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Physiological and Morphological Changes in a Cold Torpid Marine Fish upon Acute Exposure to Petroleum

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We have recently reported that cunner, continuously exposed to crude oil for six months, exhibited smaller testes, reduced plasma Cl^- and Cu^{++} concentrations and increased eye lense diameters, compared with untreated control fish (PAYNE et al. 1978, FLETCHER et al. 1979). Since some of these changes could reflect subtle sublethal effects of oil on endocrinological and other physiological control mechanisms, we felt that further studies should be carried out. Long term studies on the effects of oil or other environmental contaminants are necessary to determine whether sublethal effects do occur. However, once it has been demonstrated that effects can occur, it is difficult to use the long term exposure procedure as a routine technique to elucidate mechanisms of action. For this reason we decided to determine whether some of the effects noted after a 6-month exposure would be evident after 14 days. The experiments were performed during the winter period when the animals do not feed, thus negating any effects of feeding on the measured variables.

As far as the authors are aware some of the statistical methods used here have never been employed before in this type of study. One of the objectives of this presentation is to draw the attention of the reader to the potential scope that these analyses may have in similar studies.

MATERIALS AND METHODS

Cunner (*Tautogolabrus adspersus*) were continuously exposed to a surface slick of Venezuelan crude oil for two weeks in January. The experiment was conducted in a 240-L aquarium continuously supplied with seawater (32‰) at approximately 2 L/min. The fish were treated by adding 150 mL of crude oil to the surface of the water. The "weathered" oil was removed and replaced with fresh oil at weekly intervals. Control fish were held under identical conditions without oil treatment. All fish were maintained under ambient conditions of day

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length and water temperature (approximately 0°C). (See PAYNE et al. 1978 for further details).

After two weeks fish were removed individually from the tanks, bled by caudal vein puncture into a syringe with Na+ heparin as an anticoagulent, and killed by a blow to the head. The following morphometric measurements were then made: length, weight, spleen weight, liver weight, heart weight, gonad weight, gall bladder weight, eye lens diameters and weights, and a sample of epaxial muscle taken and weighed for muscle water determination. The muscle samples were dried in an oven at 90-100°C to constant weight. Hematocrit was determined by standard procedure on a micro sample from the main blood sample. The remainder of the blood sample was then centrifuged to separate the cells from the plasma. Plasma chloride was determined by titration (Radiometer Copenhagen Model CMT 10) and osmotic pressure by freezing point depression (Advanced Instruments Inc Model 3D). Eye lenses were measured by placing the lens on a piece of dry ice to freeze it and immediately measuring the diameter with dial calipers. The lenses were then transferred to pre-weighed aluminum weight boats and dried to constant weight in an oven at 90-100°C. Total plasma proteins were determined by the Biuret reaction (HENRY 1964).

Statistical Analysis. There were four groups of fish viz., control males (M), control females (F), exposed males (EM), and exposed females (EF). Up to 18 variables were measured in each individual. All analyses on measurements of hematocrit and muscle water were done on arc sine transformed data. Table 1 gives means, standard deviations, and sample sizes for each variable.

Differences between sexes (M ∿ F), between treatments (C ∿ E) and their interactions were first examined for each individual character by analysis of variance (anova) procedure based on cross classification (sex x treatment) layout. Sub-class numbers of individuals in this design were unequal and disproportional and this was accounted for in the anova (SNEDECOR 1959). Other contrasts which defined difference between sexes at an experimental condition and between experimental conditions for each sex were also examined.

Univariate statistical analyses are of restricted scope as these ignore correlations among variables and may sometimes be misleading (KSHIRSAGAR 1972). A multivariate analysis is more appropriate, as it employs all variables simultaneously. Usually the populations compared have overlapping measurements. Even where they

TABLE 1

Effect of 2-week crude oil exposure on cunner

Variable	Sex	Control Mean	±	S.D.	n	Oiled Mean	±	S.D.	n
Body weight (g)	M	239	±	41.1	(8)	246	±	31.5	(9)
	F	261	±	45.7	(16)	267	±	46.4	(15)
Length (cm)	M	25	±	1.1	(8)	26	±	1.4	(9)
	F	26	±	1.6	(16)	26	±	1.4	(14)
Spleen weight (g)	M	0.5	±	0.23	(8)	0.4	±	0.17	(9)
	F	0.7	±	0.37	(17)	0.4	±	0.28	(15)
Liver weight (g)	M	5.5	±	2.07	(8)	4.9	±	1.03	(9)
	F	5.1	±	1.51	(17)	4.6	±	1.13	(15)
Heart weight (g)	M	0.5	±	0.14	(8)	0.4	±	0.10	(9)
	F	0.5	±	0.10	(17)	0.5	±	0.14	(15)
Gonad weight (g)	M	2.2	±	0.82	(8)	2.2	±	0.91	(8)
	F	5.3	±	1.57	(17)	5.5	±	0.88	(15)
Gall bladder weight (g)	M	0.3	±	0.12	(6)	0.7	±	0.18	(6)
	F	0.5	±	0.13	(15)	0.8	±	0.30	(8)
Hematocrit (arc sine $\sqrt{\ }$ %)	M	36	±	3.1	(8)	37	±	2.2	(9)
	F	33	±	3.0	(15)	36	±	2.9	(15)
Plasma Osmolality (mOM)	M	373	±	7.3	(8)	366	±	5.1	(9)
	F	369	±	9.8	(15)	367	±	7.9	(15)
Plasma Cl (mM)	M	163	±	3.4	(8)	161	±	4.3	(9)
	F	163	±	4.3	(15)	162	±	5.4	(15)
Plasma Protein (g %)	M	5	±	0.5	(8)	5	±	0.6	(9)
	F	5	±	0.7	(15)	5	±	0.5	(15)
Muscle H_2O (arc sine $\sqrt{\ }$ g %)	M	62	±	0.6	(8)	62	±	0.8	(9)
	F	62	±	0.6	(17)	62	±	0.6	(15)
Eye lens diameter (mm)	M	4.2	±	0.36	(8)	4.3	±	0.31	(9)
	F	4.5	±	0.28	(16)	4.4	±	0.23	(15)

can be identified with a single measurement, a combined criterion of two or more may increase the separation between them. In each analysis presented in the following, which employs a group of variables, only those individuals which had all measurements of the variables in question were included in the analysis. Wilks's Λ values (KSHIRSAGAR 1972) were calculated for interactions of (M ∿ F), (C ∿ E) and (M ∿ F)X(C ∿ E) and contrasts.

In a study such as this where reference samples are identifiable, an extension to discriminant analysis and an examination of contributions of individual variables to discrimination would have been appropriate (KENDALL and STUART 1976). This was, however, considered unnecessary in our case, since these contrasts were not significant.

However, as the contributions of individual variables to the difference between C ∿ E in each sex were of particular interest, these were examined by an alternative procedure which employed planned Bonferroni intervals (MORRISON 1976). This procedure is independent of the multivariate test and yields short intervals when done for few comparisons such as those concerning contributions of individual variables of a group. In the present study, sample sizes were rather small and eighteen variables generated a large number of correlations. The eighteen variables were therefore split into several smaller biologically meaningful groups and contributions of individual variables to the C ∿ E difference examined for each group by Bonferroni procedure.

RESULTS

Interaction of sex and treatment were not significant ($p > 0.05$) for all variables. As interaction was not significant in each univariate analysis, main effects were also tested for significance.

Sex differences were observed in gonad size ($p < 005$) and hematocrit $p < 0.05$.

Mean difference C ∿ E was significant for spleen weight ($p < 0.025$), gall bladder weight ($p < 0.005$) and hematocrit ($p < 0.01$). A further breakdown by anova indicated that these significant differences were due to the significance of C ∿ E in the females ($p < 0.01$) for spleen weight and in both sexes ($p < 0.005$) for gall bladder and in the females ($p < 0.005$) for hematocrit. There was also a sex difference in hematocrit ($p <0.025$)

in the control group.

Using multivariate analysis which included all 18 variables, Wilks's Λ for the interaction (M $\sim$ F)X(C $\sim$ E) was 0.3734 with degrees of freedom (df) = 18, 1 and 24, yielding $p > 0.05$, therefore there was no significant interaction. None of the other contrasts referred to in the univariate analyses earlier, was significant as well at the conventional 5% probability level. The overall mean difference C $\sim$ E was significant at $p < 0.1$, however.

The Bonferroni procedure showed significant differences (C $\sim$ E) for gall bladder weight when grouped with fish length or with spleen weight ($p < 0.01$ for males, $p < 0.05$ for females). Gall bladder weight also contributed to the difference between C and E when grouped with hematocrit and plasma osmolality for each sex and was significant at $p < 0.05$ in each case. Hematocrit in females was significantly different ($p < 0.05$) when grouped with length and ($p < 0.01$) when grouped with spleen weight.

DISCUSSION

The results of the present experiment indicate that oil exposure can result in physiological changes in cunner after two weeks of exposure. The high hematocrits in the oil treated female fish was not accompanied by a rise in plasma proteins or osmolality suggesting that extra red blood cells may be coming from storage areas such as the spleen. Indeed the increased hematocrits in the oiled female group was accompanied by a decreased spleen weight. Decreased spleen weights were observed in the previous study on the effects of a six-month exposure to oil (PAYNE et al. 1978). Increased hematocrits due to spleen contraction have been reported following sustained exercise and burst activity (KICENIUK 1975, STEVENS 1968). The lack of a significant change in hematocrits following a long term exposure to oil (PAYNE et al. 1978) may be a reflection of the establishment of a new equilibrium state following the contraction of the spleen. The observation that plasma Cl^- concentrations were reduced following a six month exposure to oil also suggests a shift in the equilibrium state of the extracellular space (PAYNE et al. 1978). Qualitative as well as quantitative changes in the red blood cells following exposure to oil have been observed in several species of fish. However, the precise nature of the effects varies with the species (KOTOV 1976).

Cunner do not feed during the winter, therefore the increase in gall bladder size in oil treated animals is not an effect on feeding. However, it is likely that some bile flow is maintained in the absence of feeding, since bile is an important excretory route for natural and xenobiotic compounds (GAURINO et al. 1972, MELANCON and LECH 1978). Bile flow is controlled in both the rate of release from the gall bladder and in the secretion rate (DIAMOND 1968). In the absence of any reported work on secretion or flow rates of bile in a seasonally fasting animal, it seems most likely that the oil treatment could affect the secretion rate either by mimicing the action of bile salts which are known to increase bile secretion rates in mammals (WHEELER 1968) or by increasing the excretion of conjugated xenobiotics from the oil. EISLER and KISSIL (1975) have demonstrated liver enlargement in rabbit fish and McCAIN et al. (1978) have found evidence to suggest pathological changes in the livers of English sole following exposure to crude oil.

Although no significant changes were observed in the plasma Cl^- concentrations in the oil exposed cunner in the present investigation, the trend towards reduced concentrations in the oil exposed group is suggestive of the results observed in the long term study (PAYNE et al. 1978). THURBERG et al. (1978) have observed a depression of serum ion concentrations in winter flounder, yellowtail flounder and haddock collected from oil-exposed environments as compared to fish from clear areas.

The lack of an effect of oil treatment on eye lenses or testes in the present study suggests that the results observed in an earlier study only occur following longer exposure periods (PAYNE et al. 1978).

In summary, exposure of a marine fish to a surface slick of crude oil for two weeks decreased spleen weight and increased hematocrit in females and increased gall bladder weight in both sexes.

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