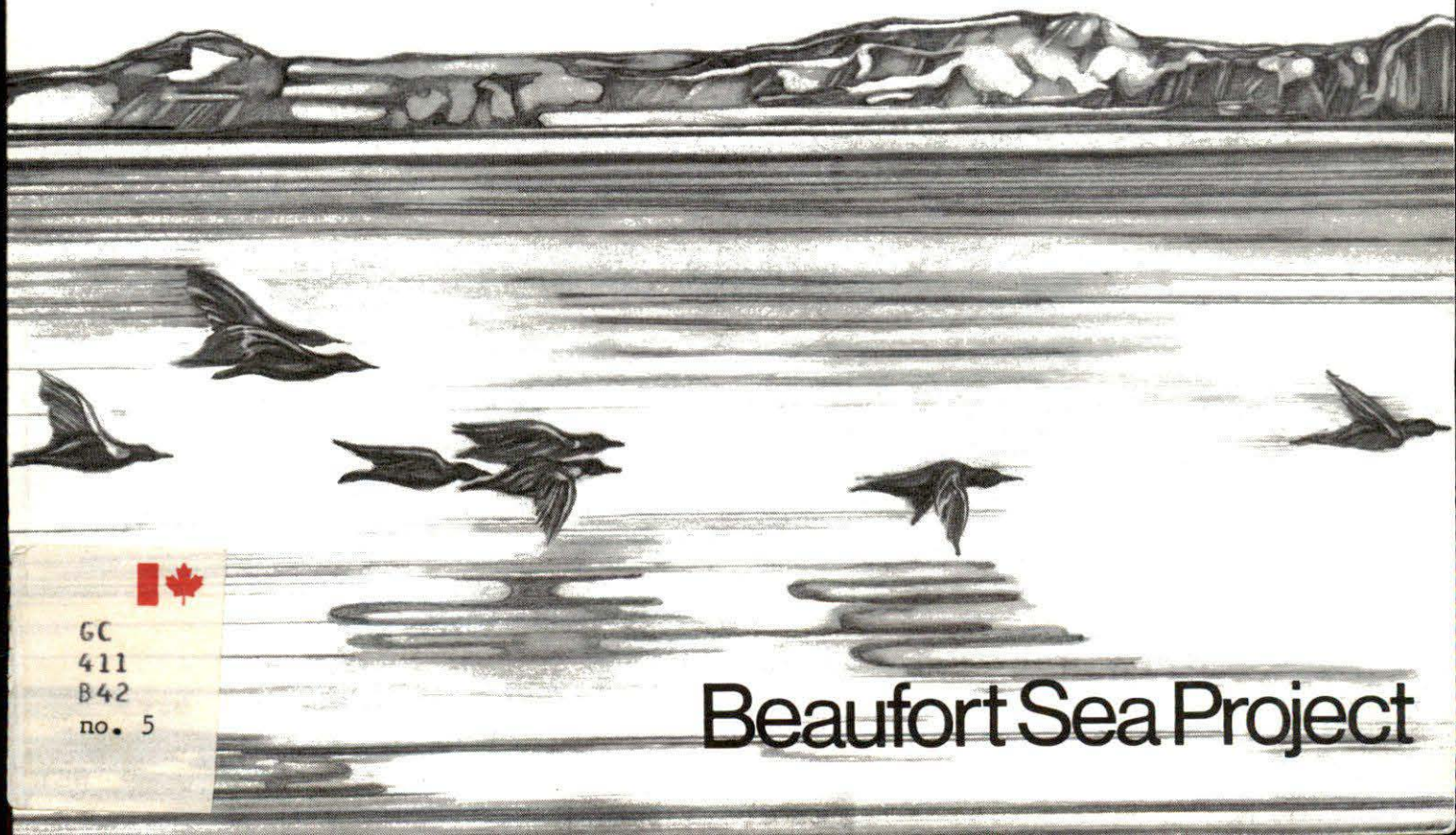
Effect of Contact and Ingestion of Crude Oil on Ringed Seals

THOMAS G. SMITH, JOSEPH R. GERACI

Technical Report No. 5



Beaufort Sea Project

THE EFFECT OF CONTACT AND INGESTION OF CRUDE OIL ON RINGED SEALS OF THE BEAUFORT SEA

Thomas G. Smith

Arctic Biological Station
Fisheries and Marine Service
Department of the Environment
P.O. Box 400
Ste. Anne de Bellevue, P.Q.
H9X 3L6

and

Joseph R. Geraci

Wildlife Disease Section
Department of Pathology
Ontario Veterinary College
University of Guelph
Guelph, Ontario

Beaufort Sea Technical Report #5

Beaufort Sea Project
Dept. of the Environment
512 Federal Building
1230 Government St.
Victoria, B.C. V8W 1Y4

December, 1975

TABLE OF CONTENTS

1. INTRODUCTION

The ringed seal, *Phoca hispida*, is the most abundant and widely distributed of the marine mammal species present in the Beaufort Sea. Because it is available to the Inuit (Eskimo) throughout the year, it has always been the basis of the coastal economy. In modern times it provides cash income from the sale of seal pelts, and is an important and constant source of food. In Canada the natives of Sachs Harbour, Paulatuk, Tuktoyaktuk and to a lesser degree Aklavik, hunt in the Beaufort Sea area. There is also positive evidence that seals in the Amundsen Gulf and Beaufort Sea are part of the same populations (Smith, 1974). The large seal catches from Holman, on western Victoria Island, must therefore be considered dependent, at least in part, on seal production in the Beaufort Sea.

This paper attempts to evaluate the effects of crude oil on ringed seals primarily, and on harp seal whitecoat pups. Studies were conducted on both the effect of immersion in oil and ingestion of oil on wild and captive seals; there exist few experimental data on the effects of oil on mammals, and none on seals.

2. MATERIALS AND METHODS

2.1 Narrative

Brown's Harbour, on the east side of Cape Parry, (70°05'30"N, 124°22'30"W) was the site chosen for capturing the ringed seals. The camp was occupied from 23 August to 9 September. During this period 96 seals, 32 of which were live, were caught using the method described by Smith et al. (1973). Only one significant change was made in the netting method which involved reducing mesh size from 30.4 to 22.8 cm stretched. All live seals were placed in holding pens, measuring 3.65 m square, constructed of pipe and chain-link fencing. The pens were located in a small salt water pond near the netting site; the location of the holding pens and other camp facilities is shown in Fig. 1.

Six seals were immersed in oil for 24 hours after which they were removed and placed in an uncontaminated holding pen in the water. Physical, hematologic and biochemical paramaters were measured throughout the study period, after which the animals were killed and necropsied.

Twenty seals were then taken to holding facilities at the University of Guelph, Ontario, and a second immersion study was conducted. There, the effects of chronic low level ingestion of crude oil were also assessed.

Harp seals, *Phoca groenlandica*, were used for the third phase of the study. Young pups were obtained from the ice in the Gulf of St. Lawrence and brought to the Magdalen Islands, Quebec, where an additional immersion and acute high dosage ingestion study was carried out.

2.2 Experimental design

2.2.1 Field oil immersion study (Ringed seals)

Six seals were used in the oil immersion experiments in the field. Prior to immersion in oil; each seal was immobilized with ketamine (Geraci, 1973), blood samples were taken and a sonic telemetry pill was administered. All the seals were placed in a plywood pen shown in Fig. 2. Pen construction allowed a free exchange with the surrounding water through an opening approximately 35 cm below the oil-water interface. The seals were kept in the pen for 12 hours before the introduction of oil. Core body temperatures were monitored every three hours during this period and blood samples were drawn to establish control values. A quantity of fresh Norman Wells crude oil, sufficient to create a layer one cm thick on the surface of the water (59.45 liters), was poured into the pen. Several small pieces of sea ice were placed in the pen at this time to cool the water which was maintained at 7° to 9°C.

The seals were left in the oil for 24 hours during which core body temperatures were monitored at frequent intervals. They were then removed from the oil, sampled for blood, clinically examined, photographed and placed in the oil-free holding pen. Two days later, two seals were removed from the pen, sampled, killed, and necropsied. The four remaining seals were sampled on day 4 post-oil. Three of them were then sampled again and killed on day 6, and the last one on day 7. Body temperature recordings continued up to the time the seals were killed. All six animals were killed by gunshot and a detailed post-mortem examination was performed in the field. Tissue samples were preserved in formalin or frozen and brought back to the University of Guelph for further studies.

2.2.2 Laboratory oil immersion study (Ringed seals)

The oil immersion study on the captive seals at Guelph was carried out in a Fibreglass holding pool measuring 3.0 x 3.6 x 1.2 m. The pool contained approximately 7,500 liters of water with sufficient sodium chloride to maintain a salinity of 24 ppm. The temperature of the water was held at between 12°to 14°C and a haul-out platform was provided . Three seals were transported from the central holding facility (described below) to the pool for the purpose of the study. At the time, they were in apparent good health and had been eating herring, *Clupea harengus*, and marine smelt, *Osmerus mordax*.

The seals were acclimated in the experimental pool for four hours. This was followed by the introduction of oil sufficient to provide a surface layer of one cm. The experiment was designed to include frequent analyses of various parameters but sudden death of all three seals precluded such studies. Post-mortem examination and blood samples were drawn immediately after death.

2.2.3 Field oil immersion study (Harp seals).

Nine, three to four week old, whitecoat harp seals were used to assess the effects of oil coating on temperature regulation in pups, which presumably are more dependent on hair coat protection than adults. The pups were taken by helicopter in two groups to the Gros Cap provincial Campground at Grindstone, Magdalen Islands, Quebec. Seven of the nine seals were held for five days in an exposed pen measuring 8 m by 15 m. They remained in the enclosure until the experimental group was completed with the arrival of two more seals. At that time each animal was placed in an airline transportation cage (Triplex Engineering, Pointe Claire, Quebec). The cages are of stiff wire construction and allow for complete exposure to the snow-covered ground and to prevailing weather conditions. The cages containing the seals were placed on an exposed cliff which afforded minimal wind protection only from the northwest.

Core body temperatures were monitored with the aid of a YSI telethermometer (Yellow Springs Instrument Co. Inc., Yellows Springs, Ohio) fitted with an internal rectal probe. Temperatures were recorded every four hours for 48 hours prior to oiling and continued for up to four days after. Six seals were designated as experimentals and three as controls. The experimental seals were coated by brushing Norman Wells crude oil onto the hair over the entire body. The next day, they were recoated in the same manner using crude oil from Mildale, Saskatchewan. Four experimental seals and two controls were killed three days post-oiling and the remaining ones a day later. All the animals were weighed before and at the end of the experiment. Complete post-mortem examinations were carried out on the dead seals.

2.2.4 Laboratory oil ingestion study (Ringed seals)

Seals for this study were maintained in the Guelph central holding facility. Five animals were acclimatized for two months in Fibreglass holding pools measuring 2.1 m x 3.0 m x 1.8 m, each containing 9,900 liters of water. The water contained sodium chloride at a level of 18 ppm; it was held at 12°to 15°C and filtered through high-rate sand filters, with a turnover rate of 1.4 changes/hr. The animals were feeding on herring and smelt throughout the acclimatization period.

For the purpose of the study, 0.547 mg of ^{3}H labelled benzene, having a total activity of 25m Ci, was added to 125 ml of Norman Wells crude oil. One ml of the final solution, having a specific activity

of 0.2m Ci/ml, was placed into each of 125 "000" gelatin capsules. The capsules were then placed into the body cavity of the food-herring and fed at a rate of five/day/seal, for five consecutive days; thus, each animal received 5 ml of labelled crude oil/day, for a total of 25 ml during the week of November 18, 1974.

Blood samples were drawn at six equally spaced intervals for 20 days prior to administering the oil in order to establish baseline values for hematology, blood chemistry and background radioactivity. Blood and tissue biopsy samples were then obtained at frequent intervals during the oil-feeding period and for four weeks thereafter.

2.2.5 Field oil ingestion study (Harp seals)

Fourteen apparently healthy harp seal pups (two to three weeks of age) were selected for an acute oil ingestion study. The two experimental dosage groups consisted of six animals each with two seals serving as controls. Individual seals were marked using numbered jumbo rototags (Nasco, Fort Atkinson, Wisconsin) applied through the web of the hind flippers.

The experimental groups contained equal numbers of males and females and the animal weight composition was similar in each group. One control seal was placed in each group. The groups were kept in separate open air enclosures measuring 3 m x 3 m, for one day following intubation to facilitate observation; after this time, they were allowed to move about freely within a larger pen. The pups were allowed 24 hours after capture to adjust to the new circumstances prior to any further handling. The animals were fasted for the duration of the experiment and weight loss was recorded.

The seals in the high dose group were intubated with 75 ml of Norman Wells crude oil, while those in the low dose group received 25 ml. A stomach tube was inserted into the control animals for a period of time equal to that experienced by the dosed seals.

One animal from each group was sacrificed on days 1, 2, 4, 6, 8 and 10 after ingestion. The control animals were killed on the final day of the experiment. Blood samples were drawn from all seals six to eight hours before intubation and again immediately prior to death. Additional blood samples were obtained from the controls on day 5. The seals were killed by a blow on the head using a regulation sealing club.

The blood was collected in Na-heparin tubes by the methods described by Geraci and Smith (1975). Plasma was removed within 1 hour, and maintained at ambient temperature (-5° to -10°C) until placed in a freezer unit 2 to 24 hours later. Enzyme analyses were performed after six weeks of storage at -20°C.

A complete post-mortem examination of each seal was made within one to two hours of death. Tissues were preserved in 10% buffered formalin for histopathologic examination.

2.3 Techniques

2.3.1 Blood sampling and processing

Table 1 lists the abbreviations and unit designation used in the blood chemistry, hematology and enzyme studies. Blood was drawn from the extradural intravertebral vein (Geraci and Smith, 1975). Blood from the harp seals was drawn from either the same site or from the hind flipper (Geraci, 1971). Samples for hemograms were placed in tubes containing the disodium salt of ethylene-diamine-tetra-acetic acid (EDTA) as the anticoagulant and maintained at about 4°C until analyzed, within 4-24 hours. Blood smears were prepared and packed cell volumes (PCV) were determined on the day of sampling. The remaining hemogram analyses were performed in Guelph two to four weeks later. The delay has been shown to have no adverse effect on the analyses (Geraci and Engelhardt, 1974). The PCV were measured with a capillary-tube microhematocrit. The hemoglobin (Hb) was determined by the cyanmethemoglobin method using Drabkin's solution. Total red and white cell counts were made using appropriate Unopette disposable pipettes (Becton, Dickinson Co., Columbus, Ohio). Blood smears were stained with Wright-Giemsa stain and 100 cells were tallied for each differential count.

Blood for plasma chemical determinations was placed in tubes containing Na-heparin as the anticoagulant, and centrifuged within one hour. The plasma was transferred to vials and frozen at 0°C until analyzed. With the exception of enzymes, plasma chemical analyses were performed with the aid of multichannel autoanalyzers (Technicon Instruments Corp., Ardsley, N.Y.), using prescribed methodology outlined in Technicon bulletins.

2.3.1.1 Enzyme analyses

Enzyme activities were determined on a Coleman 55 spectrophotometer equipped for kinetic enzyme analysis. The reagent kits used were as follows:

GPT - BMC[1] kit # 15956
GOT - BMC kit # 15955
CPK - BMC kit # 15721
Gamma-GT - BMC kit # 15794
LAP - BMC kit # 15952
SDH - Sigma[2] kit # 50-UV

In addition, colorimetric techniques were used for the following:

OCT - Sigma kit # 108
Ald - Sigma kit # 750

[1]Boeringer Mannheim Corp., St. Laurent, Quebec
[2]Sigma Chemical Co., St. Louis, Missouri, U.S.A.

Procedural information is available in the technical bulletins accompanying these kits. Abbreviations and units are listed in Table 1.

2.3.2 Hydrocarbon uptake

Blood for total hydrocarbon content from the ringed seal field oil immersion study was placed in Na-heparin tubes and processed for plasma. Tissues for hydrocarbon analyses were wrapped with aluminum foil and frozen immediately. Determinations were carried out by an extraction procedure described by Ackman and Noble (1973) and Ackman (1974). The method is outlined as follows:

a) homogenization of the tissue.
b) steam distillation of the homogenate and a small amount of hexane carrier to bring across the more solid hydrocarbons greater than C_{20}.
c) hexane extraction of the condensate.
d) preliminary analysis by fluorometry and u.v. absorption spectrophotometry.
e) concentration of extract with nitrogen for gas chromatography.
f) gas chromatography with flame-ionization detectors, using a support-coated open-tubular stainless steel column coated with Apiezon-L. Temperature programming is required from 60°C to 220°C at a rate of 5°C/min.
g) identification and quantitation of peaks by duplicate assays of standard hydrocarbon mixtures were made. Fresh and weathered samples of Norman Wells crude oil were also used.

Blood and tissues for uptake of ^{3}H-benzene from the ringed seal oil ingestion study were processed and analyzed by the method of Hansen and Busch (1967) and Amarsham-Searle Corp. (1973). Tissues for this study were obtained by biopsy sampling of liver, muscle and blubber. The seals were immobilized with physical restraint and biopsied using a tru-cut disposable biopsy needle #2N2702 with an 11.4 cm cannula (Travenol Laboratories Inc., Morton Grove, Illinois). Three to 37 mg tissue samples were placed in glass vials and frozen until analyzed.

2.3.3 Temperature recordings

Temperature transmitter pills were administered to each of the seals in the field oil immersion study. Two of the seals were immobilized with ketamine. The cylindrical pills which measured 6.5 cm x 1.5 cm in diameter were introduced into the first two seals via a two cm bore flexible tube which had been placed into the stomach. The pill was "blown" out of the tube using air pressure from a stomach pump. The procedure was discarded in favour of the more effective and less traumatic method of manually placing the pills well back onto the tongue, closing the seal's mouth and inducing swallowing by gently stroking the pharyngeal area. All the transmitters were retrieved from the stomachs at the post-mortem examination.

Each of the six pills transmitted on an inaudible frequency from 120 KHz to 170 KHz in increments of 10 KHz. The signals were received with a hydrophone and converted to a strip-chart recording. The recording in volts was transferred into temperature in degrees centigrade using a pre-calibrated graph (Bay Shore System, Springfield, Virginia).

2.3.4 Clinical evaluation

Clinical evaluation included observations on general behaviour, and physical examinations. Mucous membrane surfaces: eyes, ears, mouth, anus, and genital openings were examined. The nictitating membrane of each eye was everted and the conjunctiva scrutinized. Fluoroscein impregnated strips were moistened and applied to the eye of each animal. After 10 to 20 seconds, the eyes were examined for dye retention on the corneal surfaces. Disruption of corneal integrity, either by pinpoint shallow erosions or large ulcers was easily observed and photographed.

2.3.5 Post-mortem examination

Necropsy examination of the experimental animals was performed immediately after death. The animals which died in the netting operation served as control material and were examined within 24 hours. Tissues were placed in 10% neutrally buffered formalin and transported to Guelph. Routine histological sections were cut at five μ and stained with hematoxylin-eosin stain.

2.3.6 Measurements, biological specimens and age determination

Standard measurements, including whole body weights, were taken from all dead animals as described by Smith (1973a). Estimates of the age of the live seals were made using the ridges on the claws of foreflippers (McLaren, 1958). All dead animals were aged from the dentinal annuli of the lower canine teeth as described by McLaren (1958) and Smith (1973a). All live animals were weighed shortly after arrival at Guelph and periodically thereafter. Exact ages of animals which died in captivity were determined by counts of the dentinal annuli.

3. RESULTS

3.1 Description of the catch

A total of 96 ringed seals, 64 dead and 32 live were caught using two 100 meter nets during the period 24 August to 13 September 1974. The sex ratio of the catch did not depart significantly from unity (47 males:49 females). The age structure of the catch is shown in Fig. 3 and is compared to the age distribution of 208 seals caught in the same place in August and September 1972. The 1974 catch had significantly lower proportions of yearling (0+) seals to older seals (22:61) than the 1972

catch (147:61), (Chi square = 23.2, $P < 0.005$). In the 1972 catch, only 4% of the animals caught were adult ($\geq$ 6 years old), whereas the 1974 catch contained 40% adult seals. A significantly higher proportion of adults, (Chi square = 4.02, $P < 0.005$) is also seen in the 1974 Brown's Harbour catch when it is compared to the age structure of the net sample of 124 seals taken in August and September 1971 by Smith et al. (1973) at Herschel Island, Yukon Territory.

Figure 4 shows the mean maximum girth of netted seals from Herschel Island in 1971, Brown's Harbour in 1972 and from Brown's Harbour in 1974. The mean girths of yearling seals (0+) and all seals six years and older (adult) were computed for each net sample, and tested for significant differences using Student's t-test (Table 2). Girths of the Herschel Island seals, in both age categories, were significantly greater than those from the Brown's Harbour 1972 or 1974 samples. Comparisons between two different samples, from Brown's Harbour, show that the 1972 yearling (0+) seals had a significantly larger girth. The adult girths ($\geq$ 6 years) were not significantly larger, but this could be due in part to the relatively small number of adults in the 1972 sample.

A comparison between the two catches from Brown's Harbour shows a significantly high number of moulting seals in 1974 (Chi square = 15.21, $P < 0.005$).

3.2 Field oil immersion study (Ringed seals)

3.2.1 Clinical observation

The oil formed a uniform film on the surface of the water as soon as it was introduced into the holding pens. For the first two to three minutes, the animals continued to move about and surface with no apparent recongition of the oil. Because of the swimming movements, the oil quickly became churned into the whole water column, though most of it still remained on the surface. Within three minutes, the heads of the seals were darkened with oil. As they continued to swim about, the hair of the back became oiled, and after some time the abdominal hairs became stained.

Seven or eight minutes after the oiling, one of the seals began to lacrimate (tear) excessively, and would open and close its eyes. It jumped from the surface and shook its head violently. Soon, eye irritation became apparent in the other seals. They lacrimated profusely, yet at first there was no attempt to close their eyes and avoid the oil. Twenty minutes into the study however, some of the seals seemed to have difficulty keeping their eyes open; the conjunctiva of the eyes were obviously reddened and inflamed. The breathing rate of these same seals appeared to increase, and two of them stretched their necks out of the water and shook their heads. The animals were also observed to force air through their nostrils making an audible sound when at the surface. This general behaviour seemed to persist throughout the first four hours by which time all of the seals were lacrimating and squinting.

Throughout the remaining exposure period, five of the seals remained submerged most of the time, the sixth and most aggressive of the group would remain on the surface and continue its agonistic behaviour towards approaching persons or other seals. When on the surface all of the seals showed varying degrees of arching of the back, a behaviour which was not observed in the control group nor in the experimental group prior to oiling.

Twenty four hours after the introduction of the oil, the seals were removed from the pen and examined. The results of the examination are summarized in Table 3. All showed obvious signs of eye disturbances, characterized by blinking, squinting or closing of the lids, lacrimation, and moderate to severe conjunctivitis with swollen nictitating membranes; some evidence of corneal erosions and ulcers were also noted.

After the seals were examined, they were placed into the clean water holding pen. Within three hours, most of the eye squinting activity subsided, and there was considerably less lacrimation. They remained quiet and calm; body quivering and arching of the back was no longer detectable. Twenty hours after the transfer, they all appeared to be in good health. Their hair coats had been cleaned of much of the oil, and the eyes showed no signs of irritation. The situation improved progressively and rapidly. By the third and fourth days, there was scarcely visible evidence that the seals had been oiled.

Temperatures were monitored throughout the field oil-exposure study (Table 4). Midway through the pre-oiling period, the values dropped abruptly from a range of approximately 37.7°C to 34-36°C. This change was later shown to be caused by an offset recording needle which had not been detected in the field but was verified upon laboratory examination of the data. The numbers have been re-interpreted after recalibration of the temperature pills. The values show a stable trend with no pattern of change from one day before oiling to the end of the experiment.

Findings from gross and tissue examinations of dead seals show the presence of renal tubular necrosis in seals No. 9 and 16, as well as fatty change in the liver of ringed seal No. 16.

3.2.2 Blood findings

Results of hematologic and plasma chemical analyses taken throughout the oil-immersion study are shown in Tables 5-10. There were few consistent patterns of change in plasma enzymes (Table 11). In seals No. 7,9, and 10, glutamic oxalacetic transaminase (GOT), an enzyme of muscle and liver and creatine phosphokinase (CPK), a muscle enzyme, both decreased steadily from significantly high pre-oil levels to normal levels at the end of the study. GOT, and liver enzymes GPT, SDH, and gamma-GT showed a mild upward trend in seals, No. 11, 14, and 16. None of the remaining blood cellular and plasma

parameters showed consistent or biologically significant trends.

3.2.3 Hydrocarbons in tissues and fluids

Table 12 shows the concentrations of petroleum in tissues throughout the six day period following immersion. The highest values observed were those of blubber, kidney and muscle in the 48 hour post-oil samples of seal No. 9, and the 168 hour liver sample of seal No. 16 as shown in Table 13. Analyses of body fluids showed that whole blood had higher levels than plasma, likely due to blood cell binding of hydrocarbons. Urine and bile had the highest absolute values suggesting excretion by renal and hepatic routes, with more prolonged excretion by the liver.

3.3 Laboratory oil immersion study (Ringed seals)

3.3.1 Clinical observations

Seals No. 24, 30, and 35 were used in this study. Fresh Norman Wells crude oil was poured into the holding tank and came into contact with the seals within two minutes. Almost immediately, all three animals began to shake noticeably; eye blinking and frequent audible exhalations were observed. This behaviour continued for 15 minutes during which time the seals remained under water for unusually long periods.

Twenty minutes after the oiling, a haul-out platform was provided. The seals made no attempt to climb onto it. Swimming movements were uncoordinated.

Animal No. 24 made determined attempts to leave the pool, thrashed on the surface along the edge, dove and died 21 minutes after oiling.

Beginning at this time, the two remaining seals stopped thrashing and became quiet on the surface of the pool, but mild trembling and forced exhalation continued. Seal No. 35 died 60 minutes after the oiling. The behaviour of No. 30 remained essentially unchanged until it thrashed about briefly and died 71 minutes after contact with the oil.

3.3.2 Blood analyses

There were significant differences in glucose, uric acid, hydrocortisone, and potassium levels between pre- and post-immersion samples in all seals (Table 14). Glucose levels rose between 4 and 30%. Uric acid nearly doubled in seal No. 24. Potassium more than doubled in seals No. 24 and 30, and hydrocortisone decreased between 13 and 43%. The most dramatic changes occurred in seal No. 24 which died first.

The blood cellular changes were as follows: the total white cell count decreased in seals No. 30 and 35, reflecting a proportional decrease in all cellular elements except circulating eosinophils which showed a disproportionately greater decrease.

3.4 Field oil immersion (Harp seals)

3.4.1 Temperature recording

There were no significant changes or patterns of change in temperature between pre- and post-oiled seals or between the experimental and control groups. Values from control seals were between 35.7°C and 37.9°C throughout the study, whereas the temperatures of the experimental seals ranged between 36.0°C and 38.6°C.

3.4.2 Post-mortem examinations

On gross examination, there were no significant lesions in any of the experimental seals which differed from the controls, or which could be attributed to oil immersion.

3.4.3 Body weight changes

All seals showed a progressive weight loss throughout the study period (Fig. 5) during which time they were maintained without food.

3.5 Laboratory oil ingestion study (Ringed seals)

3.5.1 Clinical observations

There were no untoward effects or behaviour alterations in any of the seals throughout the course of the study.

3.5.2 Plasma enzyme activity

Results of the plasma enzyme studies are shown in Table 15. There was no significant change in release of liver or muscle enzymes into the plasma. Creatine phosphokinase (CPK) levels were elevated above those in the field oil immersion study and there was some increase in concentration in the seals after oil ingestion. The highest values for gamma-GT, a kidney-based enzyme were found in the post-ingestion samples of seals No. 34 and 37. They show a trend, but are not truly significantly different from control or pre-ingestion samples. LAP, also a kidney enzyme, did not change throughout the study.

3.5.3 Tritium activity in blood, plasma, and tissues

Activity in plasma rose throughout the five-day oil administration period and began to drop immediately after (Table 16). Whole blood levels rose and began to taper off during oil administration. By the end of the study, on day 28, activity was still detectable in blood; more so than plasma. All three tissue types (Table 17) taken

by biopsy showed pronounced activity at the two-day sampling; liver and blubber more so than muscle. Liver and blubber levels declined to very low activity by 28 days; muscle showed a less rapid decline.

3.6 Field oil ingestion (Harp seals)

3.6.1 Clinical observations

The presence of oil was noted on the hind flippers of the seals one and one half hours after ingestion of oil, in both the low and high dosage groups, as a result of gastro-intestinal excretion. The oiled animals grunted and vocalized more than the controls. Most of the oiled seals fell asleep within six to eight hours after ingestion, at which time the controls had already been asleep for four hours. At 10 hours post-oil, harp seal No. 6, a high dose animal, appeared to be unusually unresponsive to manipulation. This behaviour was no longer apparent on subsequent observations. For 12 hours beginning from the time oil excretion was first noted, the experimental seals had yellow stained pelts as a result of rolling in the oil-covered snow. When they were moved to clean snow, the pelts became white again within 24 hours. Aside from these findings, all observations made hourly up to 24 hours post-oil, and at three hour intervals thereafter, revealed no significant differences in behaviour or health between the control and experimental seals.

3.6.2 Post-mortem examinations

Gross examination of the oiled seals revealed no consistent pathologic lesions which distinguished them from the control animals.

3.6.3 Plasma enzyme analyses

Results of all plasma enzyme studies on harp seals which had ingested oil are shown in Tables 18 through 25. Liver based enzymes OCT,GPT, and SDH showed little or no consistent pattern of change which could be attributed to oil ingestion. SDH was elevated in a high-dosage seal 48 hours after oiling. As this was a terminal sample, it is not known whether the high value represented a peak, or was part of a continuing trend. No similar high values were noted in any of the other seals. Other liver enzymes did not follow the SDH change of seal No. 6, possibly indicating that either the value is spurious or that SDH was a highly sensitive indicator of liver damage in this instance.

Muscle-based enzymes CPK, GPT and ALD showed a definite and significant pattern of change from the beginning to the end of the oil ingestion experiment. This pattern of decreasing levels occurred in the high dose and low dose groups as well as the controls.

Gamma-GT, and LAP are primarily kidney-based enzymes. LAP showed no significant change throughout the experiments. All of the post-oil values for gamma-GT, appeared to be higher than respective control values for each animal. The control animals receiving no oil

also showed a significant increase in one case. The most dramatic increase was seen in the later samples of the high dose group, notably the 96 through 240 hours samples.

3.6.4 Hematology

Significant hematologic findings were confined to packed cell volume which increased with time in control as well as experimental animals (Table 26). The increases, which reflect hemoconcentration or dehydration were in the order of 0.5 to 50.6% within the 10-day experimental period.

3.6.5 Changes in body weight

The weight loss of the harp seals during the experimental period is given in Table 27. The animals were not fed during this time.

4. DISCUSSION

A heavy concentration of shifting, broken ice persisted throughout the summer of 1974 along the mainland coast of the eastern Beaufort Sea and southern Amundsen Gulf. In more typical years the ice disappears completely from these areas during the period from approximately mid-July to early October. The 1974 net catch of ringed seals at Brown's Harbour was different in several respects from catches made during ice-free years at Brown's Harbour and Herschel Island, Yukon Territory. The proximate causative factors are not known but are probably a combination of lowered seal productivity resulting in a lesser availability of food and a change in the patterns of their movement along the coast because of presence of an ice barrier.

The 1974 Brown's Harbour catch contained less yearling (0+) seals than either catches taken at Herschel Island in 1971 or at Brown's Harbour in 1972. It also contained a significantly higher proportion of adult seals ($\geq$6 years) than the other two samples. Adult animals which normally move to areas of good feeding during the open-water period (Smith, 1973b) probably remained in the vicinity of the netting site where they had spent the winter under the ice. The reduced number of yearling seals in the catch could either be caused by a change in their movements because of the heavy coastal pack ice, or by a much lower recruitment that year. There are good indications, from other studies being conducted by one of the authors (T.G.S.), that 1974 was a low seal production year because of absence of suitable breeding habitat. There was also high predation of seal pups by a peak population of foxes *Alopex lagopus* in areas bounding the eastern Beaufort Sea. Comparison of the mean girths of seals from the three catches indicate that both the yearling and adult seals in the 1974 sample were in a poorer nutritional state. There was also a higher proportion of moulting seals in the 1974 catch.

The effects of oil on organisms can be categorized as physical, physiological or biochemical. The simple physical fouling of birds and fur bearing mammals and consequent mortalities have been well documented (Hartung, 1967). For marine mammals, the picture is not clear. Literature does exist indicating that mortalities have occurred in seals contaminated by Bunker C fuel oil (Warner, 1969; Anon, 1970). Neither report indicates that the mortality observed was definitely linked to contact with oil. No post-mortems were performed and no data provided for comparison of the natural mortalities occurring in years when no oil spills occurred. Other reports indicate that although large numbers of hair seals had come into contact with oil, no mortalities were observed (Hess and Trobaugh, 1970; Morris, 1970; Muller-Willie, 1974). Some evidence also exists that grey seals actively avoid oil slicks (Mansfield, 1970). LeBoeuf (1971) and Brownell and Le Boeuf (1971) in investigations of the crude oil spillage in the Santa Barbara Channel, showed that no oil-related deaths were observed. In these studies post-mortems were conducted and biochemical tests performed. Reliable figures were also given on naturally occurring mortalities which further supported their claims. In spite of this, popular press and scientific review papers (Nelson-Smith, 1970), referring to this incident strongly implied that seal mortalities were caused by the oil.

In the present study, fouling by fresh Norman Wells crude oil did not cause any mechanical damage such as sticking of the flippers to the body or plugging of the body openings. Because hair, in the Phocidae, contributes very little their overall insulation in water (Irving and Hart, 1957) no thermoregulatory problems were expected and none were observed. Core body temperatures of oiled seals showed no trends indicating increased thermal conductivity such as occurs in fur bearing mammals (McEwan et al., 1974). This also applies to the immersion studies done on whitecoat harp seals which were between two and four weeks old, even though Øritsland and Ronald (1973) showed that the lanugo appears to provide protection against skin cooling by the wind. The harp seal whitecoats used in this immersion study had already developed blubber layers of between 2.5 - 5.0 cm. Thus it appears that if any thermoregulatory problem were to be caused by oil immersion, it would occur shortly after birth.

A separate study (Appendix A) attempted to evaluate the effect of exposure to fresh Norman Wells crude oil on the insulation properties of dry seal pelts. The major effect of the oil was to increase the solar heating of the skin. This likely resulted from an increase in transmittance of lightly pigmented hairs while reflectance and absorbance were not significantly affected.

Harp seal pups are usually weaned within three weeks of birth in comparison to 8 to 10 weeks for ringed seals. During this experiment, the harp seal pups also had the opportunity to clean themselves on fresh snow. Traces of oil on the pelts had all but disappeared within 24 hours after coating. In the event of an under-ice oil spill the ringed seal pups, which are born in early April and occupy subnivean birth

lairs (Smith and Stirling, 1975), would be exposed to an oil-fouled ice surface and a layer of oil at the water-ice interface for the whole of the suckling period. It is unlikely that the adult female seal would be able to move the pup away from the contaminated area until it had been weaned.

Divergent results were obtained from the ringed seal field and laboratory oil immersion studies. Apart from transient behavioural changes, the seals in the field study showed enzymatic and histologic evidence of kidney damage, as well as enzyme trends pointing to liver involvement. The liver changes appear to be mild and more than likely are reversible. The kidney lesions seem to be related to an unsuccessful attempt to concentrate and/or excrete the oil or its metabolites via the urinary system. This excretory route has been confirmed by the high kidney and urine oil concentrations which seemed to persist at decreasingly lower levels throughout the six day post-oiling period. The ultimate consequences of the kidney lesions can only be assessed by a longterm study.

The question arises as to the route of entry of oil within the body. Some quantity was probably swallowed during the early thrashing and churning behaviour of the seals. Absorption through the skin and mucous membranes must also be considered, as well as the very likely absorption of highly volatile fractions through the respiratory tract. Serial chromotographic analysis of the water in the seal pen showed that most of the volatile benzenes were dissipated within the first 20 hours; thus most respiratory absorption would likely occur only in animals which come in contact with oil during the first 24 hours of a spill, or much longer in the event of a blowout which continues to pour oil into the sea.

Eye damage was a significant physical finding in the field oil study. At least some of the damage appears to have been done by volatile components of the oil. Nearly all of the investigators experienced eye irritation when exposed to the pungent fumes in the seal pen. The eye inflamations in the seals subsided soon after they were placed in clean water; it is reasonable to assume that continued exposure to oil may have resulted in more severe and possibly permanent eye disorders. Nelson-Smith (1970), quoting an unidentified source, states that oil damage in seals frequently includes severe eye irritation, and makes reference to a female seal, now blind, which was rescued during an oil spill. Eye damage and blindness are observed in wild and captive seals (King, 1964; Ridgweay, 1972), and the occurrence in nature need not be linked with oil or other noxious substances. Nevertheless oil is irritating and

damaging to eyes and the severity of damage is likely to be related to exposure time.

The ingestion experiments were carried out in order to assess the effects of accidentally swallowed crude oil. Effects of hydrocarbon ingestion in mammals have been well documented (Cornelius and Kaneko, 1963). If sufficient quantities of these usually hepatotoxic substances are administered, liver enzymes are released into plasma and are detectable. The degree and duration of enzyme release is generally a function of the quantity and toxicity of the substance(s). Geraci (1972a) induced measurable liver damage in grey seals, using five and 10 ml quantities of carbon tetrachloride, a rather potent fraction. The release of hepatic enzymes continued to increase throughout the eight day grey seal study. In the present studies, there was only transient enzyme release. If there was damage, it was negligible; indicating that 25 ml to 75 ml ingested crude oil was not irreversibly harmful, at least to the liver. These quantities probably represent the upper limit of what an animal might ingest. The kidney seems to be a more sensitive target organ. Little oil was swallowed in the laboratory oil immersion studies. Furthermore, any live oil-contaminated food item would be unlikely to provide more than 5 ml of oil per day. Ringed seals are not known to be carrion feeders.

The seals in the laboratory oil immersion study provided a clue to some factors which might complicate the effects of a blowout. All the seals died within 71 minutes of exposure, a situation suggestive of stress. Seals often respond poorly to handling and to all of the circumstances of captivity. Geraci (1972b) observed biochemical evidence of stress in moulting harp seals, and could reproduce the same profound effects by tampering with the diet (Geraci, 1972a). Geraci and Smith (1975) showed that capture and handling stress in ringed seals is reflected by increase in circulating red cell mass, probably by the mechanism of splenic release. The seals in the present study showed an even greater circulating red cell mass than was present in the Herschel group. Values for PCV, Hb and RBC were significantly higher in all cases ($t = 3.35$, $P < 0.01$). This suggests more marked stress or hemoconcentration which might be related to dehydration or stress. Evidence from studies on BUN and plasma protein levels (Geraci and Smith, 1975) point to the combination of events. Plasma protein levels in the Brown's Harbour 1974 seals were significantly higher than those of the Herschel group ($t = 3.53$, $P < 0.001$), suggesting dehydration probably nutritional in origin, but not enough to account for the unusually high red cell values. Together, these factors point to stress associated with prolonged moult and starvation.

Stress can also be related to age. Nearly all of the older seals which were taken to Guelph died within two months; the younger animals lived. These findings are consistent with stress studies in the wild animal populations. All of these data suggest that an

environmental disturbance, including a blowout, would not affect a seal population uniformly. Older seals and seals in poor nutritional condition are likely to be more sensitive than younger healthy seals. Thus, a blowout during a bad ice year, such as 1974, would be more detrimental to seals which, as a whole, were in poor general condition, and presumably even more harmfully selective to the older animals within the population.

5. CONCLUSIONS

It is evident that all studies conducted to date are of an acute nature and will provide an accurate picture of the consequences of an oil blowout on ringed seals only when exposure to oil is of a short duration. This is likely to be the case only during the open water season from approximately July to October when the seals are free to move out of a contaminated area.

During the winter months ringed seals occupy subnivean lairs in both the nearshore and offshore stable ice. In March through May adult females give birth to single pups in a subnivean birth lair. While areas of exceptionally good breeding habitat have been identified in certain sounds and bays, these are relatively few and therefore the less densely occupied vast areas of offshore ice in the Amundsen Gulf and southern Beaufort Sea contribute significantly to the overall productivity of the region. The effect of exposure of breeding animals to an oil film under the ice cannot properly be assessed experimentally. Because the birth lair is connected to the sea by a breathing hole through the ice it would quickly become fouled by oil from the frequent coming and going of the adult female. The longer suckling period of the ringed seal and slower rate of growth would probably increase the possibility of thermoregulatory problems caused by an oil covered birth lair. It is not known whether the adult females would move out of the area thus abandoning the helpless pup or try to take the pup with her. Either of these responses would tend to increase the probability of pup mortality. If the pup and female remained in the contaminated area there is a possibility that an oil fouled birth lair would melt earlier. This would expose the helpless pup both to the cold and to predation by arctic foxes or polar bears.

During this period the probablity of ingesting large amounts of oil accidentally or from oil covered food species is not great. However, it is important to determine whether any of the prey species of the ringed seal might concentrate hydrocarbon metabolites which may be more toxic to the seal than the crude oil.

The non-breeding part of the ringed seal population (69 percent)

tends to be distributed in the further offshore areas of less stable ice (McLaren, 1958). During the months of April - May in the southern Beaufort Sea large numbers of immature animals appear to be associated with the system of large leads running from Herschel Island toward the northwestern corner of Banks Island. It is not known if this segment of the population is excluded from the more stable ice by the older breeding seals or if they are dependent on the open water because of greater food abundance or availability. An oil blowout near the area of large leads would quickly be spread on the surface of the water by wind and currents (Campbell and Martin, 1973). The response of the considerable number of seals associated with areas of open water might be to move into the various productive areas under the ice thus crowding the breeding populations and resulting in intraspecific stress with a resulting rise in mortality. Immature animals which could not feed successfully without the presence of uncontaminated open water might starve.

Beginning in early May ringed seals start hauling out onto the ice surface to bask in the sun. This hauling out behaviour is associated with the annual moult. During this period the large blubber reserves of the seals are reduced drastically. The exact cause of this weight loss is not known but is definitely not caused by a complete cessation in feeding since recent evidence has shown ringed seals to be crepuscular feeders at this time of the year. The large weight loss associated with the moulting period indicates that this is possibly the most stressful period in the life cycle of the sub-adult and mature ringed seals. Additional stress imposed by an oil blowout either by long immersion in oil, by interference with the regular hauling-out behaviour, or by necessitating large scale movements to avoid a contaminated area might have serious consequences. There is evidence from the literature that stressed seals are sensitive to environmental changes. Furthermore, we saw that older animals stressed by transportation and long captivity were killed by a short immersion in oil whereas seals freshly captured survived an even longer period of exposure with no permanent ill effects.

6. RECOMMENDATIONS

1. Because of the dependence of ringed seals on the fast ice habitat for eight months of the year and the consequent longer period of exposure to oil should an under-ice blowout occur, more studies are needed on the chronic effects of contact with oil. In particular, damage to eyes and kidneys should be thoroughly studied.

2. The main long-term effect of an oil well blowout will be contamination of food species and the reduction of food. The combined results of periods of starvation and additional stress imposed by the presence of oil in the water should be evaluated.

3. A study is needed on the reactions of young and old ringed seals to additional stress during the period of moult. Consideration should also be given to the further stress which should be induced by reduced food intake prior to and during the moulting season.

7. ACKNOWLEDGEMENTS

This study was conducted in collaboration with Dr. R. Thompson, Mssrs. David St. Aubin, Tom Austin and Andrew Gilman of the University of Guelph; Dr. R. Englehart, University of Southeastern Massachusetts, Dartmouth, Massachusetts. Hakugiak of Holman, Northwest Territories, assisted in the field studies. We wish to thank the Polar Continental Shelf Project for logistic support and Mr. John Holer, Niagara Marineland and Game Farm for providing holding facilities for the seals.

8. REFERENCES

Ackman, R. G. 1974. Fisheries and Marine Service, Department of the Environment, Halifax Laboratory. (Personal communication to F. R. Engelhardt)

Ackman, R. G. and D. Noble. 1973. Steam distillation: A simple technique for recovery of petroleum hydrocarbons from tainted fish. J. Fish. Res. Board Can. 30:711-714.

Amersham-Searle Corporation. 1973. NCS Solubilizer for liquid scintillation counting. Publ. 8832. Arlington Heights, Ill.

Anon. 1970. Report of the Task Force - Operation Oil (clean-up of the Arrow oil spill in Chedabucto Bay) to the Minister of Transport. Vol.2: 46-47.

Brownell, R. L., and B. J. LeBoeuf. 1971. California sea lion mortality: natural or artifact? p. 287-306. In D. Straughan [ed.] Biological and Oceanographical Survey of the Santa Barbara Channel Oil Spill 1969-1970. Vol I: 426 p.

Campbell, W. J., and S. Martin. 1973. Oil and ice in the Arctic Ocean: Possible large scale interactions. Science 181: 56-58.

Cornelius, C. E., and J. J. Kaneko. 1963. Clinical biochemistry of domestic animals. Academic Press, N.Y. p. 678.

Geraci, J. R. 1971. Functional hematology of the harp seal, *Pagophilus groenlandicus*. Physiol. Zool. 44(3): 162-170.

1972a. Experimental thiamine deficiency in captive harp seals, *Phoca groenlandica*, induced by eating herring, *Clupea harengus*, and smelts, *Osmerus mordax*. Thesis, McGill University, 144 p.

1972b. Hyponatremia and the need for dietary salt supplementation in captive pinnipeds. J. Amer. Vet. Med. Assoc. 161: 618-623.

1973. An appraisal of Ketamine as an immobilizing agent in wild and captive pinnipeds. J. Amer. Vet. Med. Assoc. 163: 574-577.

Geraci, J. R., and F. R. Engelhardt. 1974. The effects of storage time, temperature, and anticoagulants on harp seal, *Phoca groenlandica*, hemograms: a simulated field study. Physiol. Zool. 47(1): 22-28.

Geraci, J. R., and T. G. Smith. 1975. Environmental impact studies on ringed seals in the Canadian arctic I. Functional hematology with a description of the extradural sampling technique. J. Fish. Res. Board Can. (In press).

Hansen, D. L., and E. T. Bush. 1967. Improved solubilization procedures for liquid scintillation counting of biological materials. Anal. Biochem. 18: 320-32.

Hartung, R. 1967. Energy metabolism in oil-covered ducks. J. Wildl. Manage. 30: 564-570.

Hess, R., and L. Trobaugh. 1970. Kodiak Islands oil pollution. Event No. 26-70. Smithsonian Institution Center for Short-lived Phenomena. Annual Report, p. 150-153.

Irving, L., and J. S. Hart. 1957. The metabolism and insulation of seals as bare-skinned mammals in cold water. Can. J. Zool. 35: 498-511.

King, J. E. 1964. Seals of the world. Trustees of the British Museum (Natural History), London. p. 124-125.

Le Boeuf, B. J. 1971. Oil contamination and elephant seal mortality: A "Negative" finding. p. 277-285. In D. Straughan [ed.] Biological and Oceanographical Survey of the Santa Barbara Channel Oil Spill 1969-1970. Vol. I: 426 p.

Mansfield, A. W. 1970. Field report of seal investigations in Chedabucto Bay and at Sable Island, Nova Scotia. 2 March - 7 April, 1970. MS Rept. 3 p.

Morris, R. 1970. Alaska Peninsula oil spill. Event No. 36-70. Smithsonian Institution Center for Short-lived Phenomena. Annual Report, p. 154-157.

Müller-Willie, L. 1974. How effective is oil pollution legislation in arctic waters? The Musk-Ox 14: 56-57.

McEwan, E. H., N. Aitchison, and P. E. Whitehead. 1974. Energy metabolism of oiled muskrats. Can. J. Zool. 52: 1057-1062.

McLaren, I. A. 1958. The biology of the ringed seal (*Phoca hispida* Schreber) in the eastern Canadian arctic. Fish. Res. Board Can. Bull. 118: 97 p.

Nelson-Smith, A. 1970. The problem of oil pollution of the sea. Adv. Mar. Biol. 8: 215-306.

Øritsland, N. A., and K. Ronald. 1973. Effects of solar radiation and windchill on skin temperature of the harp seal, *Pagophilus groenlandicus* (Erxleben, 1777). Comp. Biochem. Physiol. 44: 519-525.

Ridgway, S. H. 1972. Homeostasis in the aquatic environment. p. 590-747. In Mammals of the Sea - Biology and Medicine, C. C. Thomas, Springfield, Ill.

Smith, T. G. 1973a. Population dynamics of the ringed seal in the Canadian eastern arctic. Fish. Res. Board Can. Bull. 181: 55 p.

1973b. Management research on the Eskimo's ringed seal. Can. Geogr. J. 86: 118-125.

1974. Biology of the Beaufort Sea. Northern Perspectives. Can. Arctic Resources Comm. 2: 2 p.

Smith, T. G., B. Beck, and G. Sleno. 1973. Capture, handling and branding of ringed seals. J. Wildl. Manage. 37: 579-583.

Smith, T. G., and I. Stirling. 1975. The breeding habitat of the ringed seal (*Phoca hispida*). The birth lair and associated structures. Can. J. Zool. 53(9): 1297-1305.

Warner, R. E. 1969. Environmental effects of oil pollution in Canada. An evaluation of problems and research needs, Canadian Wildl. Serv. Manuscript Rept. p. 16-17.

Table 1. List of Abbreviations and Unit Designations

	Chemistry	
Ca	total calcium (mg%)	
BUN	blood urea nitrogen (mg%)	
Bili	total bilirubin (mg%)	
Na	sodium (mEq/l)	
K	potassium (mEq/l)	
Cl	chloride (mEq/l)	
P	inorganic phosphate (mg%)	
	Hematology	
PCV	packed cell volume (%)	
Hb	hemoglobin (g/100 ml)	
RBC	red blood cells (X $10^6/mm^3$)	
WBC	white blood cells ($/mm^3$)	
	Enzymes	Specificity
A.P.	alkaline phosphatase (Bodansky units)	Liver > Kidney
LDH	lactic dehydrogenase (international units)	-
GPT	glutamic pyruvic transaminase (milliunits /ml)	Liver
GOT	glutamic oxalacetic transaminase (milliunits /ml)	Muscle
SDH	sorbitol dehydrogenase (Sigma units /ml)	Liver
CPK	creatine phosphokinase (milliunits /ml)	Muscle
Gamma-GT	gamma glutamyl transpeptidase (milliunits /ml)	Kidney
LAP	leucine amino-peptidase (milliunits /ml)	Kidney
OCT	ornithine carbamyl transferase (Sigma units /ml)	Liver
Ald	aldolase (Sibley-Tehninger units /ml)	Muscle

Table 2. Comparison of maximum girth (centimeters) of seals captured by nets at Herschel Island in 1971, Brown's Harbour 1972 and 1974.

Age Category	Herschel Island 1971	Brown's Harbour 1972	Brown's Harbour 1974
Yearlings (0+)	$\bar{X}$ = 76.58 S.D.= 4.85 N = 41	$\bar{X}$ = 74.32 S.D.= 5.99 N = 148	$\bar{X}$ = 71.88 S.D.= 10.82 N = 17
	t = 2.22, P < 0.05	t = 2.82, P < 0.01	
	t = 2.30, P < 0.05		
Adult (6)	$\bar{X}$ = 96.37 S.D.= 6.60 N = 50	$\bar{X}$ = 84.66 S.D.= 13.46 N = 10	$\bar{X}$ = 80.34 S.D.= 8.28 N = 32
	t = 4.19, P < 0.001	t = 1.23, P < 0.2	
	t = 13.74, P < 0.001		

Table 3. Condition of seals at the time of removal from 24 hours contact with crude oil.

Seal #	Lids	Lacrimation	Conjunctivitis	Cornea	General Comments
7	blinking	moderate	severe	shallow ulcer 1 cm x 1.5 cm right eye	most severe conjunctivitis of all seals
9	closed	severe	moderate	2 mm shallow ulcer left eye	head drawn back; arching of back
10	open	moderate	severe	diffuse control capacity right eye	slight body quivering
11	blinking	moderate	moderate		
14	squinting	slight	moderate		
16	closed	moderate	severe	old ulcer right eye	arching of back some body quivering

Table 4. Core body temperatures (°C) of ringed seals throughout the field oil immersion study

Date	Time	Seal Numbers RT 7	RT 9	RT 10	RT 11	RT 14	RT 16
		core temperature of seals prior to oil exposure					
Aug. 29/74	1730	37.5		36.0			
	1900	38.0	37.5	36.5	38.0	37.7	
	2200	38.8	37.4	37.7	37.0	38.2	
Aug. 30/74	0100	38.8	37.2	37.7	37.5	37.5	
	0400	38.3	37.1	37.7	38.1	38.2	
	*						
	0700	34.5	34.9	35.6	33.5	35.7	
	1000	35.1	34.9	34.8	35.4	35.7	
	1300	1 cm of oil placed on surface of seal pool					
Aug. 30/74	1300	35.1	34.9	34.9	34.7	35.7	36.0
	1630	35.1	34.9	34.9	33.5	35.7	35.6
	1730	35.1		35.4	35.4	35.7	36.0
	1900	33.9		35.4	34.1	35.3	35.0
Aug.31/74	0100	36.3	34.9	36.0	35.4	34.7	35.6
	0400	35.4		34.9	35.4	36.0	36.0
	0700	35.6	35.5	34.9	35.4	36.3	36.0
	1100	35.4	35.0	35.4	35.8	35.7	36.0
	1300	35.4	35.0	35.4	35.4	35.7	36.0
		removed from oiled H_2O, placed in oil-free H_2O					
Aug. 31/74	1900	35.6	34.9	34.9	34.7	36.3	35.0
	2200	34.1	33.8	34.9	34.1	35.3	34.8
Sept. 1/74	0100	35.5	35.5	36.0	35.8	36.3	36.3
	0500	34.5	34.4	34.9	34.9	35.7	35.0
	0700	35.1	35.0	35.4	35.0	35.7	35.4
	1100	33.5	33.8	34.2	34.1	35.1	34.7
	1600	35.6		36.5	35.5	36.3	35.9
	1900	35.4		35.6		36.3	36.0
Sept. 2/74	0100	35.6		35.4		35.7	36.0
	0700	35.4		34.5		35.6	35.3
	1330	34.9		35.2		35.7	35.3
	1800	35.6		36.0		36.4	36.0

*Between 0400 and 0700 on Aug. 30/74, the strip chart recording needle became offset resulting in a decrease in expressed temperature values. Recalibration of two unused pills showed that the expressed values were from 1.6° to 2.0° lower than actual values. There was no significant change in temperature on any of the seals throughout the experiment.

Table 5. Hematologic and plasma chemical findings in ringed seals during the 24 hour oil immersion study.

RINGED SEAL RT 7

Date	24 hr. pre oil	3 hr. pre oil	24 hr. after oil	96 hr. after oil	144 hr. after oil
			CHEMISTRY		
Ca mg%		10.8	8	8.8	10
BUN mg%		50	48	54	56
Glucose mg%		206	181	172	232
Chol mg%		360	262	298	322
Bili mg%		1	1	0.8	0.6
Uric Acid mg%		2.6	2.4	1.8	4.8
Na mEq/l		152	154	158.5	153
K mEq/l		4	4.3	4	3.4
Cl mEq/l				113	106
Protein g%		10.6	9.4	8.8	9.8
Albumin g%		2.4	2.2	2	2.4
P. Inorg. mg%		6.2	5.0	5.2	7.4
			HEMATOLOGY		
PCV %	74	73.5	79	73	
Hb g/100 ml	33.2	33.4	34.8		
RBC x 10^6 /mm^3	5.4	5.6	5.9	6.1	6.4
WBC /mm^3	6,000	6,000	7,900	6,100	7,900
			ENZYMES		
A.P.B.U.		50	48	40	72
LDH IU		994	674	772	876
SGPT mU/ml		9	8	8	7
SGOT mU/ml		41	31	27	22
SDH S.U./ml		116	58	232	174
CPK mU/ml		41	27	29	17
Gamma-GT mU/ml		9	8	0	8
LAP mU/ml		11	12	4	11

Table 6. Hematologic and plasma chemical findings in ringed seals during the 24 hour oil exposure study.

RINGED SEAL RT 9

Date	24 hr. pre oil	3 hr. pre oil	24 hr. after oil	48 hr. after oil
		CHEMISTRY		
Ca mg%		9.4	7.2	7.6
BUN mg%		42	32	38
Glucose mg%		214	190	166
Chol mg%		436	360	318
Bili mg%		1	0.6	0.6
Uric Acid mg%		5.2	2.1	1.8
Na mEq/l		136	153	159
K mEq/l		3.9	4.7	3.9
Cl mEq/l				107
Protein g%		10.6	7.4	7.6
Albumin g%		3	2.4	2.2
P. Inorg. mg%		7.4	5.0	5.4
		HEMATOLOGY		
PCV %	73	73	79	76
Hb g/100 ml	33	32.8	34.6	32.2
RBC x 10^6 /mm^3	5.8	5.7	5.9	7.1
WBC /mm^3	8,300	5,300	6,700	6,250
		ENZYMES		
A.P.B.U.		88	49	58
LDH IU			64	1090
SGPT mU/ml		10	11	10
SGOT mU/ml		86**	54	32
SDH S.U./ml		116	116	116
CPK mU/ml		127	50	16
Gamma-GT mU/ml			7	7
LAP mU/ml		3	6	7

** excessive hemolysis

Table 7. Hematologic and plasma chemical findings in ringed seals during the 24 hour oil exposure study.

RINGED SEAL RT 10

Date	24 hr. pre oil	3 hr. pre oil	24 hr. after oil	96 hr. after oil	168 hr. after oil
			CHEMISTRY		
Ca mg%		8.4	7.8	7.6	7.4
BUN mg%		38	36	34	28
Glucose mg%		196	160	170	154
Chol mg%		318	328	256	236
Bili mg%		0.8	0.6	0.4	0.4
Uric Acid mg%		2.6	2.6	2.2	1.8
Na mEq/l		143.5	146.5	157	145
K mEq/l		3.5	3.8	4	3.8
Cl mEq/l			104	111	105
Protein g%		8.2	8.8	8.2	7.6
Albumin g%		2	2	2	2
P.Inorg.mg%		4.8	4.6	4.8	5
			HEMATOLOGY		
PCV %	67.5	70	74	70.5	
Hb g/100 ml	29.2	30.6	32.3		
RBC x 10^6 /mm	5.26	5.19	5.5	5.3	5.28
WBC / mm^3	8,200	12,600	7,550	6,500	7,900
			ENZYMES		
A.P.B.U.		50	54	38	42
LDH IU		742	870	532	410
SGPT mU/ml		9	8	8	7
SGOT mU/ml		109	88**	48	22
SDH S.U./ml		116	116	116	116
CPK mU/ml		48	53	40	1
Gamma-GT mU/ml		7		8	9
LAP mU/ml		9	5	11	9

** excessive hemolysis

Table 8. Hematologic and plasma chemical findings in ringed seals during the 24 hour oil exposure study.

RINGED SEAL RT 11

Date	24 hr. pre oil	3 hr. pre oil	24 hr. after oil	48 hr. after oil
			CHEMISTRY	
Ca mg%		7.6	8	8.2
BUN mg%		36	34	32
Glucose mg%		166	162	174
Chol mg%		262	304	262
Bili mg%		.4	0.6	0.8
Uric Acid mg%		3	2.8	4.2
Na mEq/l		167.5	149	152.5
K mEq/l		4.6	3.9	4.1
Cl mEq/l		114	109	110
Protein g%		7.2	8	7.4
Albumin g%		2	1.8	2
P.Inorg.mg%		6.2	5.4	5.6
			HEMATOLOGY	
PCV%	73	73	75	72
Hb g/100 ml	31	31	32.2	31
RBC x 10^6 /mm^3	6.75	6.49	6.66	6.1
WBC /mm^3	11,250	11,250	9,700	8,500
			ENZYMES	
A.P.B.U.		50	56	64
LDH IU		668	814	938
SGPT mU/ml		12	10	31
SGOT mU/ml		69	66*	165
SDH SU./ml		116	116	232
CPK mU/ml		4	2	3
Gamma-GT mU/ml		3	4	13
LAP mU/ml		7	9	6

*hemolysis

Table 9. Hematologic and plasma chemical findings in ringed seals during the 24 hour oil exposure study.

RINGED SEAL RT 14 .

Date	24 hr. pre oil	3 hr. pre oil	24 hr. after oil	96 hr. after oil	144 hr. after oil
			CHEMISTRY		
Ca mg%		7.2	8	7.6	6.8
BUN mg%		38	46	36	36
Glucose mg%		176	156	174	172
Chol mg%		304	356	272	268
Bili mg%		0.6	0.6	0.4	0.6
Uric Acid mg%		2.4	2.2	2	1.8
Na mEq/l		165.5	143	160.5	150.5
K mWq/l		4.4	3.7	4.6	3.9
Cl mEq/l					106
Protein g%		7.4	7.8	7	6.4
Albumin g%		1.6	1.4	2	1.8
P. Inorg. mg%		5.6	4.8	5.4	5.8
			HEMATOLOGY		
PCV %		76	73	77.7	70.7
Hb g/100 ml		30	32	33.4	
RBC x 10^6 /mm^3		5.20	5.6	5.6	5.7
WBC /mm^3		6,800	8,050	8,900	10,200
			ENZYMES		
A.P.B.U.		56	56	44	46
LDH IU		768	812	698	680
SGPT mU/ml		9	10	12	16
SGOT mU/ml		48*	38	38	42
SDH S.U./ml		116	174	174	232
CPK mU/ml		33	16	7	8
Gamma-GT mU/ml		8	6	8	8
LAP mU/ml		13	6	6	10

*hemolysis

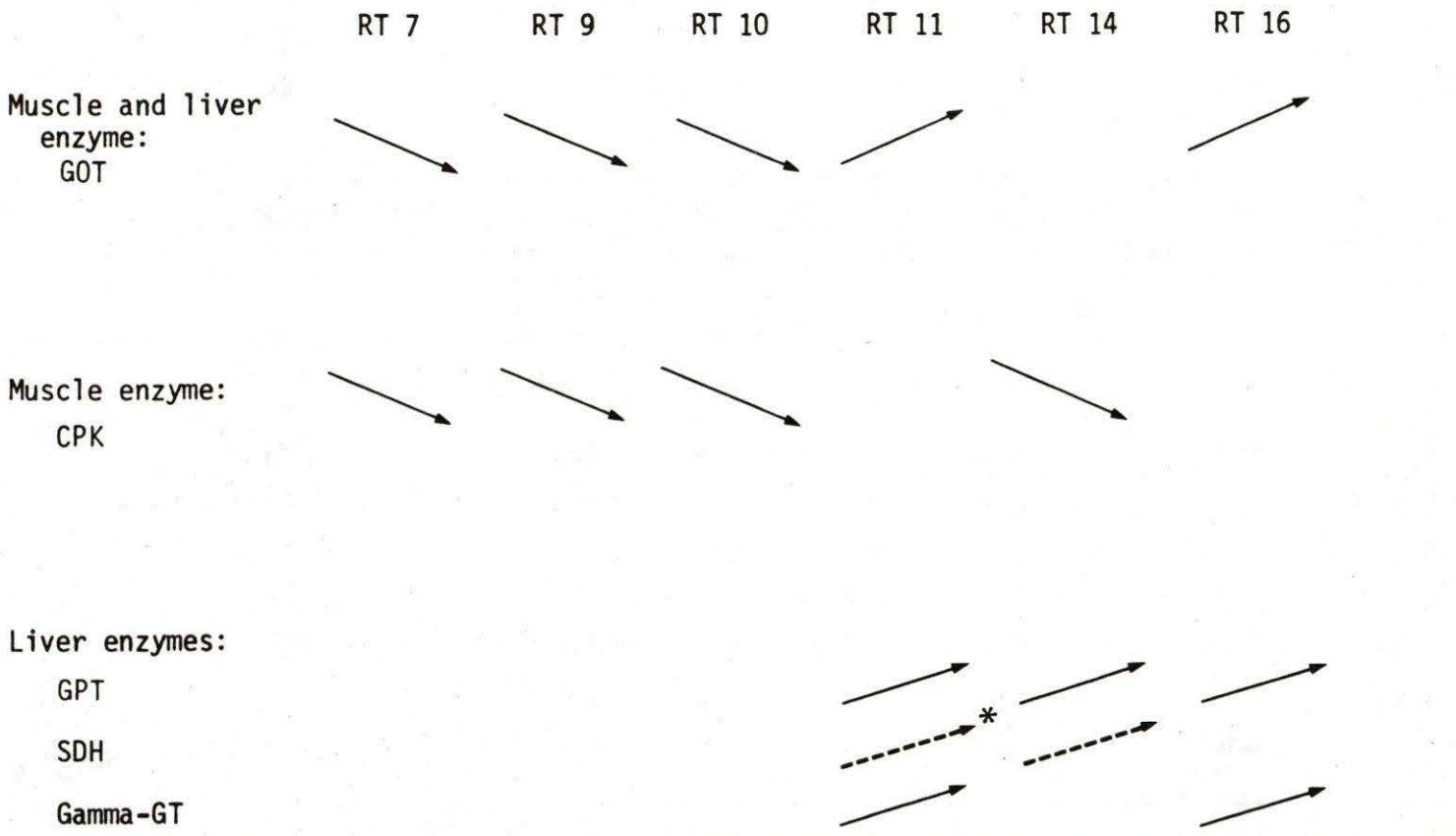
Table 10. Hematologic and plasma chemical findings in ringed seals during the 24 hour oil exposure study.

RINGED SEAL RT 16

Date	3 hr. pre oil	24 hr. after oil	96 hr. after oil	144 hr. after oil
		CHEMISTRY		
Ca mg%	7.8		8.8	8.4
BUN mg%	24		24	22
Glucose mg%	156		162	214
Chol mg%	204		216	186
Bili mg%	0.2		0.4	0.6
Uric Acid mg%	2.6		1.8	2.4
Na mEq/l			157.5	153.5
K mEq/l			4.5	3.9
CL mEq/l			109	102
Protein g%	7.6		8.2	7.6
Albumin g%	2		2	1.8
P. Inorg. mg%	7.6		6.6	7.4
		HEMATOLOGY		
PCV %	67.5	70.2	68	
Hb g/100 ml	28.8	29.4		
RBC x 10^6 /mm^3	5.7	6.1	6.2	6.02
WBC /mm^3	10.650	11,250	11,350	8,700
		ENZYMES		
A.P.B.U.	78	66	82	
LDH IU			157.5	153.5
SGPT mU/ml	9	14	28	28
SGOT mU/ml	38	62*	72	60
SDH SU./ml	116	232	232	174
CPK mU/ml	20	34	11	4
Gamma-GT mU/ml	7	1	13	13
LAP mU/ml	8	4	11	7

*hemolysis

Table 11. Plasma enzyme trends in ringed seals during the field oil immersion study.



*questionable significance

Table 12. Concentration of petroleum in tissues of ringed seals (*Phoca hispida*) during the field oil immersion study.

Seal #	Hours after oiling	Petroleum concentration (ppm)					
		Liver	Kidney	Brain	Blubber	Skeletal muscle	Lung
Composite control sample	pre oil	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
7	48	1.2	1.9	2.3	2.3	<0.5	<0.5
9	48	1.4	4.9	2.3	4.0	14.3	<0.5
10	144	-	1.1	0.8	3.2	3.6	<0.5
11	144	2.5	1.3	0.6	1.0	<0.5	<0.5
14	144	0.5	1.3	0.6	1.0	<0.5	<0.5
16	168	8.4	2.6	1.0	<0.6	<0.5	<0.5

Table 13. Concentration of petroleum in body fluids of ringed seals during the field oil immersion study.

Seal #	Hours after oiling	Petroleum concentration (ppm)			
		Plasma	Whole blood	Urine	Bile
7	48	4.4	4.7	39.0	-
9	48	2.4	11.6	30.2	58.1
10	144	1.3	3.1	1.9	32.1
11	144	-	8.0	6.3	-
14	144	1.3	1.6	-	39.2
16	168	1.0	0.7	<6.0	-

Table 14. Blood cellular and chemical parameters during laboratory oil immersion study (ringed seals).

Parameter	Seal No. 24		Seal No. 35		Seal No. 30	
	Pre oil	Terminal (21 min)	Pre oil	Terminal (60 min)	Pre oil	Terminal (71 min)
Glucose mg%	124	149	128	167	138	143
Uric acid mg%	2.8	5.4	4.2	5.7	3.3	5.7
H. cortisone µgs%	105	60	80	55	80	70
K mEq/l	3.7	7.4	4.1	6.6	3.6	7.8
Na mEq/l	157.0	161.0	151.0	153.0	150.5	152.5
Cl mEq/l	101	99	107	102	99	95
BUN mg%	47	48	45	47	55	59
Protein g%	8.8	9.3	7.7	7.3	8.6	9.0
A/G	0.5	0.6	0.6	0.7	0.6	0.6
Albumin g%	3.1	3.4	2.9	3.0	3.3	3.4
Globulin g%	5.7	5.9	4.7	4.4	5.3	5.6
Alpha 1 g%	1.2	1.2	1.1	1.2	1.4	1.5
Alpha 2 g%	0.9	1.0	0.8	0.8	0.8	0.8
Beta g%	-	0.7		0.7	1.8	1.8
Gamma g%	3.6	1.8		1.2	1.3	1.5
PCV %	58	61	52	51	55	55
Hb g/100 ml	24.2	24.4	20.0	20.8	22.8	23.3
RBC $x10^6/mm^3$	5.08	5.17	4.27	4.19	4.65	4.70
MCV μ^3	114	118	122	122	118	117
MCH µµg	47.6	47.2	46.8	49.6	49.0	49.6
MCHC %	41.7	40.0	38.5	40.8	41.5	42.4
WBC $/mm^3$	20,000	20,300	23,000	14,000	14,700	5,100
N band %	0	2	1	5	3	1
N seg %	53	48	58	63	62	55
L %	26	23	22	21	24	35
M %	0	7	10	6	5	4
B %	6	8	2	4	3	4
Eos %	15	12	7	1	3	1
Total Eos $/mm^3$	2,286	2,784	2,325	766	1,792	272

Table 15. Results of plasma enzymes studies in ringed seals during the oil ingestion study.

	Days pre-oil						Days post-oil				
	20	17	14	10	5	0	2	4	7	16	28
GPT mU/ml											
Seal No. 15	12	8	12	10	-	14		-	14	10	-
23	6	4	4	6	-	8	12	11	6	6	5
29	16	15	8	12	8	12	16	14	12	11	16
34	-	8	11	7	-	12	10	10	8	12	10
37	8	4	4	8	8	14	12	12	8	-	-
SDH (x58 = SU/ml)											
Seal No. 15	3	3	3	2	-	2	-	-	1	2	-
23	2	3	2	2	-	1	2	1	1	2	1.5
29	3	2	2	3	2.5	3	2.5	2	1	1	2.0
34	-	1.5	1	1	-	3	1	2.5	2	1.5	2.0
37	2	2	2	1	2	2	1	2	2	-	-
OCT SU/ml											
Seal No. 15	100	500	50				-	-	0	250	-
23	50	100	900				100	100	0	0	0
29	100	100	600				0	50	100	-	0
34	-	350	400				150	200	450	-	0
37	200	150	250				200	0	500	-	-

Table 15 (cont'd.)

	Days pre-oil						Days post-oil				
	20	17	14	10	5	0	2	4	7	16	28
Gamma-GT mU/ml											
Seal No. 15	8	7	9	6	-	5	-	-	6	11	-
23	9	8	9	12	-	14	14	13	0	10	12
29	12	10	12	10	6	8	8	7	13	7	10
34	-	8	10	9	-	9	18	14	9	15	17
37	12	9	6	10	5	10	11	15	11	-	-
LAP mU/ml											
Seal No. 15	9.3	8	6	6	-	8	-	-	-	-	-
23	10.3	8	4	6	-	7	8	8	6	5	7
29	9.3	8	4	7	5	6	9	9	7	7	8
34	-	5	5	6	-	6	8	9	8	8	9
37	11	10	9	10	6	8	9	10	8	-	-
CPK mU/ml											
Seal No. 15	84	91	104	41	-	58	108	-	172	264	-
23	67	53	23	46	-	48	184	213	106	84	235
29	23	92	14	112	73	34	-	150	53	50	433
34		63	50	16	-	242	62	25	124	50	122
37	19	20	21	9	17	86	115	240	73	-	-

Table 16. Tritium activity in ringed seals (*Phoca hispida*) whole blood and plasma following the addition of 3_H-benzene in Norman Wells Crude oil to their diet (1mCi/5ml day for five consecutive days from onset of experiment).

Sample	Seal	Activity (DPM x 10^4)					
		Pre oil*	2 days	4 days	7 days	16 days	28 days
Blood	15	0	38.7	-	6.9	5.8	-
	23	0	21.9	17.8	6.9	4.6	3.6
	29	0	30.2	20.4	9.9	5.1	8.0
	34	0	23.4	14.5	12.5	6.0	7.8
	37	0	-	16.3	7.9	-	-
Plasma	15	0	-	-	5.0	1.8	-
	23	0	17.7	17.8	4.9	1.5	0.4
	29	0	9.7	31.0	8.5	1.6	0.4
	34	0	16.4	15.1	4.3	1.0	0.3
	37	0	14.6	15.9	4.3	-	-

*5-6 pre oil values were obtained from each seal.

Table 17. Tritium activity in ringed seal (*Phoca hispida*) tissues following the addition of ^{3}H-benzene in Norman Wells Crude oil to their diet (1mCi/5ml day for five consecutive days from onset of experiment).

Sample	Seal	Activity (DPM x 10^4 above background levels		
		2 days	16 days	28 days
Blubber	15	0.65	0.14	-
	23	7.60	0.33	0.09
	29	7.95	0.08	0
	34	2.19	0	0.02
	37	6.22	-	-
Liver	15	9.77	1.07	-
	23	7.06	0.66	0.08
	29	2.58	0.38	0.14
	34	5.57	0.43	0.64
	37	6.82	-	-
Muscle	15	2.42	0	-
	23	1.72	0.55	0.43
	29	1.79	0.24	0.18
	34	1.53	0.14	0
	37	1.68	-	-

Table 18. Plasma GPT activity[1] during field oil ingestion study (harp seals)

Regime	Seal No.	Hours before oil	Hours after oil						
		6	24	48	96	120	144	192	240
Low dose (25 ml)	7	4.6	6.8						
	4	9.4		8.6					
	11	5.5			5.7				
	8	7.3					12.0		
	12	5.8						2.6	
	14	7.0							2.8
High dose (75 ml)	10	8.0	7.0						
	6	5.5		5.2					
	5	5.6			5.3				
	2	11.0					6.4		
	13	4.6						2.6	
	3	4.6							3.2
Control	1	12.0				4.3			4.0
	15	5.5				8.0			5.6

[1]Enzyme designation, units and organ specificity are shown in Table 1.

Table 19. Plasma OCT activity[1] during field oil ingestion study (harp seals)

Regime	Seal No.	Hours before oil	Hours after oil						
		6	24	48	96	120	144	192	240
Low dose (25 ml)	7	110	0						
	4	0		0					
	11	0			0				
	8	0					280		
	12	170						0	
	14	170							0
High dose (75 ml)	10	466	0						
	6	122		0					
	5	0			0				
	2	520					226		
	13	0						90	
	3	0							0
Control	1	10				50			149
	15	0				140			0

[1]Enzyme designation, units and organ specificity are shown in Table 1.

Table 20 . Plasma SDH activity[1] during field oil ingestion study (harp seals)

Regime	Seal No.	Hours before oil	Hours after oil						
		6	24	48	96	120	144	192	240
Low dose (25 ml)	7	197	116						
	4	197		104					
	11	162			174				
	8	232					87		
	12	145						58	
	14	116							58
High dose (75 ml)	10	87	128						
	6	116		522					
	5	104			116				
	2	220					162		
	13	116						116	
	3	203							93
Control	1	191				174			116
	15	174				116			116

[1]Enzyme designation,units and organ specificity are shown in Table 1.

Table 21. Plasma Ald. activity[1] during field oil ingestion study (harp seals)

Regime	Seal No.	Hours before oil	Hours after oil						
		6	24	48	96	120	144	192	240
Low dose (25 ml)	7	42	26						
	4	18		9					
	11	36			22				
	8	40					32		
	12	34						16	
	14	36							22
High dose (75 ml)	10	18	11						
	6	80		38					
	5	9			3				
	2	12					5		
	13	39						17	
	3	7							4
Control	1	13				12			2
	15	12				5			9

[1]Enzyme designation,units and organ specificity are shown in Table 1.

Table 22. Plasma CPK activity[1] during field oil ingestion study (harp seals)

Regime	Seal No.	Hours before oil	Hours after oil						
		6	24	48	96	120	144	192	240
Low dose (25 ml)	7	120	54						
	4	160		78					
	11	120			65				
	8	103					52		
	12	124						25	
	14	75							34
High dose (75 ml)	10	189	185						
	6	97		65					
	5	70			80				
	2	412					196		
	13	194						108	
	3	143							88
Control	1	397				55			115
	15	121				80			29

[1]Enzyme designation, units and organ specificity are shown in Table 1.

Table 23. Plasma GOT activity[1] during field oil ingestion study (harp seals)

Regime	Seal No.	Hours before oil	Hours after oil						
		6	24	48	96	120	144	192	240
Low dose (25 ml)	7	60	39						
	4	52		24					
	11	59			41				
	8	51					20		
	12	47						15	
	14	33							11
High dose (75 ml)	10	47	44						
	6	40		27					
	5	29			13				
	2	76					21		
	13	27						11	
	3	43							17
Control	1	61				26			14
	15	31				21			13

[1]Enzyme designation, units and organ specificity are shown in Table 1.

Table 24. Plasma Gamma-GT activity[1] during field oil ingestion study (harp seals)

Regime	Seal No.	Hours before oil	Hours after oil						
		6	24	48	96	120	144	192	240
Low dose (25 ml)	7	5	9						
	4	5		8					
	11	2			12				
	8	7					13		
	12	9						12	
	14	0							10
High dose (75 ml)	10	0	3						
	6	6		12					
	5	6			15				
	2	6					13		
	13	1						17	
	3	7							19
Control	1	0				*			12
	15	4				3			7

*hemolyzed

[1]Enzyme designation, units and organ specificity are shown in Table 1.

Table 25. Plasma LAP activity[1] during field oil ingestion study (harp seals)

Regime	Seal No.	Hours before oil	Hours after oil						
		6	24	48	96	120	144	192	240
Low dose (25 ml)	7	4	3						
	4	4		6					
	11	4			4				
	8	6					3		
	12	5						4	
	14	6							6
High dose (75 ml)	10	6	5						
	6	6		6					
	5	3			3				
	2	4					4		
	13	3						2	
	3	3							3
Control	1	5				1			6
	15	4				10			2

[1]Enzyme designation, units and organ specificity are shown in Table 1.

Table 26. Changes (increase) in packed red cell volumes during field oil ingestion study on harp seals.

	Pre-oil sample	Post oil sample		Increase in PCV	
Seal #	PCV % ($\bar{x}$)	post-oiling day	PCV % ($\bar{x}$)	absolute (%)	percent increase
7	45	1	47	2.0	4.4
10	44	1	54.5	10.5	23.9
4	48.5	2	56.3	7.8	16.0
6	51	2	52	1.0	2.0
11	43	4	56.8	13.8	32.0
5	40.8	4	59.8	19.0	46.6
8	44	6	56.3	12.3	27.8
2	46	6	54.8	8.8	19.0
12	53.5	8	53.8	0.3	0.5
13	40	8	60.3	20.3	50.6
14	48.8	10	57.3	8.5	17.4
3	45.5	10	57.5	12.0	26.4
Control					
1	37.3	5	50.0	12.8	34.2
	45.8	10	53.8	16.5	44.0
15	45.8	5	55.3	9.5	20.8
		10	56.0	10.3	22.0

Table 27. Changes in body weight during field oil ingestion study on harp seals.

Seal #	Pre-oil weight (kg)	Post oil Day	Post oil Weight (kg)	Weight loss absolute (kg)	Weight loss percent
7	15.8	1	-	0	0
10	18.0	1	-	0	0
4	17.1	2	15.8	-1.3	7.89
6	15.8	2	14.9	- .9	5.71
11	18.0	4	15.8	-2.2	12.50
5	15.8	4	14.9	- .9	5.71
8	19.4	6	15.8	-3.6	18.60
2	19.4	6	15.8	-3.6	18.60
12	21.6	8	18.0	-3.6	16.67
13	21.6	8	18.0	-3.6	16.67
14	23.9	10	18.0	-5.9	24.53
3	23.9	10	18.0	-5.9	24.53
Control					
1	26.1	10	20.3	-5.8	22.41
15	23.9	10	18.0	-5.9	24.53

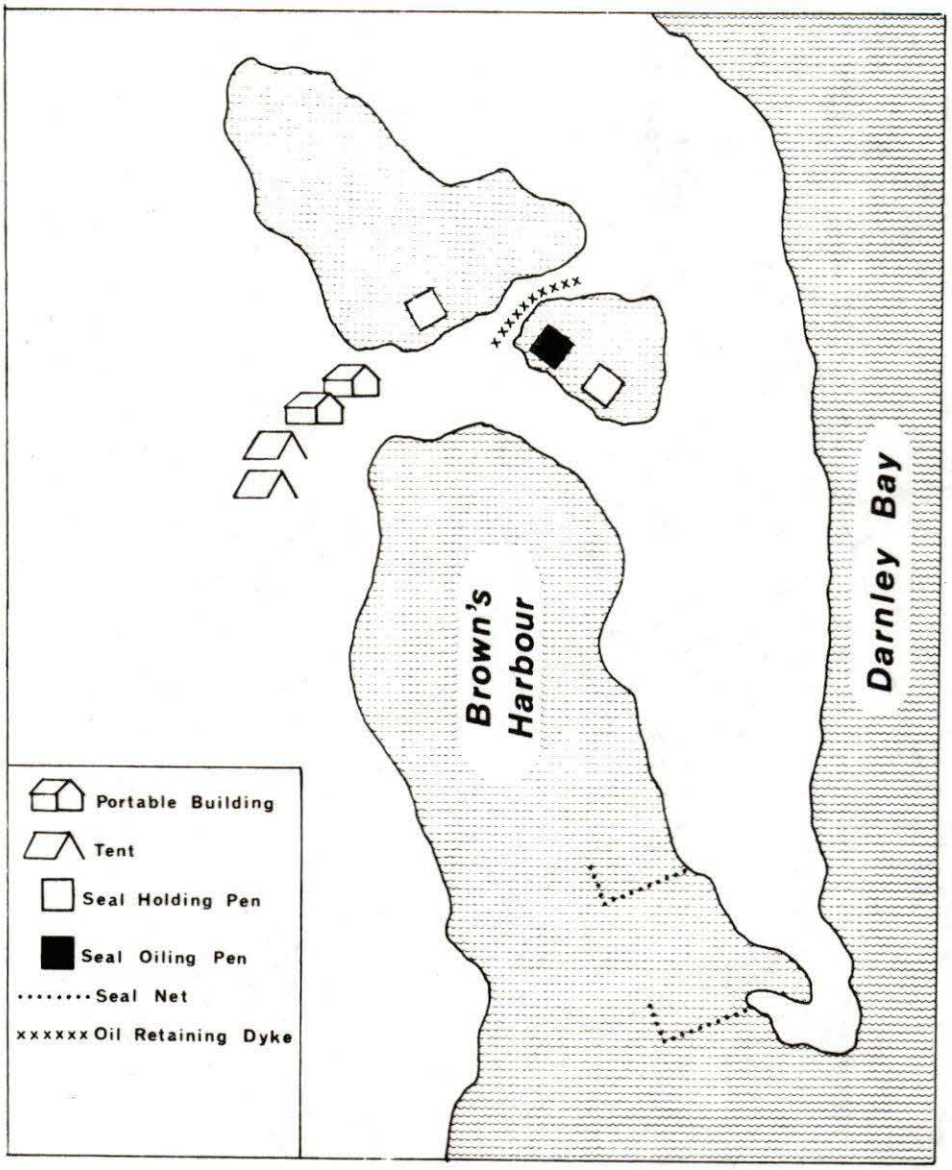


Figure 1. Seal netting site, holding pens and camp facilities at Brown's Harbour, N.W.T.

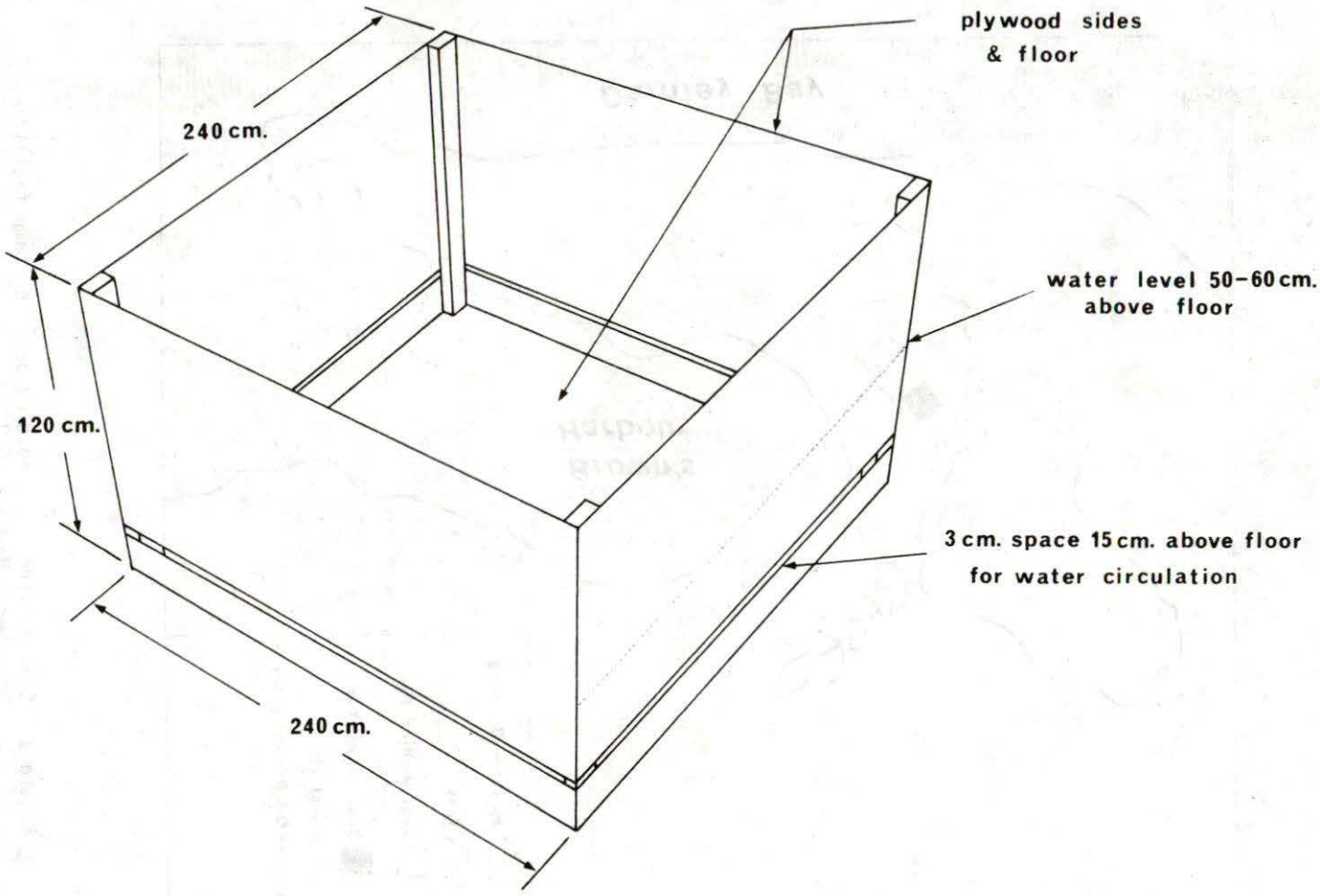


Figure 2. Plywood holding pen used in the field oil immersion study.

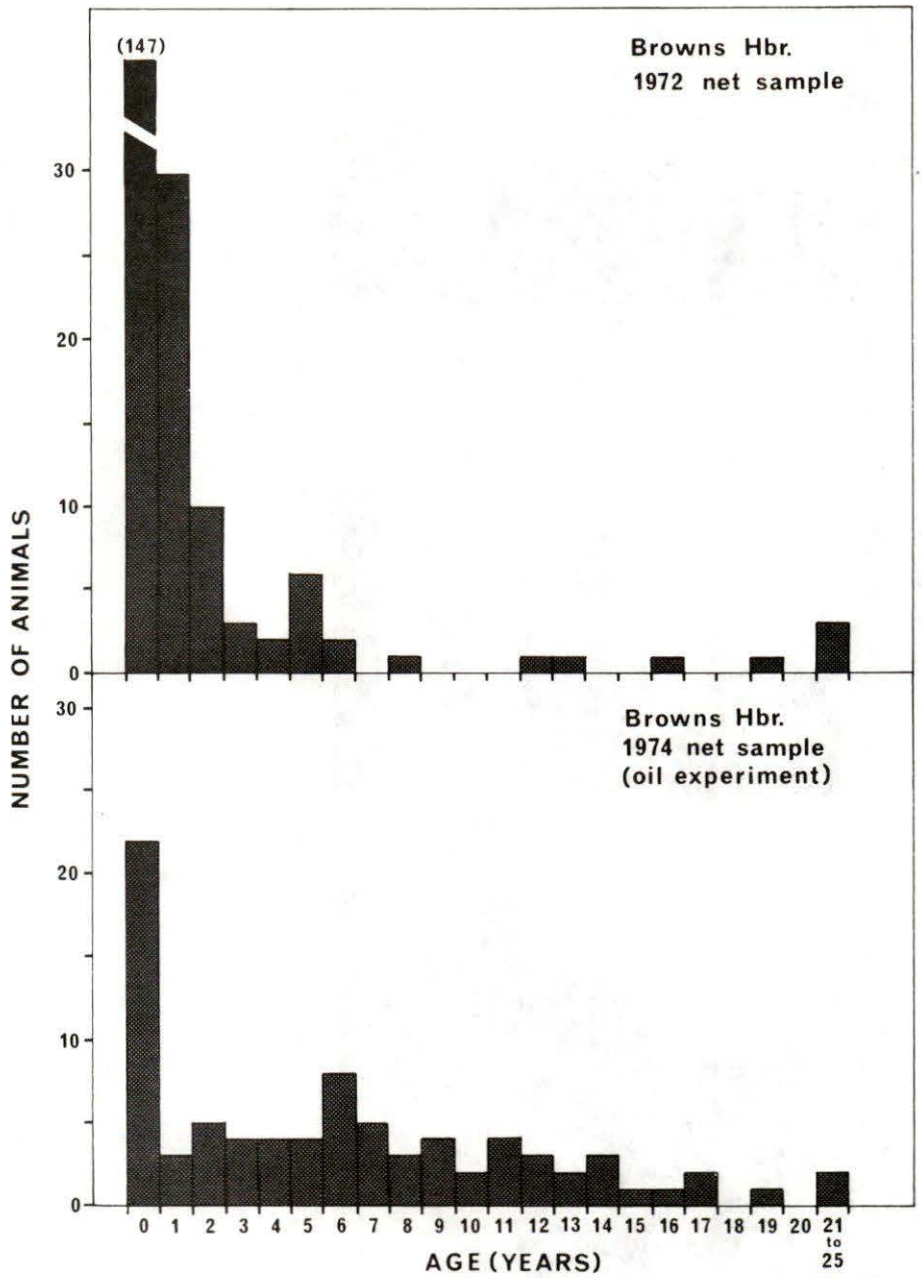


Figure 3. Age frequency distributions of seals netted at Brown's Harbour.

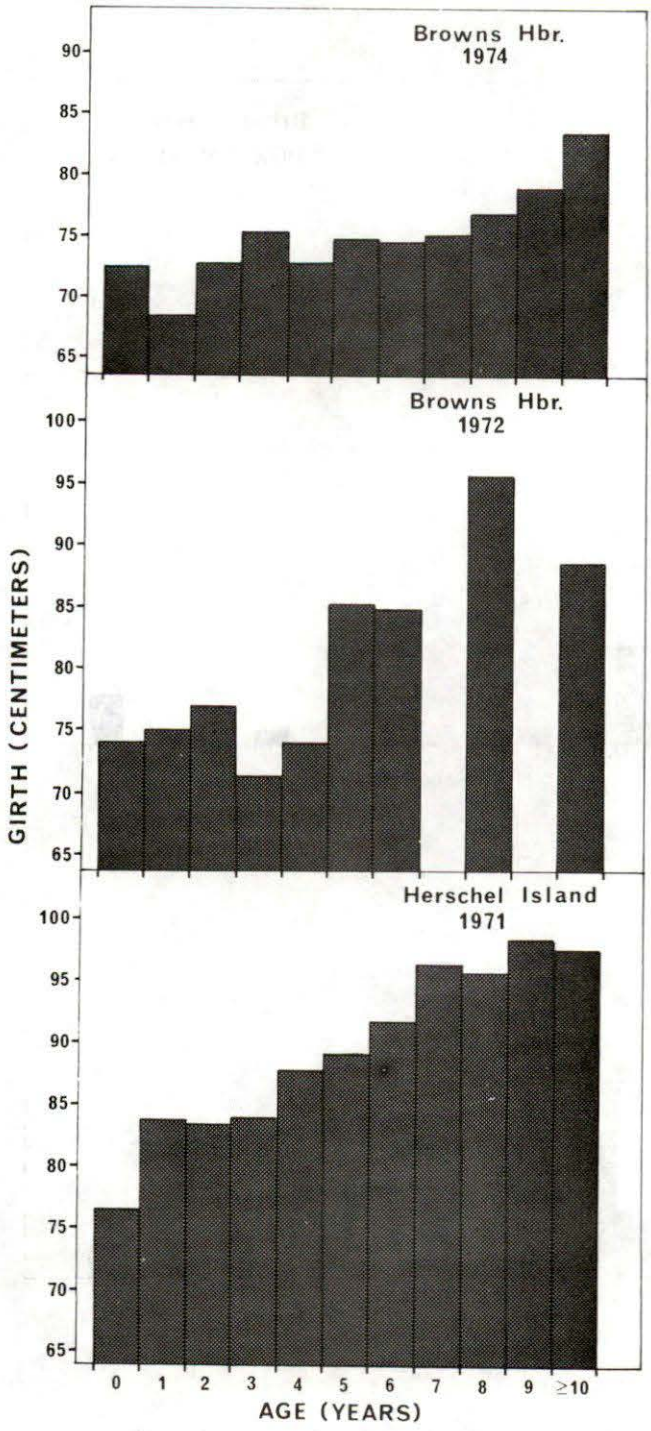


Figure 4. Girth per age class of seals netted at Brown's Harbour in 1972 and 1974 and at Herschel Island, Yukon Territory in 1971.

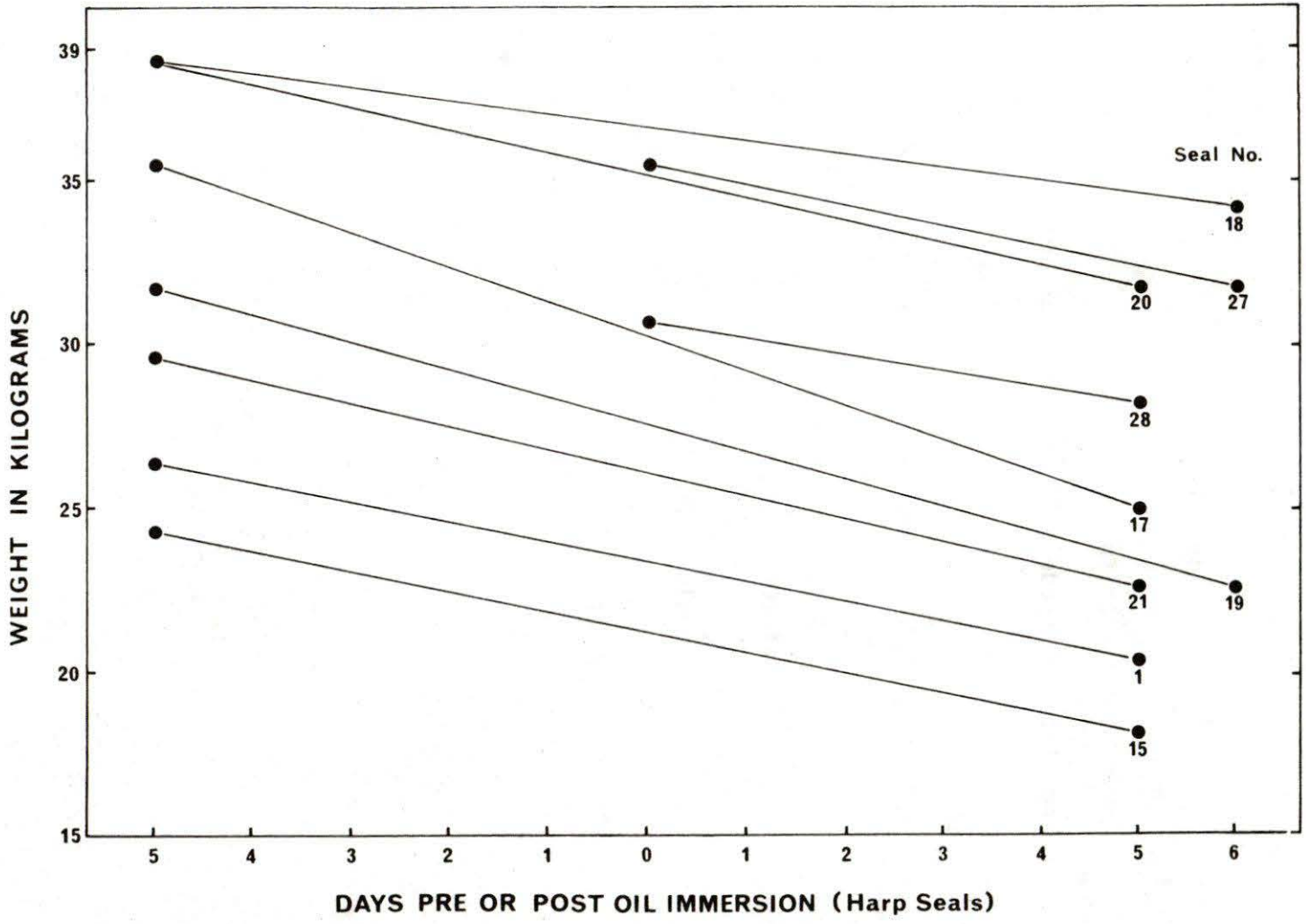


Figure 5. Weight loss of fasted whitecoat harp seals prior to and during the field oil immersion study.

Appendix A

Insulation in marine mammals:

The effect of crude oil on ringed seal pelts

by

N. A. Øritsland

Institute of Zoophysiology
University of Oslo
P. O. Box 1051, Blindern
Oslo 3, Norway

TABLE OF CONTENTS

1. INTRODUCTION

The ringed seal *Phoca hispida* will, prior to and during the moult, haul out on the fast ice for periods of up to 24 hours at a time. Whether or not this haul out pattern is an obligatory part of the moulting process is not yet known. However, it may be speculated that the higher skin temperatures following existence in air may speed up the enzyme activities necessary for the process. During the haul out season (mid May to the end of July) the seals lose much of their fat reserves and are also subject to significant hunting pressure (Smith, 1973 a,b). The haul out behaviour is also strongly influenced by weather (thermal) conditions (Burns, and Harbo, 1972; Smith, 1973 b,c). Therefore, environmentally induced changes in the seals' insulation may have consequences beyond short term bioenergetics. We know that pelt insulation is of significance in the heat balance of seals in air (Hart and Irving, 1959; Øritsland, 1970 a,b, 1971) while it is a negligible part of the total insulation in water (Frisch et al., 1974). The present work concerns a quantitative evaluation of the ringed seal fur insulation in air and how it is influenced by crude oil.

2. MATERIAL AND METHODS

2.1 Narrative

Fresh seal pelts were cleaned, stretched onto a plywood plate, and shipped to the University of Oslo, where special instrumentation from various departments was available. The Institute of Odontology provided a Zeiss microspectrophotometer; the Institute of Nutrition Research made a Shimadzu spectrophotometer available; the Laboratory of Electron Microscopy allowed the use of a Scanning electron microscope and the Institute of Zoophysiology provided laboratory facilities and some of the instrumentation for thermal measurements.

Since purely thermal and optical measurement on dead pelt samples at a first glance may seem irrelevant to the understanding of ringed seal biology, the principal factors are outlined below:

The hauled out ringed seal regulates its deep body temperature near 37°C. Heat is produced in the body and, by regulating its insulation against heat loss, the seal is able to maintain the body temperature within quite narrow limits. Heat production in the resting animal is normally kept at an even rate and the heat loss must also therefore be kept at the same rate in order to maintain a balance; i.e. a constant body temperature. At the same time the cooling power of the environment is variable and the seal will counteract the changes in weather conditions by a corresponding regulation of its insulation.

The seal's insulation consists of fur and a subskin layer of fat or blubber. The insulation of the blubber is determined by its thickness, the thermal properties or conductivity of the fat and the amount of blood circulating through the blubber. Rapid changes in insulation are obtained by the regulation of blood circulation while changes in blubber thickness occur slowly and the conductivity of fat itself remains constant. The insulating quality of the fur is virtually constant. However, the fur is an extremely important modifier in air of the thermal load imposed by the weather on the seal and it should be kept in mind that it is the heat load at skin level that is of significance for the survival of the animal and not the thermal conditions which may exist within or outside the fur. Thus, the ringed seal must, by circulatory means, continuously buffer the heat load at skin level. Because of the buffering effect of the blood circulation on the skin, the thermal properties of the fur cannot be properly evaluated by measurements on the living animal.

The fur will modify the weather at the skin by acting as a barrier against the wind. It also keeps the cold air temperature at a distance from the skin and acts as a filter against solar and thermal radiation. For practical purposes the capacity of the air temperature and the wind to cause heat to flow through the fur can be lumped in a convective heat transfer expression as follows:

$$HF = H_c (T_s - T_a)$$

where HF = heat flow W/m^2,

H_c = convective heat transfer coefficient in $W/m^2°C$, characteristic for the fur,

T_s = skin surface temperature in °C,

and

T_a = air temperature in °C.

Wind will have an effect on the convective heat transfer coefficient such that:

$$H_c = h + \alpha V^{\beta}$$

where h = calm air heat transfer coefficient,

V = wind speed in m/sec,

and

α and β are experimentally determined factors.

Solar radiation causes heat to flow towards the body. The amount of solar heat reaching the skin will thus depend on the filter action of the fur. Since the fur is an open structure, the filtering effect of the fur to incident radiation will be complex compared to

normal optical filtering. The solar energy reaching the skin will depend mainly on the following fur properties:

hair transmittance (t)	=	the fraction of incident radiation that will pass through a hair;
hair absorbance (a)	=	the fraction of incident radiation that will be absorbed by the hair;
hair reflectance (r)	=	the fraction of incident radiation that will be reflected away from the hair (for a single hair a + t + r = 1);
fur density	=	the number of hairs per unit surface area;
and the fur thickness	=	distance from skin to fur surface.

Finally, further complications are introduced by recognizing that hair porosity, pigmentation and surface structure will also affect the values of a, t and r.

In order to obtain results of practical significance within the time limits of this work, gross insulating values only were determined on intact fur samples while a detailed examination of other relevant structural, chemical and physical paramaters was dealt with fleetingly.

Fresh Norman Wells crude oil was used to study the effect of oil on the insulating properties of ringed seal fur.

2.2 Techniques

2.2.1 Heat flow measurements

A fresh fur sample measuring 25 cm by 40 cm was mounted over a heat flow disc (Thornthwaite soil heat flux disc model 610) embedded in a layer of silicone sealant (Fig. 1). The heat flow disc with sealant was placed on a steel plate, 2 mm thick, on top of a steel box within which water circulated at a temperature constant within ·2°C. Uniform thermal contact between the fur sample and silicone, and steel plate and water container was achieved with thin layers of grease. Skin surface

temperatures were measured with copper-constantan thermoelements inserted from below, up to skin level. Air temperature was measured with a thermoelement placed about 30 cm above the fur surface. Wind speeds up to 6 m/sec were generated using a variable speed fan directed away from the sample such that the wind was set by suction rather than blown onto the sample.

The windspeed profile above the fur, measured with a hot wire anemometer (Wallac Oy, Turku, Finland), was uniform within ± 0.5 m/sec at distances greater than 2 cm above the fur surface. Consequently windspeed values were recorded 10 cm above the fur surface.

Solar irradiance was measured with an Eppley solarimeter mounted parallel to the skin surface. A general view of the experimental arrangement for the heat flow measurements is presented in Fig. 2.

2.2.2 Reflectance measurements

Incident light is reflected in two ways, specularly from the surface of a sample or diffusely. With an integrating sphere (Fig. 3a) the sum of the diffusely reflected light, I_{dr}, and the specularly reflected light, I_s, can be measured. This was done using a Beckman DB-G Spectrophotometer with an integrating sphere coated with MgO. Also, MgO was used as reference to the fur samples.

For measurement of the specular component of reflected light a special reflectometry unit (Fig. 3b) using a Shimadzu MPS-50L S spectrophotometer was employed.

2.2.3 Microphotometry

Single hair absorbance was measured by using a Zeiss microspectrophometer. Fig. 4 shows the principle of measurement. A sliding interference filter was inserted between the field stop and the condenser in order to allow spectral analysis.

3. RESULTS

3.1 Heat flow and fur conductance

Experimental conditions and the resulting heat transfer coefficients for normal and oiled fur are presented in Table 1 and 2, respectively. Multiple regression analysis of the results gave the following equations:

for normal fur:

$H_c = 5.2 + 0.31\ V - 0.020\ SR$

for oiled fur:

$H_c = 6.1 + 0.30\ V - 0.045\ SR$

For the normal fur, 69 percent of the variation in the conductance was accounted for by the solar radiation; for the oiled fur, 74 percent.

3.2 Reflectance

Two types of samples were used: one with a bright appearance where bright hairs predominated and one with a dark appearance where dark hairs predominated. The integrated reflectance of 6 samples, 3 of each type, is presented in Fig. 5. The apparent difference in darkness between the two types was detectable in the intergrated reflectance of wavelengths above 850 nm. Crude oil in the fur produced no significant effect on the integrated reflectance. However, the oil caused changes in diffuse reflectance as shown by the Shimadzu spectrophotometer (Fig. 6) while the isolated specular component of the reflectance could not be detected.

A comparison of the results from use of the integrating sphere with the results from use of the attachment for diffuse reflectance measurements indicates differences in curve slopes for short ($\lambda < 400$ nm) and long ($\lambda > 100$ nm) wavelengths.

3.3 Single hair transmittance

Exposure to crude oil had a noticeable effect on the single hair transmittance (Fig. 7). For dark hairs the oil caused a reduced transmittance while in the bright hairs such a reduction occurred only for wavelengths shorter than $\sim$ 450 nm.

4. DISCUSSION

It may have been expected that oil exposure would affect the resistance of the ringed seal's fur to heat loss in air. However, the oil did not significantly change the insulating values in air and at varying wind speeds. It seems that the fresh Norman Wells crude oil used in this study does not matt down the hairs due to its low viscosity. The fur's insulation is determined mainly by the thickness of the

stagnant air layer between the hairs and not the thermal properties of the hairs per se (Hammel, 1955; Tregear, 1966). Thus the oil increases the effective hair diameter, as observed by an electron scanning microscope, without disturbing the protective air blanket in the fur. Also, the fur seemed to maintain its erect posture over a period of 10 months after being exposed to oil. The single hair properties were not examined on this "old" fur.

The major effect of Norman Wells crude oil on the fur of the ringed seal is to increase the solar heating of the animal's skin. Solar heating is an important factor in the haulout behaviour of the ringed seal (Burns and Harbo, 1972; Smith, 1973 b,c). In vivo physiological examinations should be performed in order to assess the seals' ability to buffer the oil induced changes in the insulating properties of its fur.

The oil probably increases the solar heating of the seal's skin by increasing the hair transmittance of lightly pigmented hairs while reflectance and absorbance values are not significantly affected in the wavelength band 400 to 700 nm. The present work indicates that attention should be directed in future to heating effects caused by solar radiation in shorter ($\lambda < 400$ nm) and longer ($\lambda \geq 700$ nm) wavelengths. Long-waved radiation produces a significant part of the total solar heating (Gates, 1962) while the short-waved radiation is of minor significance in the heat balance of the seal.

5. RECOMMENDATIONS

5.1 Solar heating of seals exposed to oil should be examined in vivo both in relation to the animals' total heat balance and yearly moult. This is very important since the moulting period appears to be the most stressful part of the animal's life cycle and interference with its regular haul-out behaviour might be serious.

5.2 Further spectral analysis of the optical properties of fur and single hairs should be performed with special emphasis on wavelengths shorter than 400 nm and longer than 700 nm.

6. ACKNOWLEDGMENTS

We wish to thank Mr. Jimmy Memogana, Holman, Northwest Territories, for obtaining the pelt samples.

7. REFERENCES

Burns, J. J. and S. J. Harbo. 1972. An aerial census of ringed seals, northern coast of Alaska. Arctic 25: 279-290.

Frisch, J., N. A. Øritsland and J. Krog. 1974. Insulation of furs in water. Comp. Biochem. Physiol. 47A: 403-410.

Gates, D. M. 1962. Energy exchange in the biosphere. Harper Row, N.Y. 151 p.

Hammel, H. T. 1955. Thermal properties of fur. Am. J. Physiol. 182: 369-376.

Hart, J. S. and L. Irving. 1959. The energetics of harbor seals in air and in water with special consideration of seasonal changes. Can. J. Zool. 37: 447-457.

Øritsland, N. A. 1970a. Energetic significance of absorption of solar radiation in polar homeotherms. In Arctic Ecology, Academic Press, London 1: 464-470.

1970b. Liceburning of seal pelts. Fiskets Gang. 38: 689-691.

1971. Wavelength-dependent solar heating of harp seals (*Pagophilus groenlandicus*). Comp. Biochem. Physiol. 40A: 359-361.

Smith, T. G. 1973a. Population dynamics of the ringed seal in the Canadian eastern Arctic. Fish Res. Board Can. Bull. 181: 55p.

1973b. Management research on the Eskimo's ringed seal. Can. Geogr. J. 86: 118-125.

1973c. Censusing and estimating the size of ringed seal populations. Fish. Res. Board Can. Tech. Rept. 417: 18 p.

Tregear, R. T. 1966. Physical functions of skin. Academic Press, N. Y. Theoretical and Experimental Biology Ser., Vol. 5: 185 p.

Table 1. Results from heat flow (HF) measurements of normal ringed seal fur exposed to varying air temperature (T_a) wind speed (V) and solar irradiance (SR). Fur conductance is $H_c = HF/(T_s - T_a)$ where T_a is the skin surface (bottom of fur) temperature.

H_c W/°C	V m/sec	SR W/m^2
6.1	2.7	0
6.1	2.7	-
6.5	3.5	-
6.7	3.5	-
7.3	4.3	-
6.7	4.3	-
6.8	4.3	-
6.7	5.0	-
6.7	5.0	-
6.9	5.3	-
5.8	2.7	-
5.4	1.5	-
6.1	2.7	-
6.2	3.6	-
6.3	4.4	-
5.9	1.2	-
6.2	1.2	-
6.4	2.0	-
5.7	1.6	-
5.9	2.2	-
6.3	2.7	-
5.5	1.5	-
5.1	1.1	-
6.1	4.5	-
6.2	3.6	-
6.7	3.0	-
5.4	2.4	-
4.8	1.5	74.4
3.4	1.5	100.1
4.5	2.7	97.9
5.1	1.9	29.7
4.2	1.5	92.0
4.1	2.4	90.5
5.0	3.5	89.0
5.1	4.3	92.0
4.7	2.7	66.8
5.5	1.2	14.8
5.0	1.7	43.0
3.9	2.2	71.2
2.8	2.7	96.4
5.4	3.5	13.4
5.5	2.7	13.4
5.1	2.1	14.8

Table 2. Results from heat flow (HF) measurements of oiled ringed seal fur exposed to varying air temperature (T_a), wind speed (V) and solar irradiance (SR). Fur conductance is

$$H_c = HF/(T_s - T_a)$$

where T_s is the skin surface (fur bottom) temperature.

H_c W/°C	V m/sec	SR W/m²
5.8	3.3	37.1
6.1	3.9	33.4
4.9	3.2	41.5
0.2	4.1	142.4
5.9	3.3	57.9
4.8	1.4	53.4
4.0	0.0	7.1
7.1	2.4	0.0
7.2	3.5	0.0
7.1	4.0	0.0
7.3	4.0	0.0
7.3	3.4	0.0
7.4	4.0	0.0
5.8	3.5	21.5
5.6	1.4	27.8
3.9	0.5	23.4
6.5	0.0	14.8
5.8	4.5	13.4
6.5	4.2	7.4
6.8	4.1	4.1
7.0	3.2	0.7
7.2	2.3	0.0
6.9	1.5	0.0

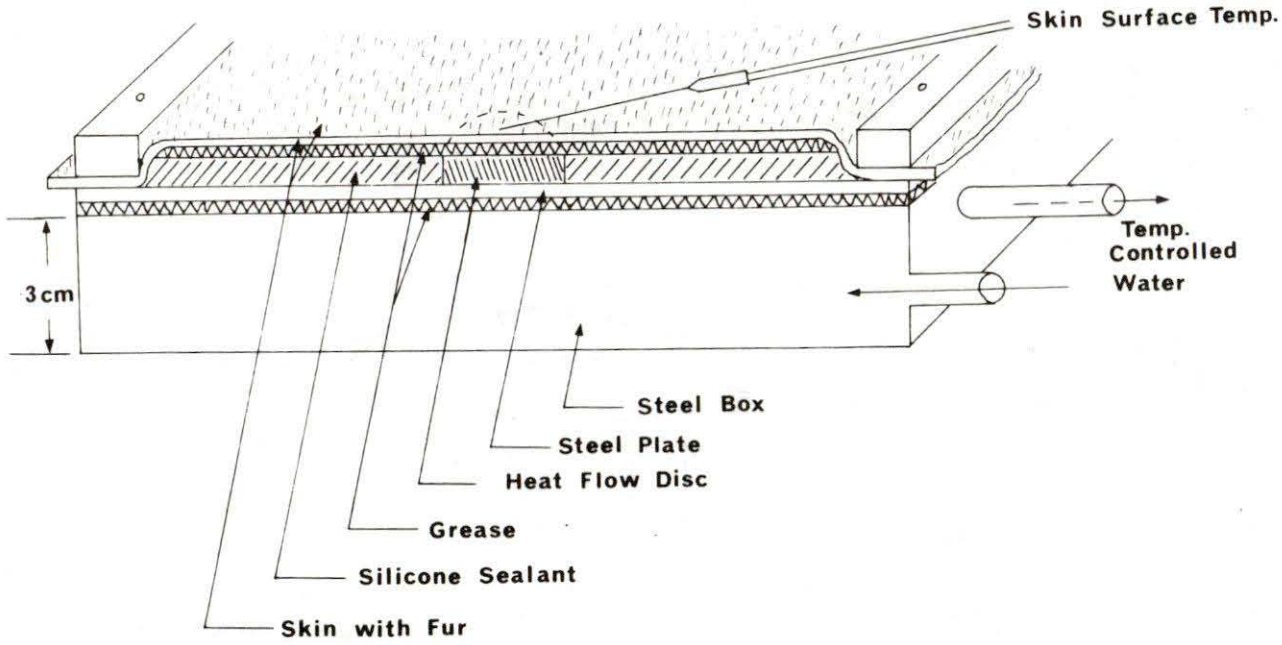


Fig. 1. Fresh fur sample mounted over a heat flow disc.

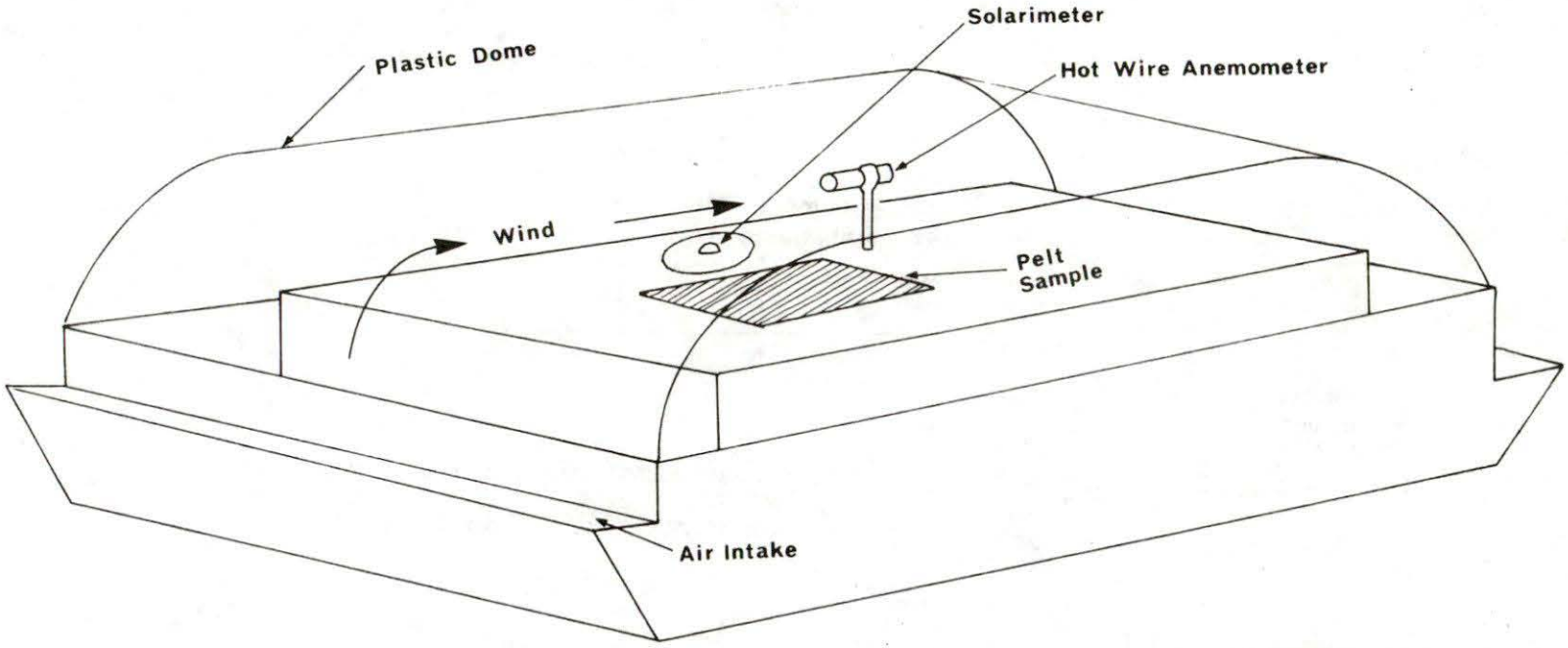


Fig. 2. Experimental apparatus to measure heat flow resulting from solar irradiation.

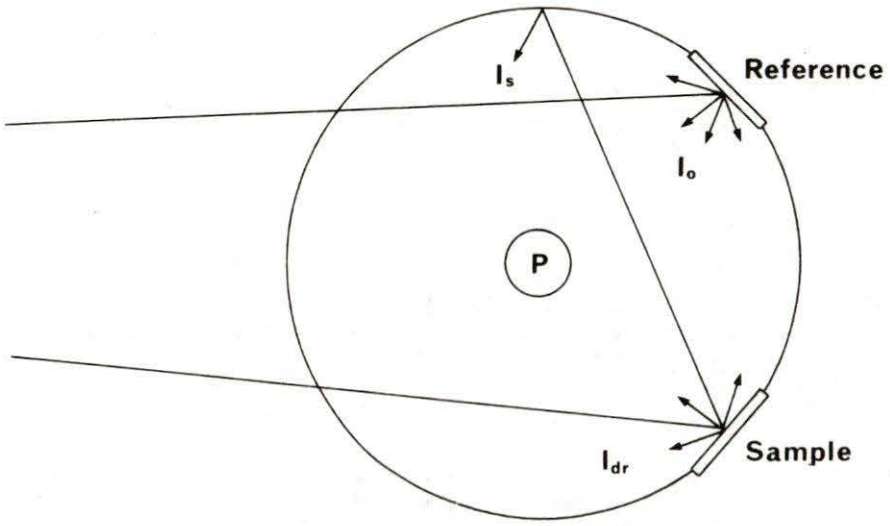


Fig. 3a. Integrating sphere. Both specularly and diffusely reflected light beams are captured by the photomultiplier. I_S is the specularly reflected light; I_{dr} is the diffusely reflected light and P is the position of the photomultiplier.

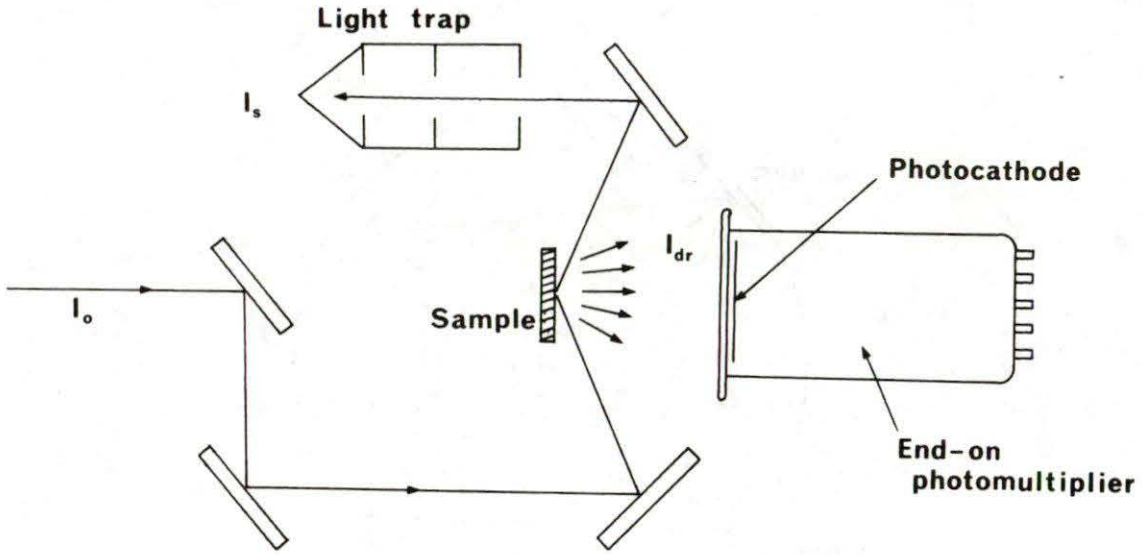


Fig. 3b. The specularly reflected light, I_S, is completely eliminated and a large fraction of the diffusely reflected light, I_{dr}, is captured by the photomultiplier.

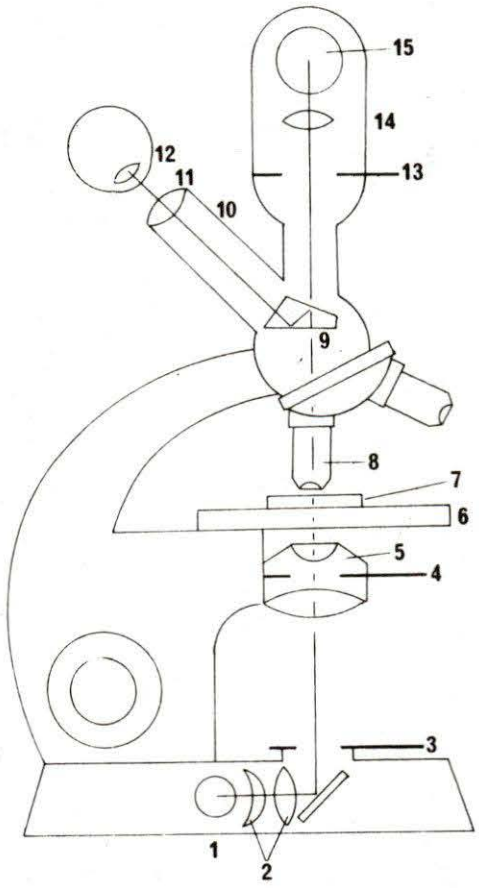


Fig. 4. Diagram of a microscope with photometer attachment. Components are: 1 light source, 2 collector, 3 luminous field stop, 4 aperture stop, 5 condenser, 6 stage, 7 specimen, 8 objective, 9 deflecting mirror, 10 plane of intermediate image, 11 eyepiece, 12 observer's pupil. With the mirror 9 removed, the light is measured in the photometer attachment: 13 measuring diaphragm, 14 projective, and 15 multiplier.

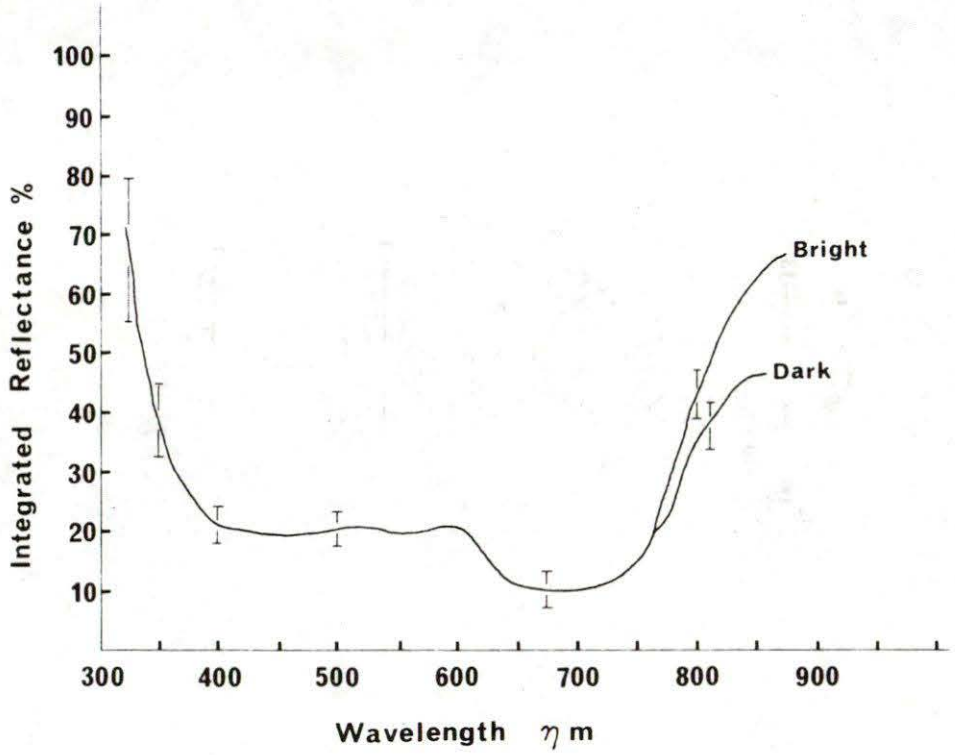


Fig. 5. Integrated reflectance of ringed seal fur. The effect of crude oil was not significant.

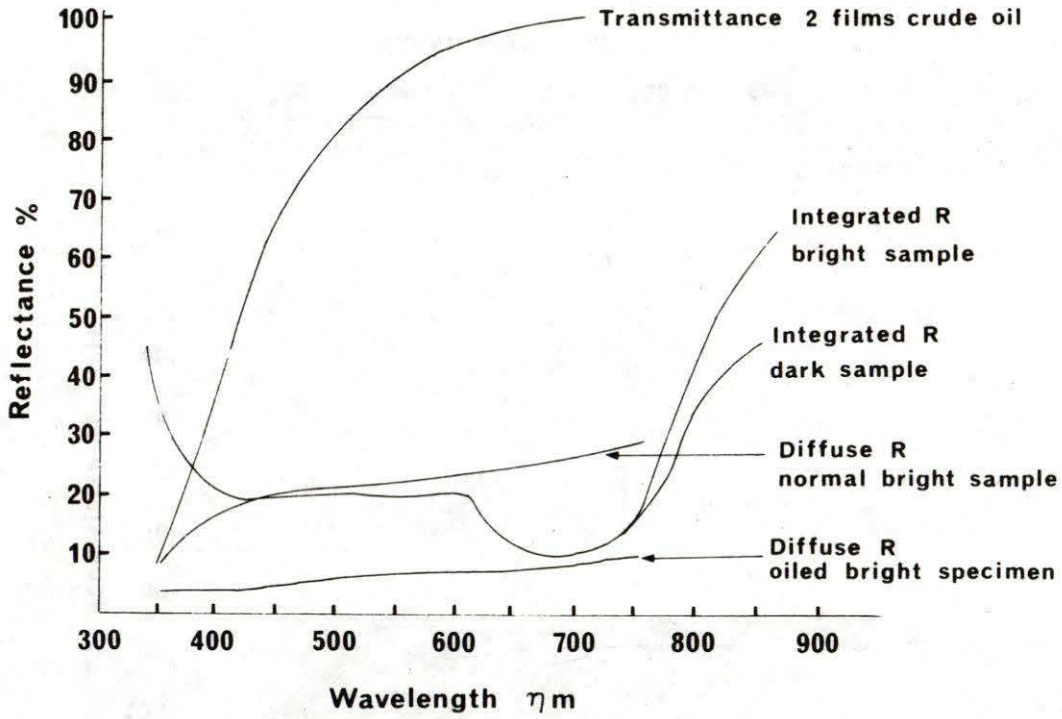


Fig. 6 Reflectance values of ringed seal fur as measured with the integrating sphere shown in Fig. 3a. Also shown, values obtained by use of a diffuse reflectance unit shown in Fig. 3b.

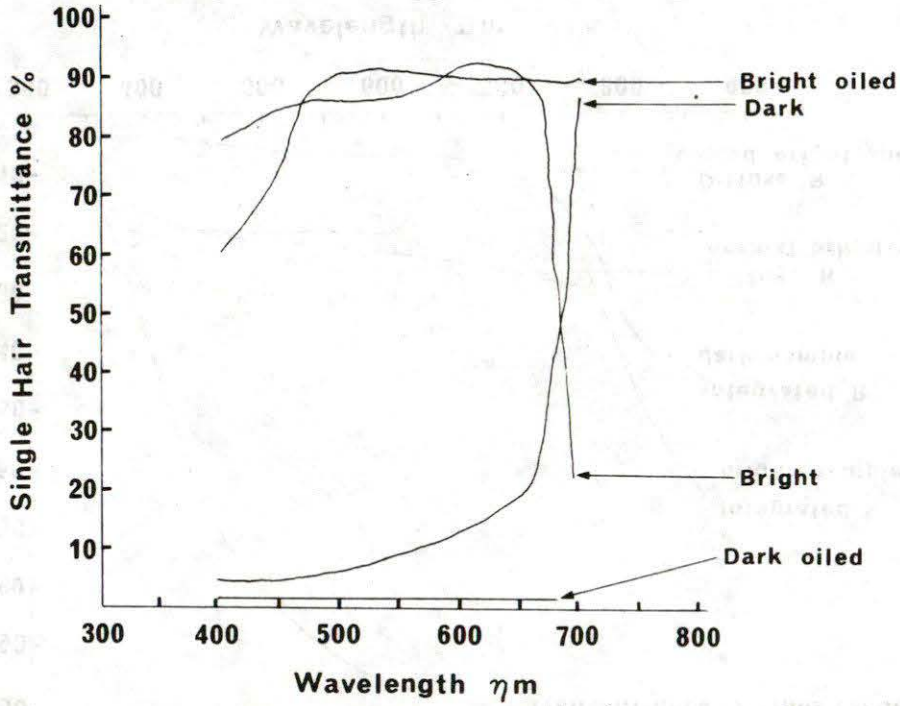


Fig. 7. Microspectrophometric values of single hair transmittance for ringed seals measured using the microscope shown in Fig. 4.