for volatiles, samples will be homogenized, freeze dried, extracted and analyzed for "total hydrocarbon burden" using the TLC technique of Hunter et al. (1974). Information from this technique is both qualitative and semiquanitative allowing evaluation of 1) ug total alkane, 2) ug total aromatics and 3) ug total hydrocarbon content with an average relative error of 17-20%. We have selected this method since other workers will also be doing detailed analysis of hydrocarbon uptake and depuration (Research Unit 72 B). We will be intercalibrating analytical methodology with the research units of Rice (72 a and b).

VI. Information Products

The product which will result from this research will be a report of the effects of four exposure regimes on the hatching success and the rate of morphological abnormalities in developing herring. Also, a section on uptake of the above-mentioned oil components will be included.

VII. Data or Sample Exchange Interfaces

This study will interface with research units 72 A and 72 B. I don't know who will require the results of our work. However, the work of this and the other "effects" studies will be useful in more clearly delineating the potential immediate and long-term impacts on the littoral biota (Task A-21a). Additionally, subsamples will be shared with appropriate groups for intercalibration on hydrocarbon analysis. We will require input (including bibliographic) from data sources such as OASIS and Environmental Data Services.

VIII. Sample Archival Requirements

Although not required, it might be appropriate to preserve samples of larval herring in case other investigators wish to examine them for effects other than those being examined in this study. Discussions with Research Unit 73 personnel suggest the possibility of sample splitting for electron microscopic investigations. This may require temporary archival of material or simply sample exchange.

IV. Schedule

An exact schedule of research progress is impossible to give in advance of the work. Herring spawning is dependent not only on season generally, but on temperature as well. There is as much as five weeks variation in spawning times recorded from Prince William Sound alone. We assume that spawning will occur in late April or early May. Egg

development proceeds rapidly after spawning occurs. Collection of eggs will, hopefully, be carried out in a cooperative effort by personnel at the NMFS Auke Bay Laboratory and shipped by air to Seward. Alternatively, collections may necessitate a research vessel capable of traveling to herring spawning locations, deploying shore parties to collect the eggs and returning the eggs to the Seward laboratory within a day of collecting.

X. Equipment Requirements

This project will require 30 aquaria of about 4 l capacity, each supplied with an aerator. The TLC equipment and gas chromatograph mentioned above will be available at the Fairbanks campus through another OCS project being conducted by the associate investigator, John Pearson.

XI. Logistics Requirements

As mentioned in IX above, a vessel may be required for collections of herring roe. If necessary, this vessel should be capable of operations in the shallow water environs of Prince William Sound. It should be capable of launching shore parties to collect the herring eggs in very shallow water. Also, the eggs, once collected, will need to be returned to Seward very quickly so that larval development does not proceed too far before the experiments begin. I would thus like to hold out the possibility of a vessel from NOAA since I do not know if the University of Alaska can provide such a vessel and since, even if it could, I would have to spend part of my $62,005 project money to buy ship time. The project cannot support this additional expense.

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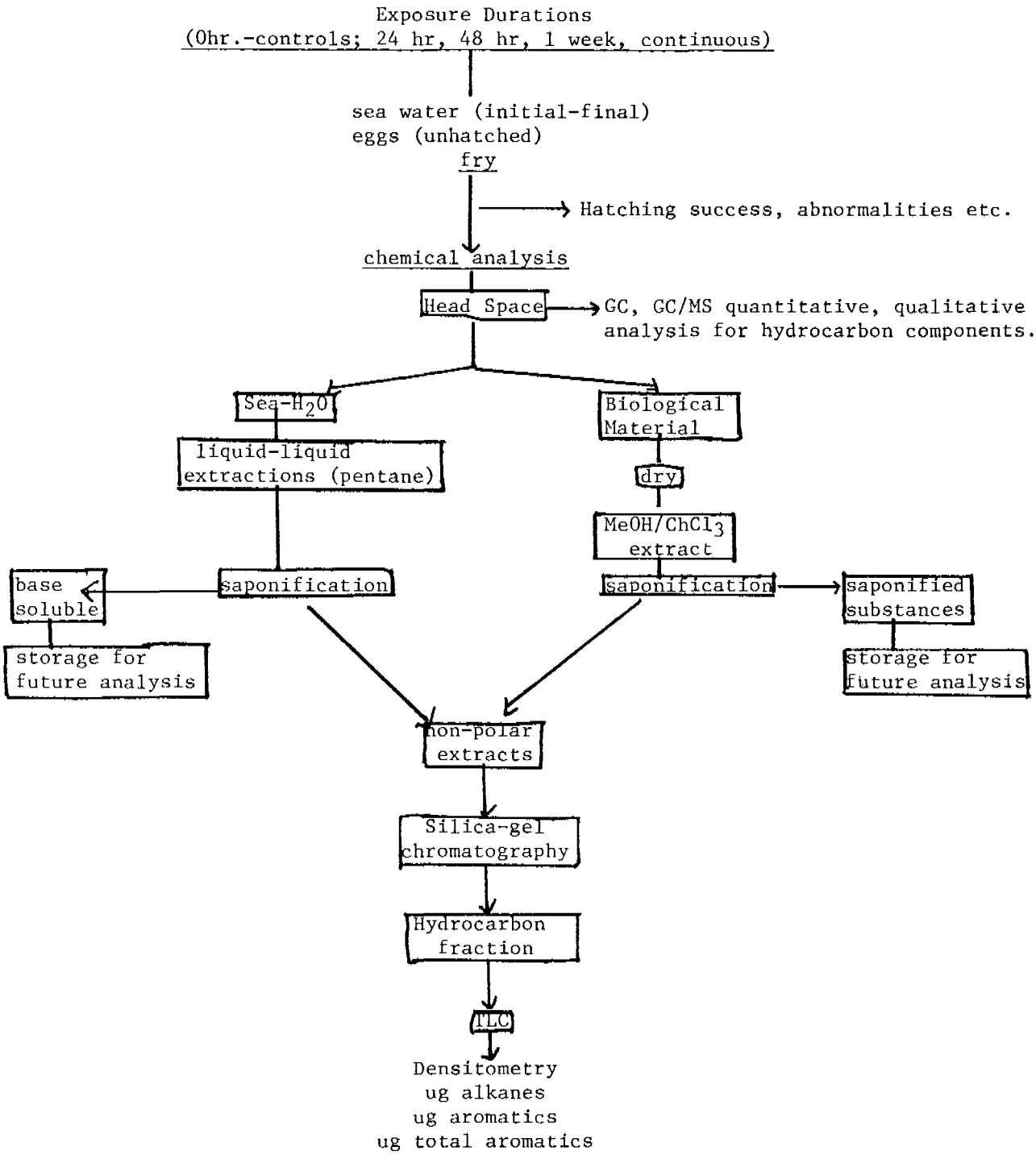
EXPERIMENTAL




Total: 40 analytical samples
160 data segments

WORK STATEMENT

(Research Unit #183)

I. TITLE

Acute and Chronic Toxicity of Seawater Extracts of Alaskan Crude Oil to Zoeae of the Dungeness Crab, Cancer magister, Dana.

II. PRINCIPAL INVESTIGATOR

Richard S. Caldwell, Assistant Professor of Fisheries
Oregon State University Marine Science Center,
Newport, Oregon 97365, (503) 867-3011

III. GEOGRAPHIC AREA AND INCLUSIVE DATES

Gulf of Alaska
July 1, 1975 - September 30, 1976

IV. COST SUMMARY

| FY 1975 7/1/75 - 6/30/75 | FY 1976 7/1/76 - 9/30/76 | Total Cost |
|---|---|---|
| $31,261.00 | $ 7,426.00 | $38,687.00 |

V. PROPOSED RESEARCH

A. Background and Objectives

The Dungeness crab fishery represents and important resource to the State of Alaska, accounting in recent years for from 10 to 40 % of the total harvest of this species from the Pacific coast of North America (Pacific Marine Fisheries Commission, 1974). Taking 1970 and 1971 as representative years, 9.7 and 3.7 million pounds were the respective annual landings of this crab at Alaskan ports (National Marine Fisheries Service, 1970 & 1971). These catches were valued at 1.4 and 0.6 million dollars, respectively. Since the value of this fishery is roughly the same for each of British Columbia, Washington, Oregon and California, areas where Alaskan crude oil may be shipped to refineries, the importance of studying the potential effects of Alaskan oil on this species is evident.

Studies of the biological effects of oil pollution in marine waters has intensified in recent years as a result of the publicity created by large oil spills such as that associated with the Torrey Canyon shipwreck. Recent reviews have summarized much of the pertinent data (Nelson-Smith, 1970; 1973). The seawater soluble components of oils have generally been considered to be the most toxic to marine organisms Dunning and Major, 1974; Struhsaker et al., 1974. Although the developmental stages of organisms are usually more sensitive to toxicants than adults, relatively little work has yet been done on these forms. Kuhnhold (1970), Struhsaker et al. (1974) and others have studied the effects of oil and water soluble components of oil on developmental stages of fish, but studies on larval stages of invertebrate species are few. Wells (1972) reported that emulsions of crude oil were lethal to lobster larvae. Larval stages of crabs and shrimp are affected by 1-100 ppb of various oil products (Mironov, 1969). Katz (1973) reported that larvae of the crab Neopanope texana showed reduced survival when exposed to seawater that had been polluted with 10 ml/liter of Venezuelan crude oil. Only larvae exposed during the earliest developmental stages were affected, however. We are unaware of any studies that have attempted to document sublethal effects of oil on crustacean larvae even though such sublethal effects have been reported for other toxicants (DeCoursey and Vernberg, 1972; Vernberg et al., 1973).

We propose in this study to evaluate the lethal and sublethal effects of seawater extractable components of Alaskan crude oil on the zoeal stages of the Dungeness crab, Cancer magister Dana.

This study will be addressed exclusively to Task C-2 of the Alaskan OCS study (Determine the acute and chronic effects of crude oil, its component fractions, and other petroleum-associated chemicals on physiological and behavioral mechanisms of selected arctic and subarctic organisms).

The specific objectives are:

1. To determine the concentrations of the total seawater soluble extract of Alaskan crude oil which causes mortalities in the first zoeal stage of the crab in 96 hr acute exposures.
2. To determine the concentration of the total seawater soluble extract of Alaskan crude oil which affects hatching success of eggs and prezoeal development.
3. To determine in continuous exposure tests, the concentration of the total seawater soluble extract of Alaskan crude oil and possible 1 or 2 specific components (e.g. naphtalene methylnaphthalene, benzene) which impairs the normal development of crab larvae from the 1st zoeal stage to at least the 5th (last) zoeal stage and, if possible, through the megalops to 1st postlarval crab. Some chronic experiments may employ toxicant exposures during only limited segments of the developmental sequence in order to identify the most sensitive stages.
4. To attempt to characterize in such variously exposed larval crabs such sublethal effects as impaired tolerance of unnatural salinities or temperatures or impaired behavior (e.g. swimming abnormalities, abnormalities of photo- or geotrophic responses, etc.).

We believe that the objectives listed above can be accomplished within the proposed project period ending September 30, 1976. During the initial months of the project we will devote our efforts to developing and perfrecting our analytical methodologies and adapting existing culture facilities for use in flowing water bioassays with seawater extracts of oil. The bulk of the actual experimental work with

crab-larvae will commence in mid-December, 1975 and continue through June, 1976; the period of the year when Dungeness crab larvae are available. We expect to be able to complete objectives 1 and 2 by the end of January, 1976. Objective 3 will be accomplished during the period January 1976 to May, 1976. We will attempt to accomplish objective 4 during the period March, 1976 to June, 1976. During the period July, 1976 through September, 1976 we will devote our efforts to data analysis and the preparation of the project final report.

Related work is planned by personnel of the NMFS, Northwest Fisheries Center, Seattle, Washington. We understand that their work with Dungeness crab larvae will emphasize analysis of ultrastructural effects of Alaskan crude oil. Their project will also entail study of other larval forms such as those of mussels, oysters, shrimp, etc. We intend to keep in close contact with that group to insure that our projects are complementary rather than duplication. We have discussed the possibility of collaboration in providing them with oil extract treated crab larvae for ultrastructural examination. The limited work planned with crab larvae at the Auke Bay NMFS laboratory will only include acute toxicity in 1st stage zoeae and will emphasize comparison with other species. We are unaware of other related work planned or presently being conducted on Dungeness crab larvae.

B. Methods

Initially, we will determine the 96 hr acute toxicity of the total seawater soluble components of the oil against the first zoeal stage of the crab. These tests will be done using standard bioassay methods under static exposure conditins as previously used in our laboratory (Armstrong et al., 1975; Buchanan et al. 1970; Caldwell et al., 1975). First stage crab zoeae will be

obtained in the laboratory from spawning female crabs collected in the nearby ocean. Examination of the effects of seawater extracts of oil on hatching success of eggs and prezoeal development will also be conducted by methods previously used in our laboratory (Armstrong et al., 1975; Buchanan et al., 1970: Caldwell et al., 1975). Unhatched eggs will be carefully removed from ovigerous female crabs in which hatching has just begun and the eggs will be placed in beakers containing various concentrations of seawater extracts of oil. After a 24 hr period, the success of hatching and prezoeal development will be compared with control treatments. We have found in studies with pesticides that the short duration prezoeal stage is one of the most sensitive of the crab larval stages.

Following the determination of acutely toxic levels of the oil extracts in seawater, we will conduct chronic toxicity experiments covering the entire zoeal period of development of the crab. These tests will be conducted using the flowing water culture methods previously developed in this laboratory (Buchanan et al., 1975; Caldwell et al., 1975). Using this method, we have successfully reared Dungeness crab larvae for up to 50 days with survivals of 85 % or better. By this time the zoeae at about 12°C are molting into the 5th or last zoeal stage. We expect to be able to improve these results through further improvements in technique. We will modify our earlier procedures appropriately to allow for isolation of individual 5th instar zoeae and expect in this way to be able to successfully rear larvae through the megalops stage to 1st postlarval crabs.

Seawater extracts of Alaskan crude oil will be prepared daily by gentle stirring of large volumes of seawater to which 1-10 % by weight of Alaskan crude oil has been added (Anderson et al., 1974; Katz, 1973). Appropriate dilutions of the seawater extract for use in the chronic experiments will be obtained either by metering the stock extract into uncontaminated seawater (Buchanan et al., 1975) or by use of a standard diluter system; probably the latter. (Mount & Brungs, 1967; Mount & Warner, 1967). After initially determining the 96 hr acute toxic level of the seawater extract to crab zoeae, a series of four to six dilutions will be selected for use in the chronic bioassay tests.

The principle criteria of toxic effect to be used in the rearing tests will be survival through the entire series of zoeal developmental stages as compared to control groups. In addition, we will attempt to characterize certain sublethal responses to the toxic materials. One of these will be to evaluate possible inhibitory effects of the oil extracts on zoeal molting and, if possible, metamorphosis to the megalopa and 1st crab stages. Katz (1973) found that exposure of the larvae of Neopanope texana to water soluble fractions of Venezuelan crude oil may enterfere with molting at least during the early zoeal stages. Similar responses have been shown with other species of crab larvae exposed to pesticides (Buchanan et al., 1970; Epifanio, 1971) and to suboptional salinities (Costlow et al., 1966). Other sublethal effects to be examined, if feasible, may include size of the resulting megalopae of 1st instar crabs, tolerance of the developed organisms to such stressful environmental conditions as high temperature or unnatural salinities, and swimming behavior and photo- and geotropic responses. We expect

to collaborate with NMFS Northwest Fisheries Center, Seattle, to examine possible ultrastructural anomalies.

During the acute and chronic exposure periods, water from the culture containers will be routinely analyzed for total toxicant concentrations by appropriate quantitative procedures. We plan to use a UV method for our routine quantitative analyses (Stan Rice, Auke Bay Fisheries Lab). We will also obtain detailed qualititative and quantitative characterization of seawater extracts in a few instances to thoroughly characterize the changes in toxicant solution during passage through the bioassay system and between daily periods of renewal of the stock extract.

Since this laboratory has not had prior experience in the detailed characterization of seawater extracts of oil, we will not attempt these analyses ourselves. Therefore, we have included a budget category for purchasing the detailed laboratory analyses of these selected samples.

C. References

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Wells, P.G. 1972. Influence of Venezuelan crude oil on lobster larvae. Mar. Pollut. Bull. 3: 105-106.

VI. INFORMATION PRODUCTS

The results of the proposed study will provide the following specific information: (1) the level of seawater extract of Alaskan crude oil which will be detrimental to the survival of Dungeness crabs through the zoeal developmental stages and (2) the level of extract which does not appear to influence these crab stages. The latter will be judged as lack of effects on survival, molting, and other sublethal criteria such as size attained at the megalopa or 1st instar crab stages, tolerance to environmental stresses of temperature and salinity, or behavioral modifications. The proposed research will not identify the specific components of oil which affected developing zoeae but based on these results future research could be aimed at this specific question.

VII. DATA OR SAMPLE EXCHANGE INTERFACES

We anticipate no data or sample needs from other investigators. However, detailed analytical data available from other projects relating to the components of Alaskan crude oil which dissolve in seawater would be of possible interest to us in evaluating our results or in the event that we were able to conduct limited toxicity experiments with selected components of the oil.

We do not know to what extent our results will be of immediate use to other projects with the possible exception of the physiological effects project of the Northwest Fisheries Center in Seattle. We will maintain regular contact with that group.

VIII. SAMPLE ARCHIVAL REQUIREMENTS

None

IX. SCHEDULE

A detailed schedule of our work is given in a preceding section. We will need an initial sample of Alaskan crude oil by September, 1975. Our final project report will be submitted by September 30, 1976.

X. EQUIPMENT REQUIREMENTS

None

XI. LOGISTICS REQUIREMENTS

The only logistics requirements of this research would be the provision of samples of Alaskan crude oil to this laboratory. Initial shipments would be required by September, 1975.

XII. COST

See attached budget pages.

WORK STATEMENT
(Research Unit #305)

I. Title: Sublethal Effects - Effects on Seagrass Photosynthesis

II. Principal Investigator:
John G. Pearson
Institute of Marine Science
University of Alaska
Fairbanks, Alaska 99701
SS#: 534-38-4946

III. Geographic Area and Inclusive Dates:

Bering Sea - July 1, 1975-September 30, 1976

IV. Cost Summary:

| FY 1975 through June 30, 1975 | FY 1976 July 1 1975-Sept 30, 1976 |
|---|---|
| | $77,677 |

V. Proposed Research:

A. Background and objectives

1. Background

Seagrass meadows are highly productive ecosystems. The primary production of these systems is to a great extent due to the productivity of the seagrasses although benthic algae, epiphytes and planktonic algae are certainly contributors. Studies show that the productivity of *Zostera marina* L. in Izembek Lagoon averages 4.8 g C/m$^2$ on an areal basis. The total production during the growing season is estimated at 812 g C/m$^2$, (McRoy 1974).

The primary thrust of this study will be directed to evaluating the effect of selected petroleum contaminants on photosynthetic uptake of carbon by eelgrass, *Zostera marina* L. Two of the petroleum components (toluene and napthalene) were selected on the basis of their solubilities and noted toxic effects on such physiological functions as osmo-regulation and respiration. (See for example, Morrow, 1974; Kauss *et al.*, 1973). The third component (dodecane) was selected as a representative non-aromatic hydrocarbon; it is expected to be relatively inert in the physiological aspects of this study.

The literature is devoid of studies related to the effects of chronic exposure to petroleum contamination on *Zostera* sp. However Baker (1970) and 1971) has noted deleterious and growth stimulating effects of petroleum on vascular plants in salt water marshes.

The total impact of chronic exposure to petroleum contamination can not be answered by this study alone. However, some questions can be raised which, if answered by this study, will aid in evaluating potential effects. These questions are:

a. Do seagrasses represent a significant sink for the contaminants? This is expected from the evidence of eelgrass density in Izembek Lagoon and the non-polar nature of leaf cuticle structure.

b. What are the rates of incorporation of the more physiologically active hydrocarbons and what are the environmental factors affecting the kinetics of uptake? Focusing on seagrass with repect to uptake will provide information on the significance of eelgrass as a sink and provide a data base for process modeling. To the extent that changes in environmental variables have concommitant effects on physiological states, and if the uptake of contaminants is an active process, alternations in the physical environment should alter uptake rates.

c. What are the primary plant surface absorption sites?

d. Are the contaminants degraded by eelgrass? Most plants are capable of extensive oxidative conversions. However, if the absorption of the contaminants is limited to cuticular wax then conversions may not occur.

e. With repect to eelgrass, what are the effects of the contaminants on the kinetics of carbon uptake in productivity?

f. Are the effects of contaminants reversible?

Directly then, this study will supply information outlined by tasks B-8, C-2, and C-4. Indirectly, information gained will feed into task A-33.

2. Specific Tasks

a. An evaluation of existing levels (if any) of toluene, naptholene, and dodecane in eelgrass, *Zostera marina* L. from Izembek Lagoon.

b. An evaluation of uptake rates by ellgrass of the selected contaminants on the basis of: (1) photoperiod; (2) light intensity; (3) salinity; (4) temperature.

c. An evaluation of the effect of the contaminants on the kinetics of photosynthetic carbon uptake in the limiting kinetic situations defined in the literature (McRoy, 1974) and by the experiments listed above (that is, at the upper limits of contaminant incorporation).

d. Formulate and apply an approach to evaluating the conversions of contaminants.

e. Determine the primary absorption tissue(s) of the contaminants.

The proposed research overlaps with Research Units 43 and 275 in as much as examination for existing levels of the contaminants will be carried out and will directly require the involvement of a member of Unit 43 (according to their proposal). It would be informative to have examinations of alterations in eelgrass surfaces and tissues in a fashion outlined by Research Unit 73.

The specific tasks outlined here should be completed and a final report avaliable by 30 September 1976.

B. Methods

Throughout the course of study, available literature will be monitored for advances in techniques and studies related to the one described here, and will require periodic assistance of bibliographic resources of OASIS. Additional information will be required on a need basis from EDS and climate agencies.

1. Sampling

*Zostera marina* L. will be collected from Izembek Lagoon on an experimental need basis. Whole plant samples will be acquired by the coring device of McRoy (1973) and returned to Fairbanks, where they can be maintained in sea water aquaria.

All sampling trips will include sub-samples for evaluation of existing levels of the contaminants utilized in this study. For this consideration, plants will be washed free of sediment and epiphytes in sea water when collected, frozen in clean glass containers as outlined by Straughan (1974) and Hertz *et al.* (1974) and returned to Fairbanks for analysis.

2. Experimental

Specific task 2a. Whole plant evaluation for existing levels of the specific contaminants will be carried out by a modified version of the head space stripping technique outlined by Hertz *et al.* (1974). A modification is necessary for sample injection into available GC/MS system. This procedure will provide information on the levels and nature of low molecular weight components of eelgrass and since samples will be acquired throughout the year, variations on a seasonal basis will be determined as incidental to the prime thrust of the project. It is possible that naturally produced molecular species will swamp those of interest (i.e. toulene, dodecane, and napthalene); if this is the case, ion monitoring will be utilized to quantitatively evaluate levels of the contaminants.

All analyses will be carried out on a replicate basis and will include calibration with plant tissues spiked with known amounts of the contaminants. Results will be reported on a wright (contaminant) to dry weight plant basis. The dry weight plant will be determined after homogenation-stripping.

Specific task 2b-general: All rate studies will be carried out in static chambers in sea water saturated with the particular contaminants under study. ($^{14}C$)-labeled contaminants will be administered in all rate studies. Each chamber will be connected to a small sea water reservoir with a $^{14}C$-doped contaminants layered on the surface to insure a constant concentration of the contaminants.

Except for those experiments in which a particular variable is under investigation, light intensities will be at one-half $V_{max}$ (referring to Milhadis-Newton experssion for saturation kinetics) for carbon uptake as identified by McRoy (1974), at 10°C, and at normal sea water salinity (35Z). For each variable, initial experiments will be carried out to evaluate appropriate temporal ranges.

Uptake will be evaluated on the amount of label recovered by utilizing the head space technique of Hertz *et al.* (1974) and cross calibrated with gas chromotography. The material absorbed on Tenax will be either (a) extracted with scintillation solutions or (b) stripped as for GC preparation into scintillation solution and then counted. The remaining plant homogenate will be dried and weighed. The rate of uptake will be in terms of the dry weight obtained at this stage. Combustion of the residue, trapping of resultant $CO_2$ in scintillation solution and subsequent counting will give a measure of $^{14}C$ incorporation by the plant. The sum of the activity by the head space sample and sample undergoing combustion will then give the total uptake of contaminant. Of course replicates will be determined for each sample time. In addition controls, and plant samples spiked with known amounts of material will be analyzed for each experimental run.

The dual approach to analysis is necessary in view of possible chemical alteration of the contaminants by plants. This cross calibration will also allow for identification of the time dependence of contaminant conversions.

2b(1) photoperiod: Seagrass samples will obtained from Izembek Lagoon in July/August for long-day studies and again in winter for short day studies. Light conditions in equaria will be held at constant intensity and with periods in phase with Izembek Lagoon.

2b(2) Light intensity: Uptake rates will be evaluated at one-half $V_{max}$, at $V_{max}$, and at inhibition intensities (with respect to carbon uptake) as found by McRoy (1974).

2b(3) Salinity: Variable salinities as found in Izembek Lagoon (29% to 32%) will be generated by the appropriate dilutions of normal sea water.

2b(4) Temperature: Temperature will be varied over the ranges noted in Izembek Lagoon (-1°C to 30°C).

Specific task 2c. Rates of $^{14}C - HCO_3^-$ will be determined at the light intensities identified by McRoy (1974) that correspond to one-half $V_{max}$, $V_{max}$, and at inhibition of carbon uptake. For these experiments non-labeled contaminants will be utilized and will be evaluated by gas chromatography. Carbon incorporation over a four hour period will be evaluated by the techniques employed by McRoy (1974). That is drying, combustion, trapping of $CO_2$ in scintillation solution and counting. Additional experiments will be carried out on plants exposed to contaminants. The duration of exposure will be determined by information derived from specific task 2b. Recovery in clean sea water will be examined after 24 hours, 48 hours, and 1 week following transfer to clean water. In addition, synergistic effects of the contaminents will be evaluated along with a sea water equilibrated with Prudhoe Bay crude oil.

## VI. INFORMATION PRODUCTS

All rate data will be presented in tabular and graphical form with appropriate statistical indices noted. When appropriate and where possible variables will be related by the appropriate mathematical models. Each portion of this study, when completed, will be reported to the appropriate agency within 120 days.

## VII. DATA OR SAMPLE EXCHANGE INTERFACES

At this time, the only apparent possibility of exchange would be with those involved in histological studies (Research Unit 73).

## VIII. SAMPLE ARCHIVAL REQUIREMENTS

Plant homogenates spiked with contaminants will be maintained frozen throughout study and longer. At this time, this research unit is attempting to enter the spiked samples into our inter-calibration pool.

## IX. SCHEDULE

The experimental aspect of this project makes it difficult to project all dates with certainty. In part, the dates will depend on acquisition of instrumentation. Preliminary schedule as follows (assuming major instrument components available).

| | |
|---|---|
| July 10, 1975 | Sample Acquisition Izembek (will need member of research unit 43). |
| August 15, 1975 | Sample Acquisition Izembek |
| October 15, 1975 | Long-day uptake, studies completed. Carbon-uptake studies on going. Possible results from autoradiography. |
| January 10, 1976 | Specimen Acquisition - Izembek |
| March 1, 1976 | Short-day uptake completed. |
| April 1, 1976 | Temperature and salinity studies completed. |
| May 30, 1976 | Light intensity studies completed |
| July 15, 1976 | Degradation - In corporation studies completed. |
| August 15, 1976 | Carbon uptake studies completed. |
| September 30, 1976 | Final Report Issued. |

Again, these dates are estimates. The first sampling acquisition should be considered firm. Additional acquisitions to those listed will certainly be necessary.

## X. EQUIPMENT REQUIREMENTS

None, other than those noted in section XII of this draft..

## XI. LOGISTICS REQUIREMENTS

No special logistics necessary. All expeditions to Cold Bay - Izembek Lagoon will be facilitated by the University of Alaska.

## XIII. REFERENCES


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