RESPIRATORY RESPONSE OF JUVENILE CHINOOK SALMON AND STRIPED BASS EXPOSED TO BENZENE, A WATER-SOLUBLE COMPONENT OF CRUDE OIL

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ABSTRACT

Interest surrounding the potential effects of crude oil on aquatic organisms has increased in recent years due to the incidence of accidental oil spills. There are few experimental results reported, however, dealing with the effect on aquatic species of water-soluble aromatic hydrocarbons contained in crude oil. Such compounds are highly toxic to mammals.

Experiments were conducted using juvenile chinook salmon, Oncorhynchus tshawytscha, *and striped bass,* Morone saxatilis. *The fish were exposed to sub-lethal concentrations of the aromatic hydrocarbon benzene, for periods ranging from 1-96 hours. Prior to exposure, and after exposure to the benzene, respiration rates of individual fish were measured. Results show increases in respiratory rate up to 115 percent above that of control fish after exposure periods of 24 hours for striped bass and 48 hours for chinook salmon. Fish exposed to benzene concentrations of 10 ppm for periods longer than those listed exhibited a narcosis that caused a decrease in respiratory rate. The narcotic state induced by exposure to benzene was shown to be reversible when the fish were placed in fresh water and kept for periods longer than 6 days. Possible biochemical mechanisms leading to this response are hypothesized.*

INTRODUCTION

Interest surrounding the potential effects of crude oil on aquatic organisms has increased in recent years due to the incidence of accidental oil spills from vessels, oil terminals, and offshore drilling operations. There is extensive documentation in the literature of the effects of tar components on aquatic organisms[1,2] and researchers are beginning to investigate the accumulation and translocation through aquatic systems of hydrocarbons associated with crude oil. There are few experimental results reported however, dealing with the effects on aquatic species of water-soluble aromatic hydrocarbons contained in crude oil.

While the holistic approach to subjecting organisms to crude oil in total is desirable, the results are difficult to analyze for cause and effect relationships and it is impossible to examine for differential toxicity of the various components. Our laboratory has therefore, begun a systematic examination of those compounds thought to be most toxic to fish: the low-boiling point aromatic compounds.

Among the aromatic compounds, benzene is high in relative abundance, constituting 20 percent or more of the total aromatics in crude oil. A second reason for choosing benzene for investigation is its high degree of water-solubility (in excess of 200 ppm).

Manifestation of the effects of environmental factors are expressed in the physiological state of the organism and in the production of the population. Respiratory metabolism as a measure of the physiological state of fish has been proved to be a sensitive parameter in evaluating stress caused by a variety of environmental variables[3,4,5,6,7]. The relationship of standard metabolism to energy utilization in fish is well represented in the balanced equation presented by Warren and Davis[8]. The cost in energy of standard metabolism is considered to be the minimal level of physiological and mechanical work necessary to maintain an organism. Any increase in this cost will reduce the amount of energy available for other fates such as growth and there will occur a resultant decrease in production over time in the absence of other compensatory mechanisms.

I have therefore chosen respiratory rate as an appropriate and sensitive parameter to evaluate potential stress caused by sub-lethal concentrations of benzene. These experiments were conducted using juvenile chinook salmon, *Oncorhynchus tshawytscha*, and juvenile striped bass, *Morone saxatilis*. The fish were exposed to sub-lethal concentrations of benzene for varying periods of time. After exposure to the benzene, standard metabolism was measured using tunnel-type continuous flow respirometers. This research was conducted in association with the Department of Animal Physiology, University of California at Davis, from April through September 1972.

Methods and Materials

Juvenile chinook salmon used in this study were obtained from the Bureau of Sport Fisheries and Wildlife's Coleman Hatchery, located near Red Bluff, California. All salmon used in this study were under-yearlings ranging in weight from 2.10 to 5.90 grams (Table 1). The fish were selectively sorted for uniform size and held in well-aerated 200-gallon tanks at 16± 1°C. During the period of acclimation (two weeks) and also during the periods of exposure to benzene, the fish were fed commercially obtained tubificid worms of the genus *Tubifex spp.*

Striped bass used in this study were obtained from the water diversion facilities of the Bureau of Reclamation, located near Tracy, California. The striped bass were not as uniform in size as the salmon, ranging in weight from 33.13-67.62 grams (Table 1). The striped bass were held under the same laboratory conditions and fed the same food type as the salmon.

Water used in the experiments and for holding fish was supplied by a well, the quality of which is reported by Kruger[9]. Water was continuously exchanged in the holding tanks at the rate of 2 liters per minute and filtered through glass wool and activated charcoal filters. Banks of daylight fluorescent lights operated through time switches provided controlled illumination and day length was adjusted weekly to correspond to the natural photoperiod.

Exposure to Benzene

All exposure experiments were conducted in 15-gallon glass aquaria under static water conditions. Each aquarium was divided into three chambers measuring approximately one cubic foot. Each chamber was aerated and used to hold

Table 1: Summary of Experimental Conditions and Experimental Animals Used in These Studies.

Benzene concentration (ppm)	Water velocity (cm/sec)	Length of exposure (hours)	Number of fish	weight of fish (grams wet) Range	Mean
Chinook Salmon					
5	7	1	5	4.32- 5.91	4.87
5	7	24	5	5.16- 5.70	5.54
5	7	48	5	3.63- 4.79	4.08
5	7	72	5	4.36- 5.24	5.02
5	7	96	5	3.30- 4.07	3.94
5	14	1	5	4.32- 5.91	4.87
5	14	24	5	5.16- 5.70	5.54
5	14	48	5	3.63- 4.79	4.08
5	14	72	5	4.36- 5.24	5.02
5	14	96	5	3.30- 4.07	3.94
10	7	1	5	4.80- 5.02	4.94
10	7	24	5	3.33- 5.10	4.51
10	7	48	5	2.15- 3.20	3.04
10	7	72	5	4.17- 4.89	4.46
10	7	96	5	2.55- 3.08	2.97
10	14	1	5	4.80- 5.02	4.94
10	14	24	5	3.33- 5.10	4.51
10	14	48	5	2.15- 3.20	3.04
10	14	72	5	4.17- 4.89	4.46
10	14	96	5	2.55- 3.08	2.97
control	7	1-96	16	4.00- 5.58	4.32
control	14	1-96	16	4.00- 5.58	4.75
Striped bass					
5	7	24	5	36.78-47.88	45.33
5	7	48	5	43.08-67.62	51.90
5	7	72	5	38.68-46.23	43.43
5	7	96	5	36.70-49.40	40.98
5	14	24	5	36.78-47.88	45.33
5	14	48	5	43.08-67.62	51.90
5	14	72	5	38.68-46.23	43.43
5	14	96	5	36.70-49.40	40.98
10	7	24	5	41.64-65.66	49.78
10	7	48	5	31.67-43.74	38.03
10	7	72	5	39.14-51.62	45.33
10	7	96	5	33.61-45.16	39.13
10	14	24	5	41.64-65.66	49.78
10	14	48	5	31.67-43.74	38.03
10	14	72	5	39.14-51.62	45.33
10	14	96	5	33.61-45.16	39.13
control	7	24-96	20	33.13-56.63	44.60
control	14	24-96	20	33.13-56.63	44.60

a single fish. Dissolved oxygen and temperatures were monitored on an hourly basis averaging 9.2 mg/l and 16± 1°C respectively. Dissolved oxygen levels never dropped below 7.8 mg/l.

Benzene concentrations used were 0, 5, and 10 ppm. Preliminary acute toxicity bioassays produced mortality at 15 ppm benzene (by volume). It is probable that concentrations in the range of 0-15 ppm would exist in the vicinity of an oil spill in fresh or salt water considering the relative abundance of benzene in most crude oils (20 percent or more of total aromatics) and its high solubility in water (in excess of 200 ppm).

Benzene concentrations were derived on a volume basis by the following procedure: Reagent benzene was introduced by syringe into a 200 ml water sample held in a glass-stoppered reagent bottle. The sample was then stoppered and shaken for 15 seconds to emulsify the benzene in the sample. The emulsified sample was then poured into the test aquaria so as to equally distribute the benzene amongst the chambers. Each chamber was stirred with a glass rod for 10 seconds to distribute the benzene in the water column. During each exposure experiment, the above procedure was repeated every 48 hours, with the exception that one-half the water volume in each aquarium was removed and replenished. Analyses for benzene concentration were performed using gas liquid chromatography.

The test fish were placed in the aquaria prior to introduction of the benzene and allowed an acclimation period of 48 hours. After exposure to benzene, which ranged in time from 1-96 hours (Table 1), the fish were removed and placed in the respirometers for measurement of respiratory metabolism.

Respirometry

Six recirculating tunnel respirometers were used in this study and were modified from similar apparatus used by Brett[10] and Averett[6]. A detailed explanation of the operating mechanisms can be found in Kruger[9]. Briefly, the respirometers consisted of a plexiglass tube, 6.3 cm long (Fig. 1). Two plexiglass baffles were situated 4 cm apart at the front end of the tube. Turbulent water entering the tube was channeled into an essentially rectilinear flow by these baffles. At the rear of the tube, a 0.6 cm mesh stainless steel screen formed a barrier to prevent fish from being sucked into the pumps. Water entered and left the respirometers through 2.5 cm openings in the expansion and reduction cones which were bolted over the ends of the respiration tube.

Oxygen consumption of the experimental fish was taken to be the difference between the dissolved oxygen content of the water in the respirometer from one point in time to another (usually 15 minutes), during which time the respirometer was sealed from any exogenous source of gas or liquid exchange. At the beginning of each test, a water sample was taken from the flushing-sampling outlets, then both the inlets and outlets were sealed. After the 15 minute time interval, a terminal water sample was taken in a 125 ml glass stoppered bottle. Analyses for dissolved oxygen was made using the Azide modification of the Winkler method[11].

After each fish was exposed for the appropriate period of time to benzene, it was removed from the aquarium, placed in the respirometer and allowed to acclimate for at least 12 hours prior to the first oxygen consumption measurement. This procedure was followed to allow excitement in the fish due to handling to subside. All fish were weighed prior to and immediately after each experiment and all weights reported are initial wet weights unless otherwise stated. Experiments were conducted at two water velocities; 7 and 14 cm/second to test the ability of the fish to increase their activity level after exposure to benzene.

The striped bass used in this study were not as uniform in size as were the salmon. This was because the salmon were supplied from a hatchery but it was necessary to collect the striped bass from the field and only a wide range in sizes was available (Table 1). Because of this range in sizes, it was necessary to determine the relationship between respiration rate and body weight of striped bass. This was done in order to derive values of standard metabolic rate for striped bass exposed to benzene. The difference between the metabolic rate exhibited by a fish after exposure to benzene and the standard metabolic rate derived from the relationship shown in Fig. 2 was taken to be the response caused by the benzene. It was then possible to calculate the percent increase in respiration by dividing the difference by the value for standard metabolic rate derived from Fig. 2.

Results and Interpretation

Exposure to 5 ppm benzene caused an increase in the respiration rates of juvenile chinook salmon irrespective of the length of time of the exposure (Fig. 3). However, the magnitude of the increase did appear to be related to the length of the exposure time. The greatest percent increase (86 percent) occurred after an exposure time of 48 hours, becoming less accentuated at exposure periods of 72 and 96 hours (Fig. 3). The same general relationship between percent difference in respiration rate of control fish and fish exposed to 5 ppm benzene was exhibited when salmon were exposed to 10 ppm benzene (Fig. 4). The increase in respiration rates was greater at every exposure period except 96 hours. The mean increase in respiration rate of salmon exposed to 10 ppm benzene for 48 hours was 115 percent. The oxygen consumption rates of all fish expressed as mg O_2/kg/hr are given in Table 2. There did not appear to be a significant difference between the response of fish held at the two different water velocities. The 14 cm/sec water velocity was near the flow capacity of the respirometers used, but well below the swimming abilities of the juvenile salmon. Dahlberg, et. al.[12] found juvenile coho salmon ranging in size from 4.3 to 6.6 grams to be able to swim at speeds greater than 66 cm/sec. I believe, therefore, that the difference between 7 and 14 cm/sec was insufficient to cause a significant increase in the activity of the salmon.

When the difference in respiration rate of striped bass exposed to 5 ppm benzene is plotted against the length of exposure (Fig. 5) the resultant relationship is different than that of the salmon (Fig. 3). The largest increase was exhibited at 24 hours by the bass where it was elicited at 48 hours in the salmon. The magnitude of the increase was not as great in the bass as it was in the salmon; the greatest increase being 45 percent. As can be seen in Figure 5, the difference in respiration rate was least at the 48 hour exposure period at a water velocity of 7 cm/sec. Contrary to the response of the salmon to the two current velocities, the striped bass appeared to have a distinct response to benzene at water velocities of 7 and 14 cm/sec (Fig. 5). The mean values have been plotted showing 2 standard errors. This separation at the two water velocities may be due to the relative poor swimming ability of the striped bass in comparison to the salmon. Painter and Wixom[13] have shown

striped bass to be poor swimmers and found fish of 41 to 70 cm in length to be able to swim at velocities of 60 cm/sec for only a few minutes. Therefore, a difference in 7 cm/sec water velocity could be sufficient to cause an increased stress on the fish after exposure to benzene.

The response of striped bass exposed to 10 ppm benzene (Fig. 6) is much different than any of those previously discussed. The respiration rate of the striped bass decreased at both 7 and 14 cm/sec when exposed for 24 hours. At 7 cm/sec and an exposure time of 48 hours, respiration rate was depressed even more but the depression was not as great with exposure times of 72 and 96 hours. At a water velocity of 14 cm/sec., the depression in respiration rate occurred only with the 24 hour exposure period, the rate being increased at the other exposure periods (Fig. 6). I believe this difference in response is due to the gill flushing rate and metabolism of the benzene within the fish and this will be treated in detail in the Discussion section.

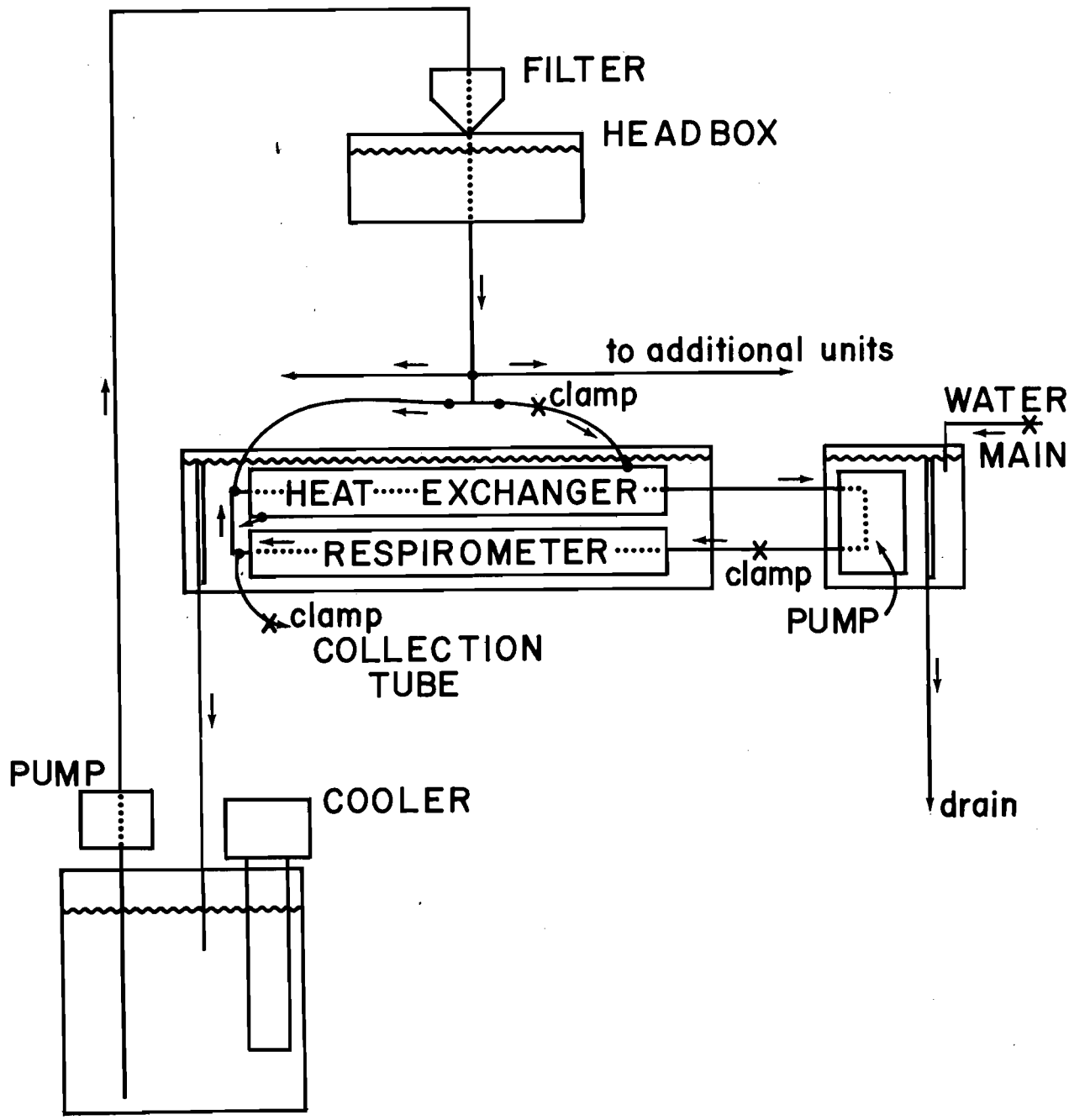

Figure 1: Diagram of One of the Respirometers Used in This Study.

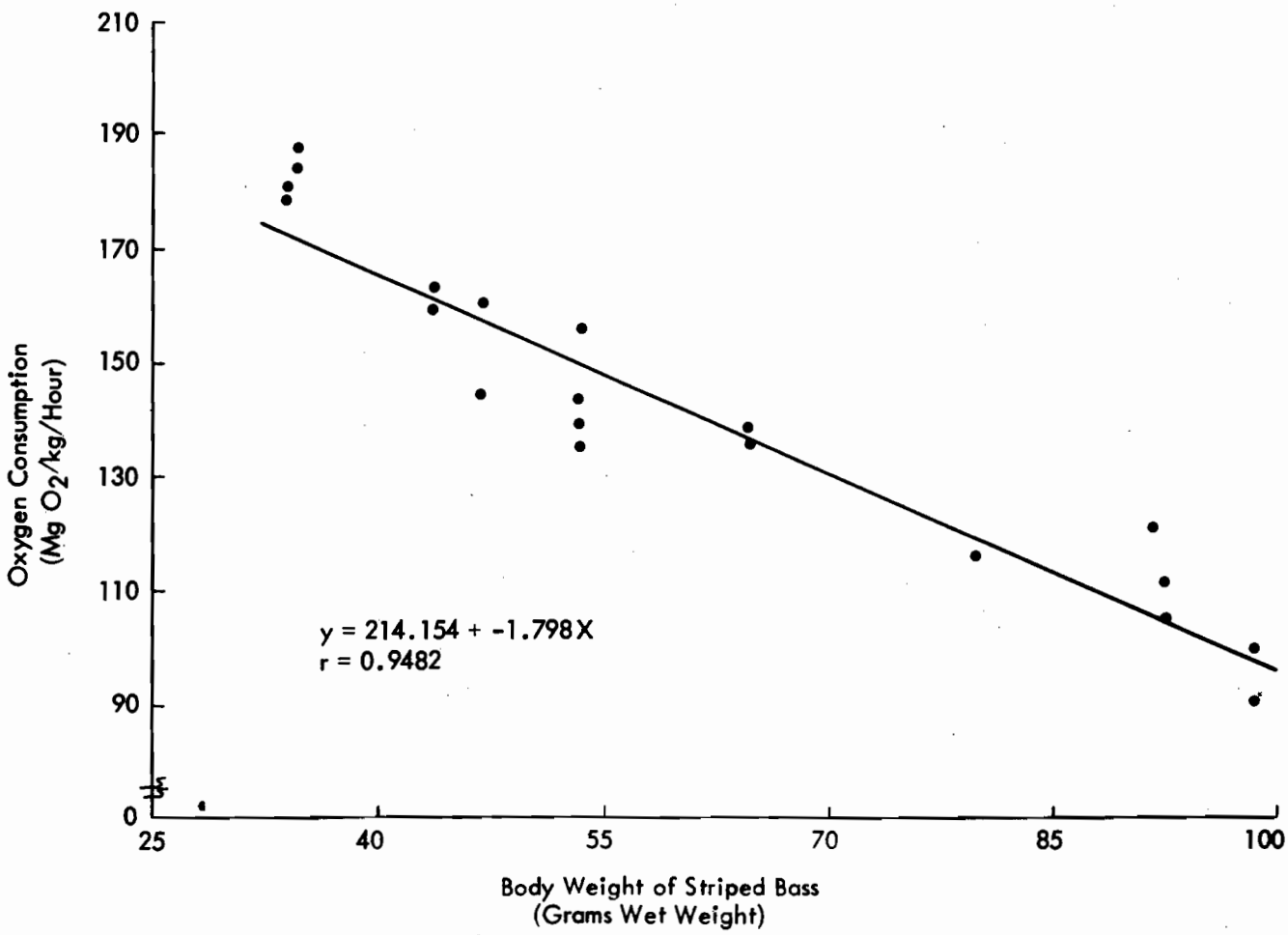

Figure 2: Relationship Between Rate of Oxygen Consumption and Body Weight of Striped Bass.

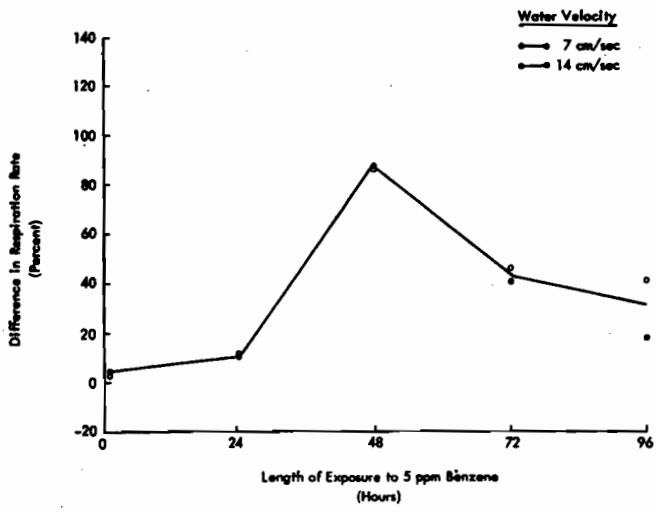

Figure 3: Relationship Between Percent Difference from Control Fish in Respiration of Juvenile Chinook Salmon Exposed to 5 ppm Benzene and Length of Exposure. Each Point Represents the Mean Value for 5 Fish.

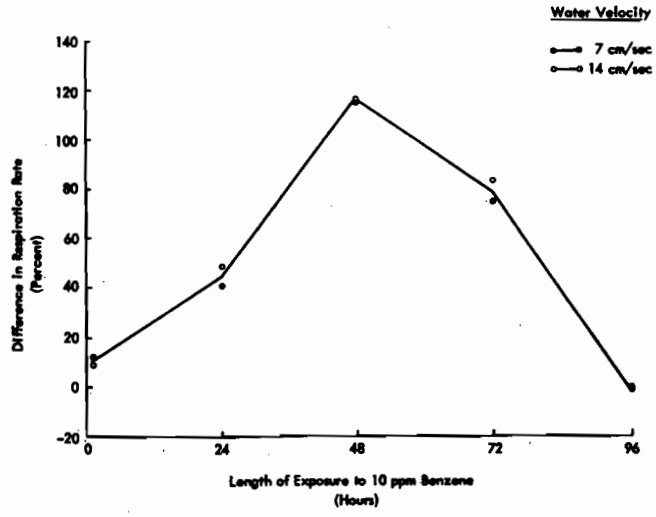

Figure 4: Relationship Between Percent Difference from Control Fish in Respiration of Juvenile Chinook Salmon Exposed to 10 ppm Benzene and Length of Exposure. Each Point Represents the Mean Value for 5 Fish.

Table 2: Summary of Respiration Rates Derived for Experimental Fish.[1]

Benzene concentration (ppm)	Water velocity (cm/sec)	Length of exposure (hours)	Respiration Rate ($mgO_2/kg/hr$) $\bar{x}$ standard	$\bar{x}$ after exposure
Chinook Salmon				
5	7	1	231.00	241.20
5	7	24	231.00	254.40
5	7	48	231.00	431.40
5	7	72	231.00	323.70
5	7	96	231.00	272.40
5	14	1	240.00	246.00
5	14	24	240.00	268.20
5	14	48	240.00	445.20
5	14	72	240.00	349.30
5	14	96	240.00	336.60
10	7	1	231.00	259.2
10	7	24	231.00	325.2
10	7	48	231.00	495.0
10	7	72	231.00	401.3
10	7	96	231.00	229.80
10	14	1	240.00	261.60
10	14	24	240.00	356.40
10	14	48	240.00	517.80
10	14	72	240.00	438.10
10	14	96	240.00	237.60
Striped bass				
5	7	24	158.67	205.50
5	7	48	150.20	140.60
5	7	72	161.20	138.94
5	7	96	164.60	169.18
5	14	24	156.80	227.13
5	14	48	151.17	150.36
5	14	72	158.80	162.12
5	14	96	164.60	181.12
10	7	24	154.00	132.40
10	7	48	167.20	137.14
10	7	72	158.20	145.14
10	7	96	166.60	164.34
10	14	24	154.00	148.86
10	14	48	167.20	179.46
10	14	72	158.20	173.96
10	14	96	166.60	190.32

[1]Each value is a mean of 5 fish. Values for individual fish based upon 8 determinations.

Gill tissue of some of the experimental fish exposed to the benzene was examined for histological damage and none was found. To examine the question of the duration of the response elicited by a single exposure to a concentration of benzene, both salmon and striped bass were exposed to a concentration of 10 ppm benzene for a period of 24 hours. The fish were then placed in clean water and held for a period of ten days, during which their respiration rates were measured every two days at a current velocity of 14 cm/sec.

The results of these tests are shown in Fig. 7. Although the initial respiratory response was different between the salmon and the striped bass, the respiration rate returned to normal in both species at the end of the ten day period following exposure.

DISCUSSION

Benzene is absorbed across the gill surface of fish directly into the blood. Being lipid-soluble, the benzene attaches to erythrocytes and, to a lesser degree, the lipoproteins in the blood[14]. It is then transmitted via the blood for deposit in the tissues or undergoes bio-oxidation to phenol in the liver and other tissues. Some benzene is excreted across the gills in unchanged form. Some hydroxylation of benzene to phenol occurs in the kidney and muscle tissue[15,16]. Common to all of these pathways is the fact that the reaction is enzymatic and requires $NADPH_2$ and O_2. This is the same biological pathway followed in the hydroxylation of steroids and pre-oxidation of lipids. Since this is the case, the amount of foreign chemical that can be oxidized is proportional to the amount of $NADPH_2$ and O_2 available. If lipid oxidation and the hydroxylation of benzene were occurring at the same time, there could exist a competitive inhibition, and neither compound would be likely to be fully metabolized, thus allowing for a build-up of benzene in lipid-rich nervous tissue. If we examine the respiratory responses of the experimental fish shown in Figures 3-7, I believe we can see evidence of the above phenomenon.

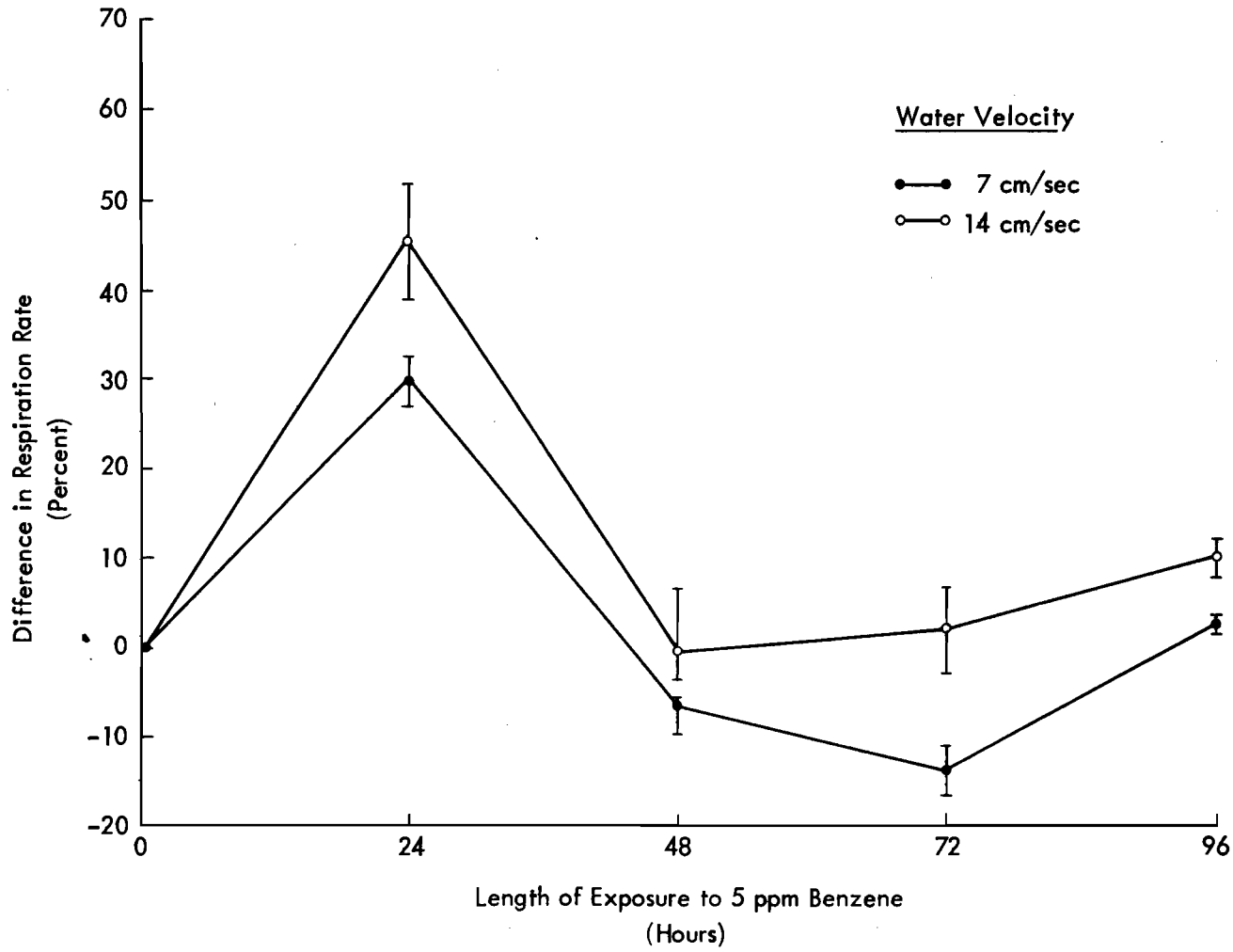

Figure 5: Relationships Between Percent Difference from Control Fish in Respiration of Juvenile Striped Bass Exposed to 5 ppm Benzene and Length of Exposure. Each Point Represents the Mean Value for 5 Fish with 2 Standard Errors Shown.

The juvenile chinook salmon exhibited an increase in respiratory rate at both the 5 and 10 ppm concentrations of benzene (Figures 3 and 4). I believe this increase in respiration to be due to the increased need for oxygen to metabolize the benzene via the pathway previously discussed. The difference in the magnitude of the increase between the two concentrations is probably proportional to the oxygen demand for metabolism. The decreased level of respiration at exposure periods of 72 and 96 hours, even though greater than control values, could be due to an inability of the fish to retrieve sufficient oxygen to fully metabolize the benzene, thus causing an accumulation of benzene in the nervous tissue and resultant narcosis. Such a narcotic effect is well-documented for benzene in mammals[14], and could act in a similar manner in fish due to the particular metabolic pathway under discussion.

In the case of the striped bass (Figures 5 and 6), the results are somewhat different but can, I believe, be interpreted in a manner similar to the salmon. After exposure to 5 ppm benzene for 24 hours, the respiration rate of striped bass increased to a maximum. Here again, the attempt by the fish to increase their oxygen supply to metabolize the benzene is evident. The narcotic effect of the benzene appears at the 48 hour exposure period at 14 cm/sec and is greatest in fish held at 7 cm/sec after an exposure time of 72 hours. A gradual recovery from narcosis is apparently being achieved at the 96 hour exposure period at both current velocities. Striped bass exposed to 10 ppm benzene show an apparent narcosis within 24 hours (Fig. 6) and, as with the fish exposed to 5 ppm benzene, appear to be able to recover from this effect rather quickly, depending upon the current velocity. A reasonable hypothesis for the difference between the fish held at 7 and 14 cm/sec is the increased flushing rate at 14 cm/sec. Even though more benzene may be carried across the gill surface at the higher water velocity, so is more oxygen available, thus decreasing the effect of the benzene at 14 cm/sec.

The differences in response to benzene between the salmon and the striped bass may be due to several variables. The salmon were smaller than the bass, thus they had smaller gill surface. The benzene would diffuse into the blood and accumulate at a slower rate in the salmon, but would also be excreted at a slower rate. Differences in the amount of lipid-rich tissue in the two species could also have been a factor. Harper[17] found that in mammals the $NADPH_2$ involved in utilizing lipids and detoxifying ben-

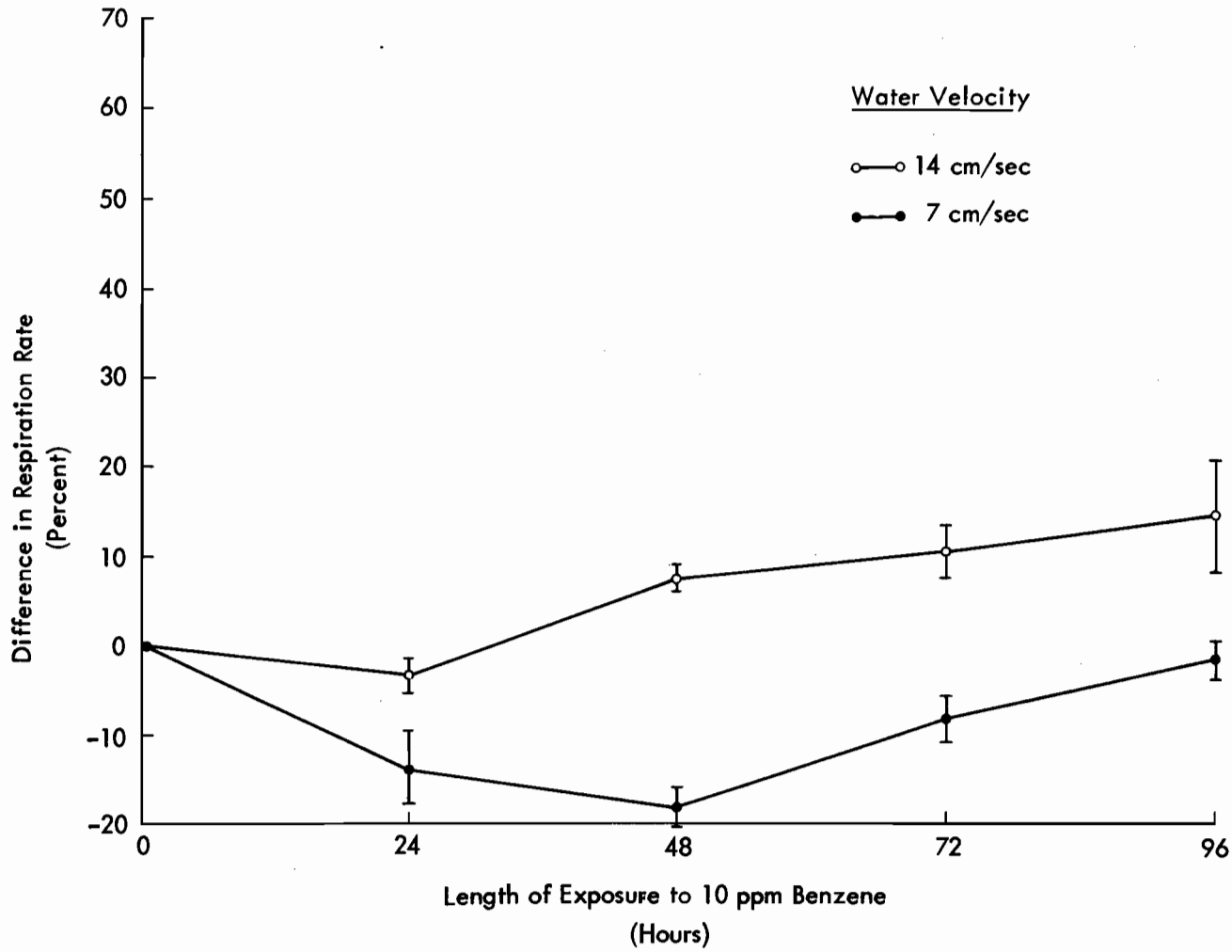

Figure 6: Relationship Between Percent Difference from Control Fish in Respiration of Juvenile Striped Bass Exposed to 10 ppm Benzene and Length of Exposure. Each Point Represents the Mean of 5 Fish with 2 Standard Errors Shown.

zene comes from the HMP shunt, part of the cycle for the catabolism of glucose. If a fish is not lipid-rich, the shunt ceases to operate and the incoming carbohydrate is changed directly to CO_2 and H_2O. The effect would be a lowering of the rate of detoxification and an increase in accumulation, thus a longer period of narcosis. Analyses of total body fat showed the salmon to have an average lipid content of 11 percent where the striped bass had a lipid content of 21 percent.

That narcosis exhibited by the fish used in these studies was reversible was demonstrated (Fig. 7). This is true for the exposure periods and the concentrations used. Investigation of potential effects of benzene on other life history stages of fishes, such as the egg and larval forms, which are not as mobile should be conducted.

Though the exposure tests in this experimentation were conducted under static conditions and extrapolation of laboratory results to the natural environment is difficult, potential effects were shown that under certain conditions could be hazardous to fish. Further investigation should be undertaken to elucidate the mechanisms of the responses to benzene and other water-soluble components of petroleum oil that can conceivably effect the production of aquatic species.

ACKNOWLEDGEMENTS

Special thanks are given to John Hiscox, Howard Bailey and Willard Johnson for their help in conducting these experiments. Mr. Richard Shomura and Drs. R. C. Averett and Jeanette Struhsaker reviewed the manuscript and their constructive criticisms are appreciated.

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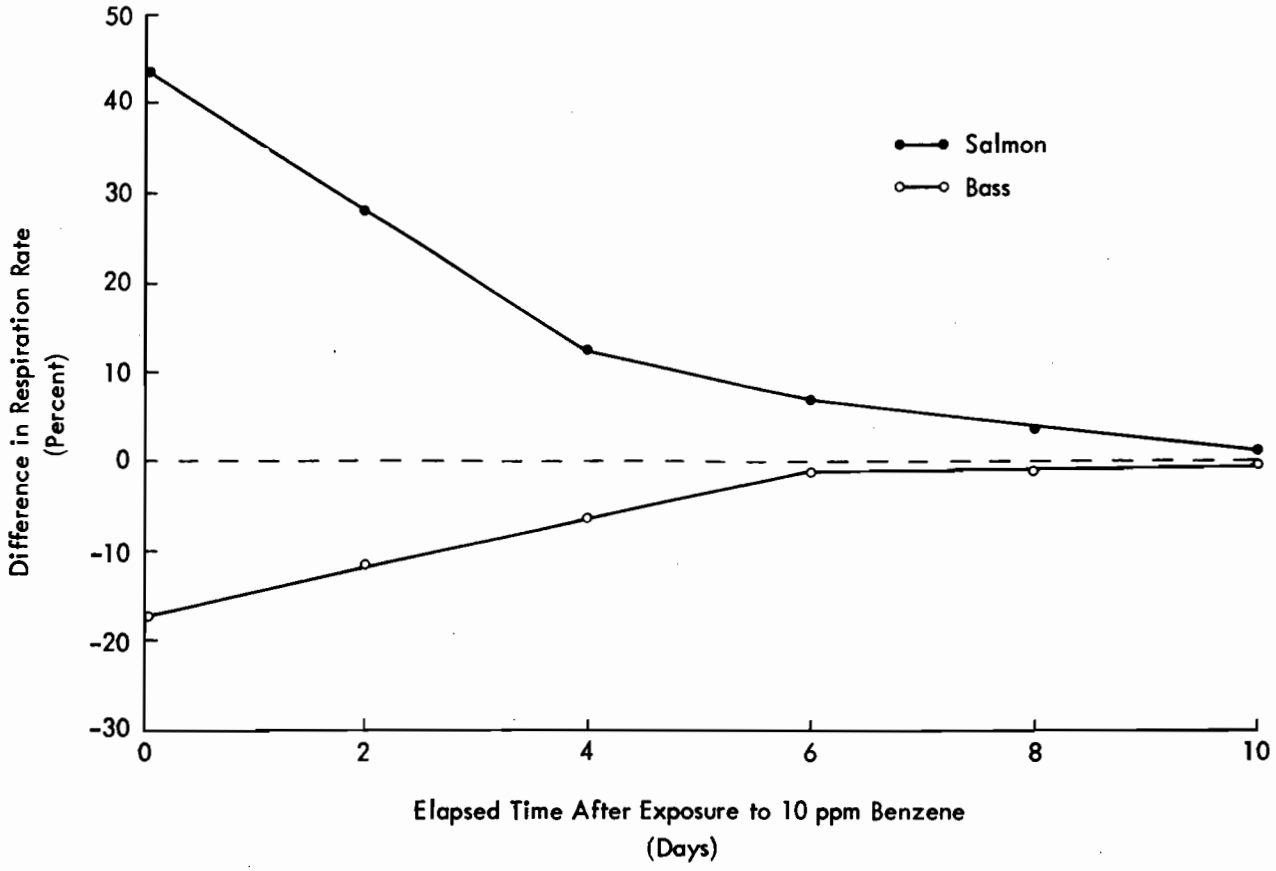

Figure 7: Relationship Between Percent Difference in Respiration from Control Fish of Juvenile Chinook Salmon and Striped Bass Exposed to 10 ppm Benzene for 24 Hours and Removed to Fresh Water. Each Point Represents the Mean Value for 5 Fish.

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Proceedings of Joint Conference on

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March 13-15, 1973
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Sponsored by: American Petroleum Institute, Environmental Protection Agency and United States Coast Guard

oil spill control

EPA API USCG

a cooperative effort

Printed in the United States of America
Library of Congress Catalog No. 74-124324

American Petroleum Institute
1801 K Street, N. W.
Washington, D. C. 20006