# Reduction of Blood Plasma Copper Concentrations in a Marine Fish Following a Six Month Exposure to Crude Oil

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Recently we reported that when cunner were exposed to petroleum for six months, they exhibited lower plasma C1<sup>-</sup> concentrations than did control fish (PAYNE et al. 1978). This slight reduction in plasma C1<sup>-</sup> suggested that crude oil affected the cunner's homeostatic mechanisms regulating C1<sup>-</sup>. This prompted us to re-examine the plasma of these cunner in more detail in order to determine whether crude oil also affected the concentrations of total proteins, and four essential elements Cu<sup>++</sup>, Zn<sup>++</sup>, Ca<sup>++</sup> and Mg<sup>++</sup>.

## MATERIALS AND METHODS

Cunner (Tautogolabrus adspersus) were continuously exposed to a surface slick of Venezuelan crude oil for six months. The experiment was conducted in a 240 l aquarium continuously supplied with seawater (32 o/oo) at approximately 2 l/min. The fish were treated by adding 150 ml of crude oil to the surface The "weathered" oil was removed and reof the water. placed with fresh oil at weekly intervals. Control fish were held under identical conditions without oil treatment. All fish were fed "ad libitum" with herring or capelin at weekly intervals and maintained under ambient conditions of day length and water temperature. (See PAYNE et al. 1978 for further details).

Blood samples were obtained by cardiac puncture and collected in sodium heparin. Concentrations of the four metals were determined in the plasma by flame atomic absorption (Varian Techtron AA5) (FLETCHER et al. 1975, FLETCHER and KING 1978). Total plasma proteins were measured by the Biuret reaction (HENRY 1964).

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TABLE I

The effects of a 6 month exposure to Venezuelan crude oil on the plasma of cunner

Protein (9%)	O۲	4.72	±0.21	(10)	4.77	±0,32	(8)	NS
	ď				5.5	-	(1)	
$\frac{\mathrm{z_{n++}}}{(\mu \mathrm{g/ml})}$	O+	9.27	±0.44	(17)	8.73	±0.39	(3) (14) (1) (8)	NS
	ъ	0.6	±1.70	(3)	9.16	0.31	(3)	SN
Mg++ (µg/m1)	O+	33.8	±1.74	(36)	31.5	1.1	(22)	SN
	ъ	32.1	±1.58	(3)	36.0	3.94	(4)	SN
$\frac{Ca^{++}}{(\mu g/m1)} \frac{Mg^{++}}{(\mu g/m1)}$	0+	122	± 2.23	(26)	126	± 2.84	(22)	NS
	ъ	112	± 5.43	(3)	117	± 4.25	(4)	NS
C1 <sup>-</sup> (mM/1)	0+	157	+ 1.1	(25)	161	± 1.24	(4) (20)	0.01
	ზ	154	± 6.33	(3)	164	± 3.9	(4)	
Cu++ (µg/100 ml)	Ot	48.8	±3.21	(13)	60.3	±2.47	(10)	0.02
	ъ	38.5		(1)	61.3	±2.95	(3)	
TREATMENT		OIL			CONTROL			

NS = not significant. (P > 0.05). The C1 values were presented (males and females combined) in Payne et al. All values are means ± standard error. Numerals in parentheses are the number of samples analyzed. (1978).

### RESULTS

At the end of the six month exposure to crude oil plasma  $\text{Cu}^{++}$  concentrations were significantly lower in oil exposed female cunner than in control females. The few values obtained for the males were similar to those observed in the females, suggesting that plasma  $\text{Cu}^{++}$  may also be depressed in oil exposed males (Table 1).

Plasma Ca++, Mg++, Zn++ and total proteins did not differ significantly between oiled and control groups (Table 1).

### DISCUSSION

There appears to be a paucity of information available regarding the chronic sublethal effects of oil on marine fish. Long term studies are restricted in number, and those which have been carried out have examined a limited number of physiological and morphological parameters (see for example PAYNE et al. 1978 and MCCAIN et al. 1978). The present report and the one presented by PAYNE et al (1978) suggests that long term exposure to oil may interfere with Cu++ and Cl- homeostasis in marine fish. However the consequences of such physiological changes to the fish are as yet unknown.

Although a considerable amount of information is available concerning Cu++ in mammals (EVANS 1973) virtually nothing is known about the homeostatic mechanisms regulating this element in fish. Studies carried out to date on fish suggest that plasma Cu++ may be influenced by the thyroid (LELOUP 1947, FONTAINE et al. 1948), methyltestosterone (PETERSON and SHEHADEH 1971) and spawning (FLETCHER et al. 1975).

Recently MILLER et al. (1978) have demonstrated that an oral dose of crude oil caused pathological changes in tissue morphology of the small intestine of gulls. These authors suggest that such changes may be responsible for impaired nutrient transport. Since the fishes' major source of copper is probably dietary (see FLETCHER & KING 1978) it would be of interest to determine whether the reduced plasma Cu<sup>++</sup> observed in the cunner of the present study was due to the effects of ingested oil on the digestive tract.

Many of the consequences of copper deficiency have been investigated in mammals where it has been shown, for example, to lead to anemia (LEE et al. 1976) and increased susceptability to toxic metals such as cadmium and lead (PETERING et al. 1977). PAYNE et al. (1978) have observed a significant decrease in spleen weight in oil exposed cunner. This response may be a reflection of the oil exposed cunner's demands for an increased blood oxygen carrying capacity, which could

be aggrevated by copper deficiency.

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