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Evidence for Metal Poisoning in Acute Deaths of Large Red Drum (*Sciaenops ocellata*)

P. T. Cardeilhac,[1] C. F. Simpson,[1] F. H. White,[1] N. P. Thompson,[2] and W. E. Carr[3]

[1]*College of Veterinary Medicine,* [2]*Pesticide Research Lab and* [3]*Whitney Marine Lab, University of Florida, Gainesville, FL 32610*

Approximately 100 large mature red drum (Sciaenops ocellata, in a size range of 7-18 kg) were found dead in the Indian River and Mosquito Lagoon on the northeast coast of Florida near Titusville between 14 June and 7 July 1980. Observers reported that some fish were seen coming to the surface and showing incoordinated movements just before death. There was complete loss of posture (abdomen up) and only twitching and opercular movements present. The purpose of this report is to present evidence for metal poisoning as the cause of this mass kill.

MATERIALS AND METHODS

Fish. Two (approximately 2%) of the affected fish (one freshly dead and the other moribund) both 93 cm in length and weighing 14 kg were found to be suitable for study. A blood sample was drawn from the moribund fish. Two captive red drum (66 and 69 cm in length and weighing 3.9 and 4.6 kg respectively from the Whitney Laboratory, Marineland, Florida) were studied as controls.

Blood and Tissue Samples. Necropsies were performed. Caudal blood, gonads, gills, spleen, muscle, abdominal fat, liver, kidneys, and stomach contents were collected from the fish. Tissue samples were fixed in 10% neutral formalin, embedded in paraffin, sectioned at 5 µm and stained with hematoxylin and eosin for histologic examination. Samples of spleen, liver, kidneys and lower digestive contents were cultured on blood, eosin methylene blue, and MacConkeys agar and also on Rimler-Shotts medium (SHOTTS & RIMLER 1973), and incubated at 25 C. Blood smears were stained with Wright-Giemsa stains.

Serum total proteins, albumin, globulins (albumin to globulin ratio), creatinine, glucose, aspartate amino transferase, lactic dehydrogenase, alanine amino transferase, alkaline phosphatase, Na, K, PO_4, Ca, Cl and CO_2 were determined using a simultaneous multiple analyzer and computer or flame photometer as previously described by CARDEILHAC & HALL (1977). Metal (Cu, Zn, As, Cr, Cd, Hg, Pb and Se) concentration in tissues was determined by atomic absorption employing procedures described by MITCHELL & RHUE (1979) after digestion of the sample with perchloric acid. Ten mg of a 10% suspension of liver and kidney (in marine saline) was filtered through a 22 µ filter and inoculated into a primary culture of follicle cells taken from a female control red drum, as described by CARDEILHAC et al. (1978). The cell cultures were ex-

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amined using phase contrast microscopy for evidence of cytotoxic agents at 3 and 5 days post inoculation. A dose of 200 µl of stomach contents from the moribund red drum was given orally to 3 pinfish (Lagodon rhomboides) weighing approximately 150 g. The pinfish were observed daily for signs of intoxication. Samples of abdominal fat, gonad and liver from the red drum were tested for pesticide residues as described by THOMPSON et al. (1974).

RESULTS

Gross Examination. The body condition of the affected fish was good as determined by the method of HILE (1936) with a coefficient of condition = $K = (10^5 x\ W)/L = 1.74$. The K value for affected fish exceeded control values ($K = 1.38 \pm 2$) and values ($K = 1.50$) reported for healthy adult red drum by BASS & AVAULT (1975). Large quantities of abdominal fat were observed in the fish. The stomach and intestinal tract of affected fish were full, suggesting an acute death. Digestive tract contents consisted entirely of partially digested large blue crabs. Kidneys and gills were congested and the gills covered with mucus. Gonads, livers, spleens, digestive tracts, hearts and other organs were similar in appearance to corresponding tissues of controls.

Microbiology and Toxicology. No organisms were cultured from the kidney, liver and spleen, and organisms considered pathogens were not cultured from the lower intestinal tract. Filtered homogenates of liver and kidney did not produce detectable cytopathic effects on the follicle cell cultures. Signs of toxicity were not observed in pinfish dosed with stomach contents from the moribund red drum. Identifiable pesticide residues were not present in samples of liver and gonad.

Concentrations of Cu were distinctly (≥ 2 S.D.) elevated in gill, liver, kidney and stomach content from the diseased red drum when compared with controls. Levels of Zn were distinctly elevated in the gill, liver and stomach contents and elevated in the kidney. Arsenic concentration was distinctly elevated in the liver and elevated in the stomach contents and gill. In the liver concentrations of Cu, Zn, As, Cr, Cd and Hg were all significantly (5 to 10 S.D.) higher than control values (Table 1). None of the organs from diseased fish had distinctly elevated concentrations of Pb or Se.

Clinical Values. Serum electrolyte concentrations from the moribund fish were significantly elevated (Na, K, Cl, and PO_4, all > 10 S.D.) above mean control values (Table 2). Total proteins, albumin, globulin and creatinine values from the diseased redfish were similar to controls. Serum aspartate aminotransferase, lactic dehydrogenase, alanine aminotransferase and alkaline phosphatase activities were elevated in the serum sample from the moribund fish. However the blood sample was partially hemolyzed, and based on control values and previous studies (CARDEILHAC & HALL 1977; CARDEILHAC et al. 1979), these changes in proteins and enzymes were not significant.

TABLE 1

Increased Metal Concentrations in Organs from Diseased Red Drum[a]

Sample	Metal					
	Cu	Zn	As	Cr	Cd	Hg
Liver						
diseased[b]	55±16	183±21	8.2±1.1	60±24	8.7±5	3.5±1.6
control[c]	10±7	51±13	2.8±0.3	<1	<0.5	0.6±0.1
increase[d]	45(6.4)	132(>10)	5.4(>10)	59(>10)	8.2(>10)	2.5(>10)
Gill						
diseased[e]	10	65	1.5	<1	<0.5	<1
control[c]	2±0.1	11±1	1.0±0.3	<1	<0.5	1.1±0.6
increase[d]	8±10	54(>10)	0.5(1.7)	---	---	---
Kidney						
diseased[e]	15	18	0.8	<1	<0.5	<1
control[c]	5±4	14±6	1.4±0.1	<1	<0.5	<1
increase[d]	10(2.8)	4(<1)	---	---	---	---
Stomach Contents						
diseased[c]	4±1	32±18	1.4[e]	<1	<0.5	<1
control[c]	2±1	7±6	1.1±0.2	<1	<0.5	<1
increase[d]	2(2)	25(4)	0.3(1.5)	---	---	---

[a]ppm wet weight ± standard deviation (S.D.).

[b]Duplicate samples from each of 2 fish.

[c]Single samples from each of 2 fish.

[d]First number = difference between diseased and control. Number in ( ) = (number of S.D. of increase).

[e]Single sample from moribund fish.

Histology. The outstanding histologic lesions were found in the gills of the moribund fish. There was edema and lymphocytic infiltration of the greatly swollen lamina propria and edematous gill lamellae. Mucus covered the epithelium of affected gills, and bacteria and an occasional unidentified protozoa were embedded in the mucus. Gills of the control fish were not edematous or coated with mucus. There was a fatty degeneration in the liver of both the moribund and control fish.

DISCUSSION

The principal target organ for copper poisoning in marine fish is reported to be the gills (CARDEILHAC et al. 1979). Gills of poisoned fish are congested and excessive mucus is secreted. The gills become blunt with thickened lamellae and dilated mucous

TABLE 2

Increased serum electrolyte values in the moribund red drum[a]

Fish	Electrolyte				
	Na	Cl	K	PO_4	Ca
Moribund	249	219	22.5	26.4	14.7
Controls[b]	183±6	160±5	3.5±1.8	11.4±1.1	14.3±0.1
Increase[c] (No S.D.)	66(10)	59(10)	19(10)	15(10)	0.4(4)

[a] meq/L ± S.D.

[b] Serum samples from 2 normal control fish.

[c] First number = difference between diseased and control and number in ( ) = (number of S.D. of increase).

cells. Clinical signs in order of appearance are: lethargy, indifference, incoordination, moribundity and death. The major changes in clinical values are pronounced ionic imbalance apparently resulting from failure of osmoregulation. A potassium ion increase is considered the most life-threatening change and the specific cause of death appears to be potassium intoxication although hypoxia can not be ruled out. Lethal concentrations of copper in seawater (8.5 ppm) produce liver copper concentrations that reach 16 ppm and gill copper concentrations reach 1 ppm in dead or dying fish (after 12 hours of exposure). COUCH (1977) has reported that Cd also causes osmoregulatory dysfunction in fish and SKIDMORE (1970) found that zinc affects the gills of teleost fish and produces a fatal hypoxia but has little effect on osmoregulation. SPEHAR et al. (1980) reported that arsenic alone does not appear to be highly toxic to fish, and mercury is known to affect development of the nervous system in marine fish. There are few reports concerning the life-threatening pathophysiology of Cr and a failure of osmoregulation has not been reported (BERNHARD & ZATTERA 1975). An apparent failure of osmoregulation in the red drum of the present investigation was indicated by dramatic increases in serum electrolyte concentrations (> 10 S.D. in all but Ca) above control values. The increase in Na and Cl of approximately 60 meq/L represent a 33% increase in total ions and was the major cause of increased osmolarity of the serum. The failure of osmoregulation apparently resulted from gill damage seen in the diseased fish. Hemolysis and dramatic elevation of serum K found in blood from the moribund red drum have also been seen in both accidental and experimental copper poisoning of other marine fish (CARDEILHAC et al. 1979).

History and data indicated that the acute deaths of the present investigation were caused by intoxication. Four possible causes of the intoxication were considered: 1) pesticides, 2) a biological toxin, 3) hypoxia, and 4) metals. Identifiable pesticide residues were not found in the tissues of diseased fish and the stomach contents were not toxic to test fish. Thus the first two possibilities were eliminated. Hypoxia caused by low oxygen tension in the seawater was considered to be unlikely, since only one size class of one species of fish was affected. Metal poisoning was then investigated and the elevated metal concentrations found. The acute episode appears to have been triggered by the ingestion of Cu, Zn and As, based on analysis of stomach contents but Cd, Hg and Cr may, at least, have been contributory by lowering tolerance to metal poisoning. Metals such as Hg, Cd, Cu and Zn are all known to bind with metallothionine, a protein associated with tolerance to metal poisoning (BROWN & PARSONS 1978). The size and feeding habits of large adult red drum make it the only species of fish in the area feeding almost exclusively on large (300 g) blue crabs at the time of the kill. If large blue crabs were the source of the metals it would explain why only large adult red drum were affected.

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