

THE PHYSIOLOGICAL EFFECTS OF ACUTE AND CHRONIC EXPOSURE  
TO HYDROCARBONS ON NEAR-SHORE FISHES OF THE BERING SEA

by

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FINAL REPORT

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## I. TASK OBJECTIVE

The main objective of this study was to establish the effect of selected petroleum hydrocarbons on the physiology of certain cold-water fishes that are year round residents of the Bering Sea.

## II. FIELD AND LABORATORY ACTIVITIES

Background. In the past several years there has been much interest in the biological effects produced when marine organisms are exposed to petroleum hydrocarbons. This interest has been in the form of studies in which organisms have been exposed to the water soluble fraction of petroleum or some of its refined products such as fuel oil (1). Generally the approach in these studies has involved determining how much of the water soluble fraction it takes to kill an organism in a specified period of time. In general the hydrocarbon toxicants responsible for death were not identified and if so their concentrations were not accurately determined because of the lack of good analytical techniques (1).

Recently there has been more emphasis on identifying the toxic components of the water soluble fraction of petroleum and attempting to elucidate the mechanisms by which they disrupt behavior and cause death (2,3). Of the many petroleum hydrocarbon pollutants studied recent results indicate that the aromatic compounds, and in particular naphthalene and naphthalene type compounds are probably the most toxic (3). Naphthalene is of particular interest because it has been demonstrated that it is rapidly taken up by organisms and in the case of fishes it has been shown to concentrate in the liver where it is metabolized (4). Recently studies have also shown **that** this hydrocarbon comprises a significant portion of some crude oils and

fuel oil fractions (3). Because of its toxicity and its relatively high concentration in certain petroleums, we decided to investigate its toxic effects on the physiology and biochemistry of selected Bering Sea fishes at lethal and sublethal concentrations. The fact that naphthalene is rapidly taken up by the liver even at low exposure levels (4) suggested to us that this toxicant might affect liver protein synthesis. The liver proteins selected for study was the secreted plasma proteins (albumins and globulins) because it is a relatively easy system to work with (see review by Haschemey (5) and the biological antifreeze proteins which have been demonstrated to be present in certain members of the fish families Gadidae and Cottidae which inhabit the Bering Sea. The species selected for long term sublethal exposure in this study was the cottid, Myoxocephalus verrucosus (Bean) because it was found to be a very hardy fish which could be handled easily in an experimental study. The biological antifreeze which protects this fish at subfreezing temperatures is a small peptide which is composed of approximately 40% alanine (6). A recent study of protein synthesis in antarctic fishes indicates that their antifreeze compounds (glycopeptides) are synthesized in the liver (7). No studies have been done to demonstrate that the peptide antifreeze is synthesized in the liver of the sculpin, however there is no reason to believe that their site of synthesis should be different than that of the antarctic fish.

#### A. Ship and Field Trips for Specimen Collection and Methods

Collection of Specimens. Ten specimens of the sculpin, Megalocottus platycephalus laticeps (Gilbert) were trawled using a 14 foot otter trawl from Safety Lagoon south of Nome Alaska during September of 1975. The

water temperature was  $+7^{\circ}$  C and the depth 3 meters. The fish were shipped to the experimental aquarium facility at Scripps Institution of Oceanography (SIO) in coolers which were equipped with air pumps. They were held at  $+1^{\circ}$  C until used.

Naphthalene Uptake. Two specimens of Megalocottus weighing 27 g each were used in two separate naphthalene uptake experiments. The experiments involved solubilizing 1  $\mu$  Ci of Naphthalene-1- $C^{14}$  (specific activity 39.8  $\mu$  Ci/ $\mu$  Ci) in one ml of ethanol and slowly infusing it into one liter of filtered seawater through a 30 gauge needle. The seawater was stirred with a magnetic stirrer to ensure mixing occurred. The water was gently aerated and radioactivity measurements of a water sample indicated that loss of naphthalene from the water by evaporation was insignificant. After the sculpin was put into the water, samples were withdrawn and analyzed for radioactivity. One ml of seawater was diluted to 3.5 ml with distilled water and then shaken with 11.5 ml of Aquasol (New England Nuclear). The resulting stiff clear gels were counted in a Beckman liquid scintillation counter. After 22 hours one of the sculpins was washed with methanol and the radioactivity determined in 100 mg samples of several of its tissues. The tissue samples were digested with Protosol (New England Nuclear), and after digestion was complete they were neutralized with Tris-HCl. They were counted in Aquasol before and after addition of an internal standard.

Collection of Specimens for Short and Long Term Naphthalene Exposure. About 200 specimens of the sculpin, Myoxocephalus verrucosus were collected by the fishing crew aboard the R/V Miller Freeman while on Leg II of cruise OCSEAP RP-4-MF-76-A in the eastern Bering Sea. The fish were caught with a

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100 foot otter trawl which was towed for 30 minutes at a depth of 50 m near St. George Island. The water temperature near the bottom was 0°C as indicated by an XBT probe trace. The fish were held on the deck of the ship in 1220 liter fibreglassed circular tanks while at sea. The tanks were closed with resined plywood covers which sealed the tanks and water was introduced through a stand-pipe. This design ensured that the tanks were full and that there was little water movement even in rough seas. Seawater from the ship's fire main was continually circulated through the tanks and few of the sculpin died while at sea. The water temperature of the fire main varied between -0 and +4°C depending on how near the ship was to the edge of the ice.

Short term acute exposures to naphthalene were done aboard the ship in tanks holding 160 liters of seawater containing naphthalene at concentration of 3, 4 and 5 ppm. Naphthalene was introduced into the water by first solubilizing it in ethanol and then infusing the ethanol into a stream of high velocity seawater which ensured that mixing was adequate. When sea conditions permitted, 10 specimens of each species were transferred to the tanks and the times at which they first showed stress and when they died were recorded. Cessation of opercular movement coincided with death as indicated by the fact that when exposed fish were removed to fresh seawater they failed to recover. Only preliminary short term studies were done aboard the ship because of the lack of aquarium space, the large size of the specimens (average weight = 500 g) and the short duration of the cruise. These preliminary naphthalene toxicity determinations were intended only to provide a rough approximation of the TLm (concentration at which 50% of specimens survive) so that sublethal concentrations could be selected for long term exposure

studies to be conducted at the aquarium facility at S10 using a flow through system.

Long Term Sublethal Exposure. Forty specimens of the sculpin collected on this cruise were shipped by airfreight to the aquarium facility at S10. They were packaged in heavy plastic bags in Igloo coolers and the water aerated using a portable battery powered air pump. Some ice was put in the coolers and after 30 hours of air travel the temperature of the water was +4°C. Of the 40 specimens shipped all survived the air shipment. After a week in the aquarium at +7°C the fish began feeding on pieces of yellow tail tuna. The fish were treated once a week with the antibiotic, furacin (30 g/100 l of seawater) to prevent bacterial infections. After two weeks acclimation at +7°C they were transferred to +14°C water and held at that temperature for 2 months. At this temperature the fish were fed twice a week and each week thereafter a few of the specimens were selected for blood samples. Blood plasma samples were assayed for ion content as well as for the disappearance of the thermal hysteresis which is a measure of the peptide antifreeze content. Upon warm acclimation most of the peptide antifreeze disappeared and then 6 specimens were transferred to a 60 liter tank where they were cold acclimated to +0.5°C seawater containing naphthalene at a concentration of 1 ppm. Naphthalene concentration in the tank was determined by measuring the absorbance at 276 mμ and the absorbance agreed with that obtained for a standard solution of 1 ppm. The naphthalene was introduced by solubilizing it in 95% ethanol and infusing it through an IS gauge needle at the rate of 0.4 ml per minute into a stream of seawater flowing at the rate of 800 ml per minute. For metering the alcoholic naphthalene and seawater, two variable

speed peristaltic pumps were used. Silicone tubing was used and it was changed one a week. The calibration of the pumps was checked daily as well as the concentration of the naphthalene in the seawater by measuring the absorbance at 276 m $\mu$ . At the beginning of the naphthalene exposure experiment, 5 specimens of the warm acclimated sculpin were also transferred to +0.5°C seawater and served as controls. Blood samples were taken periodically from the caudal vein of both the control and exposed fish using a 30 gauge hypodermic needle while the fish were under light anesthesia. The plasma levels of sodium and potassium were determined using a Corning 450 flame photometer and the chloride determined using a Buchler chloridometer. Freezing and melting points were determined according to the method of DeVries (10). The difference between the freezing and melting point is referred to as a thermal hysteresis and is a reasonably accurate estimate of concentration of the peptide antifreeze in the blood.

Measurement of Resting Metabolism. Oxygen consumption measurements were done in a 6 liter glass jar at +1°C. Water was circulated through a Rankine electrode chamber and back to the respirometer. The electrode potential was displayed on a Houston strip chart recorder and a span of one millivolt indicated an oxygen concentration change from 0 to 7.9 ml per l of seawater. At +1°C the response time of the electrode was about 10 minutes. Runs usually lasted about 2 hours. Data were recorded only after the first 15 minutes to ensure that the rate of response of the electrode was constant.

When fish are put into a chamber they often exhibit elevated rates of oxygen consumption for several hours due to the activity resulting from strange surroundings and being handled. Therefore, the sculpins were held in the

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respirometer several hours before their oxygen consumption was determined. During this acclimation period water was slowly circulated through the respirometer. The oxygen consumption is expressed as ml  $O_2$  consumed/g/hour.

Measurement of Plasma Protein Synthesis. After 6 weeks of low temperature acclimation, 4 control fish and 4 exposed fish were injected each with 40  $\mu$ Ci of L-leucine- $C^{14}$ (U) (specific activity: 320 mCi/mmol) and the incorporation into secreted plasma protein determined. Forty  $\mu$ Ci of the isotope was made up to a volume of 0.4 ml with a buffered, balanced salt solution and injected into the caudal vein. The solution was injected through a 10 cm length of polyethylene tubing (PE-10) attached to a 30 gauge needle. Once the needle pierced the vein the injection could be made without disturbing the needle. The fish were lightly anesthetized with MS 222 (0.1 g/l) both during the injection and when blood samples were drawn. The arousal time was usually about 3 minutes. At various times after the injection, 300  $\mu$ l samples of blood were withdrawn from the caudal vein and immediately centrifuged before clotting occurred. A 100  $\mu$ l aliquot of the plasma was transferred to a 2.3 cm Whatman No. 3 mm filter paper. The paper disc was allowed to dry for 5 minutes then washed twice for 10 minutes in each of the following solutions: cold 10% trichloroacetic acid (TCA), cold 3% perchloric acid, cold 95% ethanol and ether. After drying at room temperature for 15 minutes the disc was put into a scintillation vial containing 10 ml of toluene containing 4 g per liter of 2,5-diphenyl-oxazole (PPO), 0.05 g per liter of 1,4-bis(2-phenyl oxazolyl)benzene (POPOP). Another 100  $\mu$ l aliquot of the blood plasma sample was added to 100  $\mu$ l of 10% TCA, shaken and centrifuged after having been immersed for 1 hour in ice. A 100  $\mu$ l aliquot of the TCA supernatant was counted in 10 ml of Aquasol.

When it was apparent that the secretion of leucine labeled plasma protein was occurring at a constant rate, the fish were anesthetized, weighed and sacrificed. The liver was removed, weighed and homogenized in one volume of a buffer containing 0.35 M sucrose, 0.05 M Tris, pH 7.4, 0.025 M KCl and 0.01 M  $MgCl_2$  for 3 minutes at low speed in a Waring Blender. The homogenate was centrifuged at 1000 x g for 3 minutes to sediment the cellular debris and rid the homogenate of bubbles. One hundred  $\mu$ l aliquots were assayed for radioactivity using the filter disc technique described above and the free radioactivity determined in TCA soluble supernatants.

The radioactive samples were counted on a Beckman liquid scintillation counter. The recoveries of radioactivity in the forms of labeled protein and free radioactivity in the plasma and liver were calculated according to the method outlined by Hashemeyer (8).

Effect of Naphthalene on Liver Morphology. Upon completion of the leucine incorporation studies but before the livers were homogenized, small sections of liver were preserved in 10% formalin for histological examination. The liver samples were embedded, sectioned and stained with hematoxylin and eosin. They were examined by light microscopy.

### III. RESULTS AND INTERPRETATION

Naphthalene Uptake in Sculpin. Exposure of the sculpin Megalocottus to low levels of naphthalene (0.025 ppm) indicates that even at very low levels fish rapidly take up naphthalene from seawater. Figure 1 illustrates that within two hours 75% of the naphthalene in a liter of seawater had been taken up by a 37 g Megalocottus. After several hours of exposure significant amounts of radioactivity (presumably naphthalene) were found in the various body fluids

and tissues. Most of the radioactivity present in the fish was associated with the liver (Table 1). Similar rates of uptake and concentrations of radioactivity in the liver have also been found when specimens of the temperate pacific sculpin Oligocottus maculosus were exposed to low levels of radioactive naphthalene (4). Recent studies indicate that the liver metabolizes naphthalene to more water soluble products such as 1,2-dihydro-1,2-dihydroxynaphthalene which are excreted via the bile (4). In rats some of the naphthalene is metabolized to 1,2-dihydro-1-naphthyl glucosiduronic acid and excreted via the urine (9). There appears to be no data available concerning the toxicity of these metabolites,

Acute Exposure Studies. Of the several species of Bering Sea fishes exposed to naphthalene at a temperature of +1°C, the cottids and pleuronectids appeared to be more resistant to exposure than the gadids. At a concentration of 4 ppm all of the cod, Gadus macrocephalus and pollack, Theragra chalcogramma died within 2 hours while other species which included the sculpin Myoxocephalus thompsoni and the rock sole Lepidopsetta bilineata did not die until they had been exposed for 20 hours. At a concentration of 3 ppm the cods were unable to maintain their equilibrium after 3 hours had passed and after 13 hours they failed to ventilate and did not recover when put into fresh seawater at the same temperature. The cottids and pleuronectids however showed signs of stress at this concentration only after 12 hours of exposure and after 48 hours only 10% of the sculpins had died. No toxic lethal doses are given for this study because only 10 specimens of each species were exposed. The results are considered to be of a preliminary nature because the exact concentrations could not be determined as there was no spectrophotometer aboard the ship. Water

samples were collected during the course of the exposures and analyzed at the laboratory at S10 one month later, however the naphthalene concentrations were about one tenth of what was expected. Therefore they were considered unreliable and not used. However, the experiments did permit a reasonable estimate of the naphthalene dose which could be tolerated for long periods of time. On the basis of these short term exposure studies, a concentration of 1 ppm was selected for the sublethal long term exposures and at this level no fish died during a 6 week period of exposure.

The cause of death from short term exposures to high concentrations of naphthalene is not known. Exposed fish usually lost their ability to retain their equilibrium in the water column very quickly, and shortly thereafter stopped ventilating. Such behavior suggests that disruption of the nervous system may be involved. The high concentration of radioactivity in the brain (Table 1) of sculpin exposed to radioactive naphthalene lends some support to this hypothesis.

Long Term Naphthalene Exposure Studies. Although none of the six sculpin Myoxocephalus exposed to seawater containing 1 ppm naphthalene died, their condition appeared to deteriorate over the course of exposure. Previously all of these fish fed on pieces of yellow tail fillet and although the controls continued to feed during the course of cold acclimation, the naphthalene exposed fish refused to feed. During the 6 week exposure period they were lightly anesthetized twice and small pieces of fish forced into their stomach. This "force feeding" did not appear to have any adverse effects. On the specimen The reduced food intake undoubtedly had some influence on their condition, however they appeared worse than the condition of the starved controls. The exposed fish also appeared to be less active when transferred to a container of anesthetic.

Effect on Blood Chemistry. Periodic sampling of both the control and exposed fish showed that the concentrations of ions in their blood did not change significantly after the initial increase which resulted from transferring them from warm to cold water (Table 2). The changes in ion levels are in accord with what has been observed with other marine fishes upon cold acclimation (10). On the basis of the data presented in this study, it appears that naphthalene does not affect the capability for osmoregulation in *Myoxocephalus*. One important difference that was noted was the large drop in the hematocrit value of the naphthalene exposed fish which appeared to drop further as exposure continued. It should be pointed out that each time the fish were sampled about 2.5% of their blood volume was removed. Since they did not accept food the red blood cells may have been regenerated more slowly or not at all. The decrease in hematocrit did not occur in a few of the control fish which were starved. The drop in hematocrit value is similar to that observed for other higher vertebrates which have been exposed to naphthalene (11).

Effect of Naphthalene on Peptide Antifreeze. When the warm acclimated control sculpin were acclimated for 6 weeks at  $+0.5^{\circ}\text{C}$ , they produced only a small amount of peptide antifreeze. The plasma freezing point dropped from  $-1.01^{\circ}\text{C}$  to  $-1.28^{\circ}\text{C}$  during this time. The change in the difference between the freezing point and melting point (thermal hysteresis) was only  $0.13^{\circ}\text{C}$  (Table 2). This change is quite small compared to difference observed between sculpin collected during the summer and winter where it is  $1.0^{\circ}\text{C}$ . The small change in thermal hysteresis observed with this extended period

of cold acclimation was surprising, however it is in accord with the magnitude of change observed when the sculpin, Myoxocephalus scorpius was cold acclimated in the laboratory during the months of August and September (12).

No increase in thermal hysteresis was observed with the plasmas of the naphthalene exposed fish. In fact it actually decreased by  $0.06^{\circ}\text{C}$  during the 6 week acclimation and exposure period (Table 2) indicating small amounts of peptide antifreeze had disappeared from the blood. In order to decide whether this change in the level of antifreeze resulted from a decrease in the rate of protein synthesis, the incorporation of radioactive leucine into liver secreted proteins was determined (see Effect on Synthesis of Secreted Liver Proteins).

The slow rate of peptide antifreeze production during 6 weeks of cold acclimation suggests to us that the experiment should have been conducted late in the autumn season rather than during the summer. A similar cold acclimation experiment done with the closely related sculpin, M. scorpius resulted in the production of 50% of their wintertime compliment of antifreeze (12), whereas in this study cold acclimation caused only 35% of the wintertime compliment of antifreeze to appear. Acclimation regimes used in the two studies were the same except that the study described in this paper was done between mid- and late summer, while the other was done between late summer and early autumn. The difference in antifreeze production during cold acclimation in these two species suggests to us that control of production is a seasonal phenomenon and involves more than low temperature and exposure to short days. It is apparent that in order to examine the effects of naphthalene exposure on peptide antifreeze synthesis, the acclimation

experiments must be done during the late autumn, a time during which the sculpin normally produce their peptide antifreezes.

Effect of Naphthalene on Metabolism The oxygen consumption rates for the exposed and control fish are given in Table 3. Data are for fish of similar weights and are therefore comparable. They clearly show that exposed fish have lower rates of oxygen consumption. This low rate is not entirely unexpected in view of their reduced food intake and apparent poor condition. It is possible that the exposed fish are unable to use oxygen at a faster rate because of their severe anemia.

Effect on Synthesis of Secreted Liver Proteins. The time course of the appearance of labeled plasma protein at  $-0.5^{\circ}\text{C}$  after injection of radioactive leucine into the caudal vein of control and exposed sculpin is shown in Figure 2. Examination of the respective curves reveals that there is no difference in the rates of incorporation between the control and exposed specimens. The shape of the curves are similar to those obtained for the incorporation of labeled amino acids into plasma protein at  $20^{\circ}\text{C}$  in the toad fish (8).

The recoveries of labeled plasma protein ranged between 3 and 10% for both groups. These values are slightly lower than those reported for the toad fish at  $10^{\circ}\text{C}$  and for the antarctic cod, Dissostichus mawsoni at  $-1.5^{\circ}\text{C}$  (7). The recoveries of liver protein (63-76%) however were similar to those reported for the toad fish and antarctic cod. Recoveries of free radioactivity in the liver were significantly higher than those in the toad fish, however this is most likely due to the fact that twice as much isotope per unit of body weight was administered to the sculpin as was to the other species. Free radioactivity recovered from the plasma was less than 3% of the total, a value which is similar to that recovered from the plasma of the toad fish.

There appear to be no differences in the recoveries of both the labeled protein and free radioactivity between the control and exposed fish. There-

fore it appears that 6 weeks exposure to 1 ppm naphthalene does not have any detectable effect on the rate at which liver proteins are synthesized and secreted into the blood.

Effect on Liver Cellular Structure. Prior to preparation of the liver homogenates the livers were examined for gross changes and there appeared to be no difference between the control and exposed fish with the exception that one of the livers of the exposed fish was slightly hardened and pigmented. Histological examination of H and E stained sections of this liver revealed that many of its cells were shrunken, and in fact some of them lacked cytoplasm. In other cells thickening of the cell wall had occurred and in some there appeared to be deposition of fibrous material in the cytoplasm. Examination of other livers taken from exposed fish indicated similar cellular changes but not to the extent observed in the hardened liver. Examination of the livers of control fish indicated that their cells were normal.

The histological analysis of the livers indicates that naphthalene exposure does produce changes in the cells of the liver. The normal pattern of protein synthesis observed in naphthalene exposed fishes is not unusual. Compensatory mechanisms exist in the liver which allow protein synthesis to occur at its normal rate despite some cellular deterioration.

#### IV. SUMMARY AND CONCLUSIONS

Sculpins from the Bering Sea were shown to take up naphthalene from their environment however it appeared to have little effect on the biosynthesis of either the plasma protein or of the peptide antifreeze. Morphological studies demonstrated that naphthalene exposure caused deterioration of the liver, however it was not determined whether this was a direct effect of naphthalene metabolism or resulted indirectly from anemia and reduced food intake. The normal rate of protein synthesis in the naphthalene exposed fishes suggests that compensatory mechanisms exist to maintain a constant synthetic rate of liver proteins.

## References

1. Petroleum in the Marine Environment, National Academy of Sciences, Washington, D. C., (1975) .
2. Winters, K., O'Donnel, R., Batterton, J. C., and Van Baalen, C., Water-Soluble components of four fuel oils, Marine Biol., 36, 269 (1976).
3. Boylan; D. B. and Tripp, B, W., Determination of hydrocarbons in seawater extracts of crude oil and crude oil fractions, Nature, 230, 44 (1971)
4. Lee, R. F., Sauerheber, R. and Dobbs, G. H., Uptake, metabolism and discharge of polycyclic aromatic hydrocarbons by marine fish, Marine Biol. , 17, 201 (1972).
5. Haschemeyer, A. E. V., Kinetics of protein synthesis in higher organism, in vivo, Trends in Biochemical Sciences, 1, 133 (1976).
6. Raymond, J. A., Lin, Y., and DeVries, A. L., Glycoprotein and protein antifreezes in two alaskan fishes, J. Exp. Zool., 193, 125 (1975).
7. Hudson, A. P., Comparative studies of plasma protein synthesis in temperate and antarctic fishes, Biol. Bull., 151 (in press).
8. Haschemeyer, A. E. V., Kinetic analysis of synthesis and secretion of plasma proteins in a marine teleost, J. Biol. Chem., 248, 1643 (1973).
9. Boyland, E. and Solomon, J. B., Metabolism of polycyclic compounds. Acid-labile precursors of naphthalene produced as metabolites of naphthalene. Biochem. J., 59, 518 (1955).
10. DeVries, A. L., Freezing resistance in fishes, In Fish Physiology, w. S, and D. J. Randall, eds. 6, 157 (1971).
11. Marks, P. A., Drug induced hemolytic anemias associated with glucose-6-phosphate dehydrogenase deficiency: a genetically heterogeneous trait, Ann. N.Y. Acad. Sci., 123, 198 (1965).
12. Duman, J. G. and DeVries, A. L., The effects of temperature and photoperiod on antifreeze production in cold water fishes, J. Exp. Zool., 190, 89 (1974) .

Table 1. Distribution of radioactivity in various tissues and fluids of Megalocottus platycephalus laticeps after 22 hours exposure in one l of sea water containing 1  $\mu$ Ci (0.025 ppm] naphthalene C-14.

<u>Tissue</u>	<u>Dpm/100 mg tissue</u>
Liver	135,000
Bile	117,000
Brain	41,300
Gut	15,300
Kidney	8,200
Gill	4,500
Muscle	2,800
Blood Serum	1,800

Table 2. Physicochemical properties of blood plasma of two groups of Myoxocephalus verrucosus acclimated to 5°C. One of the groups was exposed to 10 ppm naphthalene during the course of cold acclimation. The number of specimens analyzed are given in parentheses.

Number of specimens and acclimation conditions	Freezing Point °C	Melting Point °C	Melting Point minus Freezing Point	Na mM/ l	Cl mM/ l	K mM/ l	Hematocrit %
CONTROLS							
60 days at +12°C (8)	-1.01	-0.68	0.33	183	171	3.5	
15 days at +0.5°C (6)	-1.25	-0.85	0.40	207	190	2.9	
27 days at +0.5°C (6)	-1.28	-0.79	0.49	203	185	3.0	
50 days at +0.5°C (4)	-1.36	-0.75	0.61	207	193	3.0	17.6
NAPHTHALENE EXPOSURES							
1.5 days at +0.5°C (6)	-1.02	-0.85	0.17	205	183	4.0	9.0
27 days at +0.5°C (6)	-1.11	-0.84	0.27	205	178	2.3	4.6
50 days at +0.5°C (4)	-1.34	-0.78	0.46	214	187	3.2	2.5

Table 3. Rates of oxygen consumption for the sculpin Myoxocephalus verrucosus after 6 weeks acclimation to +0.05°C and exposure to 1 ppm naphthalene.

	<u>Milliliters of O<sub>2</sub> / g / hour</u>
Controls	0.044, 0.059, 0.045
Exposed to Naphthalene	0.029, 0.025, 0.032, 0.025

Table 4. Recoveries of radioactivity in the form of plasma and liver protein and TCA soluble radioactivity after injection of 40 $\mu$ Ci leucine C-14 into specimens of Myoxocephalus verrucosus.

Specimen	Plasma Protein		Liver Protein		Plasma Free Radioactivity		Liver Free Radioactive		
	$\mu$ Ci	% <sup>1</sup>	$\mu$ Ci	% <sup>1</sup>	$\mu$ Ci	% <sup>1</sup>	$\mu$ Ci	% <sup>1</sup>	
CONTROL									
1	0.22	17	2.1	63	0.06	2	0.94	29	
2	0.21	11	1.5	76	0.04	2	0.25	10	
EXPOSED TO NAPHTHALENE									
1	0.19	3	3.5	64	0.14	3	0.164	3	
2	0.19	8	1.8	73	0.06	2	0.40	1	

1. Percentages of recovery are percentages of total radioactivity recovered TCA soluble and insoluble fractions.

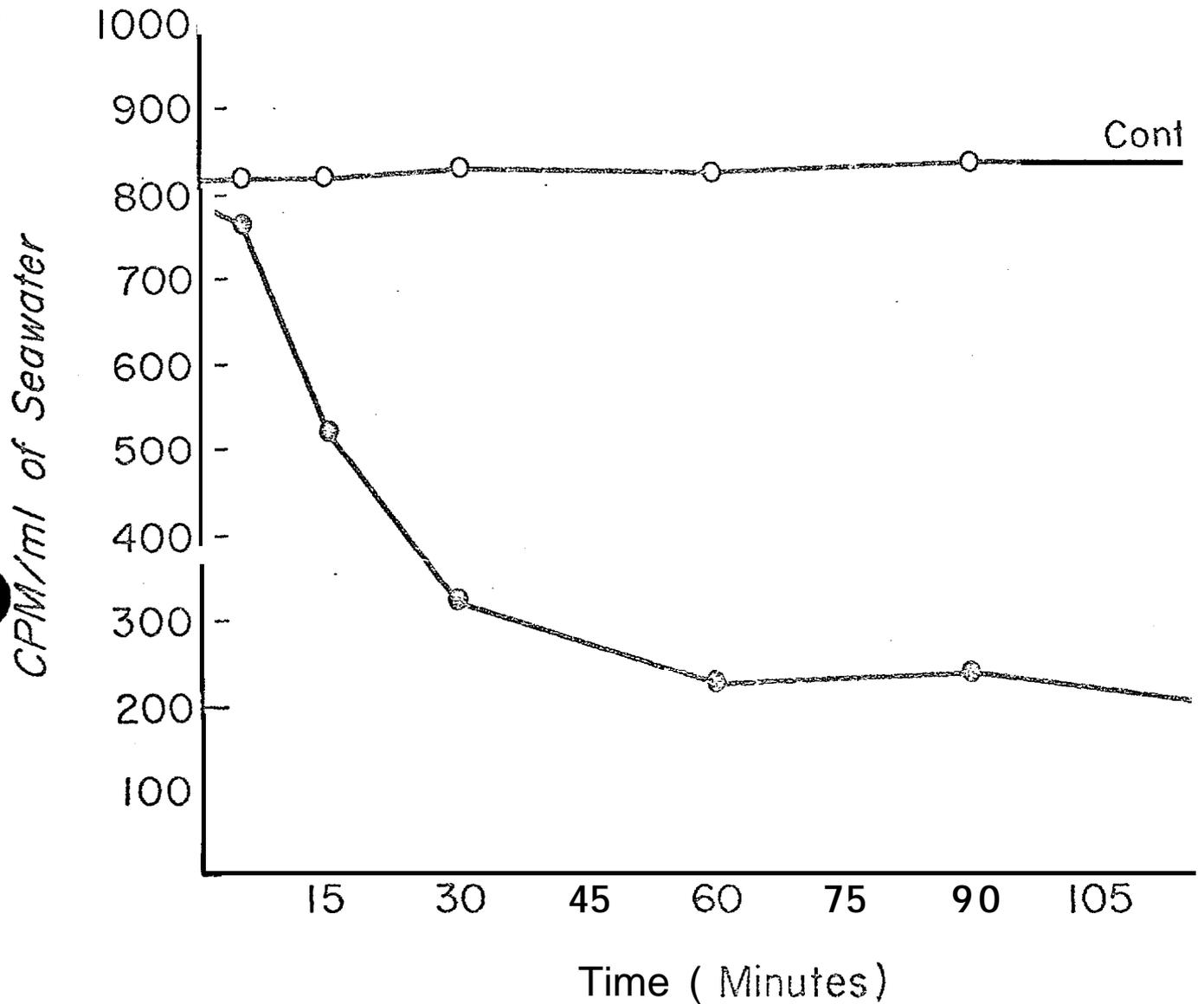


Figure 1. Uptake of naphthalene by a 37 g Megalocottus platycephalus laticeps. The fish was introduced into 11. of seawater which contained 1  $\mu$ Ci of naphthalene-1 -C14. The concentration was 0.02S mg per liter and the decline in radioactivity of one ml samples followed as a function of time.

$A/A^\alpha$

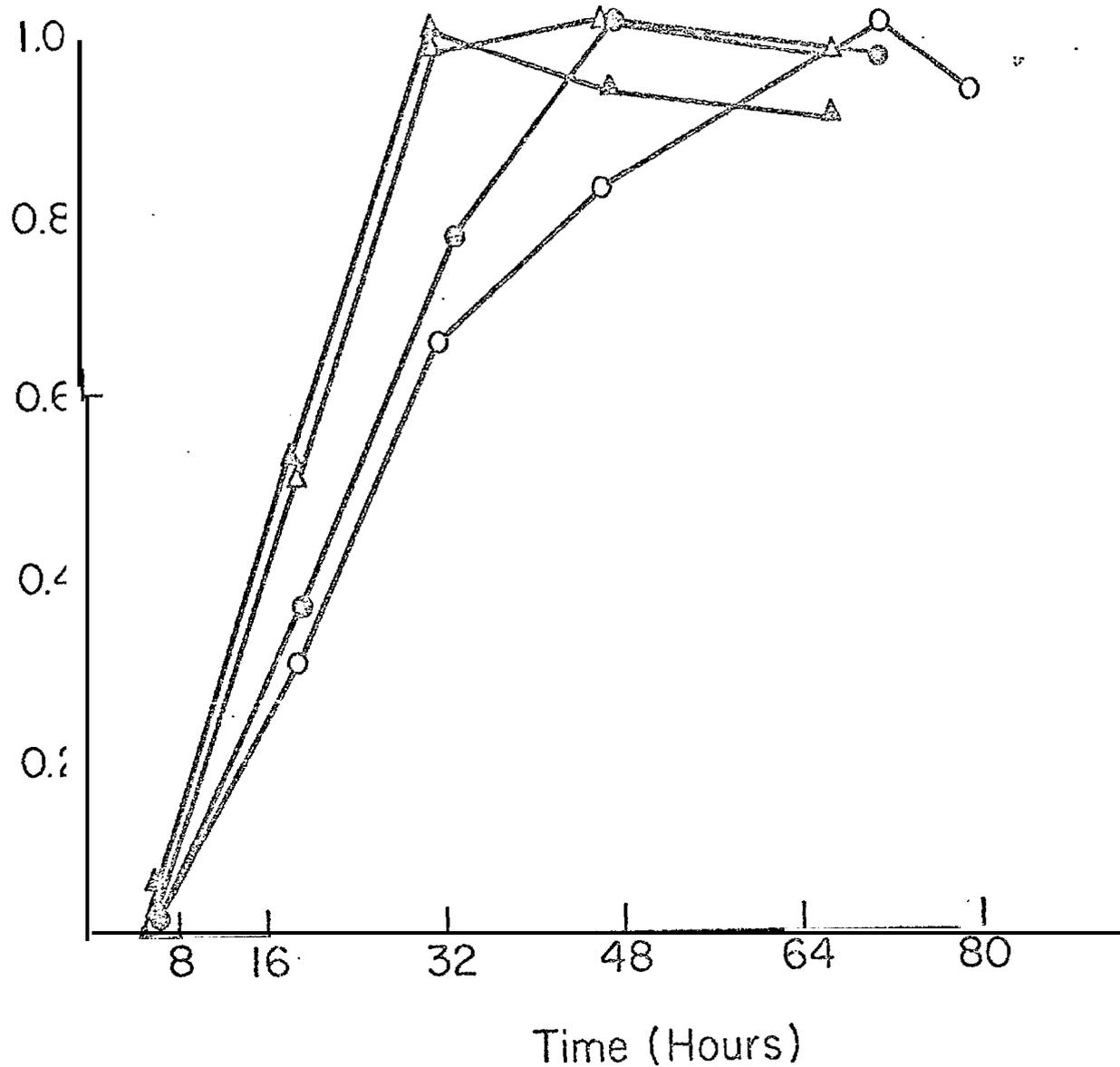


Figure 2. Time course of appearance of radioactive label in the TCA insoluble fraction of the plasma of the Myoxocephalus verrucosus at +0.5°C after 40  $\mu$ Ci of leucine C-14 was injected into the cauda vein. The control values are given by ( $\blacktriangle$ ) and ( $\bullet$ ) while the values for the specimens exposed to 1ppm naphthalene are indicated by ( $\triangle$ ) and ( $\circ$ ). The data are given as dpm divided by the dpm value of the plateau ( $A/A^\alpha$ ).

V. FUNDS EXPENDED \$47,671