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Frequency Distribution Patterns And Partitioning Of Copper, Iron And Zinc In Selected Tissues Of The Black Mullet (Mugil Cephalus)

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FREQUENCY DISTRIBUTION PATTERNS AND PARTITIONING OF COPPER, IRON AND ZINC IN SELECTED TISSUES OF THE BLACK MULLET (*MUGIL CEPHALUS*)

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ABSTRACT: *Copper, iron and zinc concentrations were determined for muscle, gill and liver tissue from 45 black mullet* (Mugil cephalus) *collected in Turkey Creek, an embayment of the Indian River Lagoon, Florida. In the liver, Fe concentrations (2420 ± 1040 μg/g, dry wt) were higher than for Cu (510 ± 350 μg/g) and Zn (330 ± 120μg/g). Gill tissue iron levels (380 ± 140 μg/g) were greater than those for Zn (96 ± 26 μg/g) and Cu (6.3 ± 4.4 μg/g). Muscle tissue concentrations were lowest with Fe values (19 ± 15 μg/g) greater than those for Zn (13.5 ± 4.9 μg/g) and Cu (1.1 ± 1.6 μg/g). Significant correlations were observed for Cu, Fe and Zn in the gills, and Fe and Cu, and Fe and Zn in the liver. Metal concentrations generally did not covary with whole fish wt or length. Cu, Fe and Zn in the muscle and Cu in the gills show differing degrees of non-normal, positive skewness.*

COPPER, Fe and Zn are trace metals required by many organisms. The margin between need and tolerance for these metals is often small. Bowen (1966) believes that these elements have a high potential as pollutants due to the improper discharge of metal-laden wastes. Biota, including man, are the ultimate victims of such improprieties. Thus, environmental scientists are challenged to understand the fine line that may separate metal need from stress in aquatic organisms. This study examines the distribution of Cu, Fe and Zn in black mullet (*Mugil cephalus*) from the Indian River Lagoon, Florida, to evaluate natural variability in tissue metal concentrations, and establish a baseline for identifying perturbations due to pollution.

Black mullet are an abundant and commercially important fish. From

1972 to 1975, commercial fishermen in Florida brought in a yearly average of 12,500 metric tons of mullet with an annual value of $3,466,000 (Prochaska and Morris, 1978). Mullet are also an important food source to aquatic predators such as sea trout and porpoises. Mullet travel in large schools and are often found in bays, lagoons and other sheltered waters. They are found in both the freshwaters of the St. Johns River, Florida, and the saline waters of the Indian River Lagoon (Jordan and Everman, 1969).

Mullet, like most other aquatic organisms may assimilate metals well above environmental levels. They do so via ingestion of suspended particulates, ion exchange, consumption of food, and adsorption on tissue and membrane surfaces (Phillips and Russo, 1978). When metal levels are extremely high in the environment, accumulation may be to lethal doses. Because the sediments are the major sink for most pollutants introduced into aquatic systems, the omnivorous benthic feeding habits of mullet make them vulnerable to sediment and water contaminants. Establishing trace metal concentrations in selected tissues of *Mugil cephalus* is thus important because mullet are utilized as a food source by man and other fish and because their habitats and feeding habits make them susceptible to coastal zone pollutants.

Study Area—The 45 mullet analyzed herein were collected at the mouth of Turkey Creek (Fig. 1). Turkey Creek receives discharges from a secondary wastewater treatment plant, the leaching of anti-fouling paints from boats in local marinas, effluents from a semi-conductor manufacturing facility, and non-point runoff from local municipalities.

Recent studies of trace metals in Turkey Creek (Wieckowicz, 1980) suggest instances of elevated concentrations of Cd, Cu, Ag, Cr and Zn. Barber and Trefry (1981) showed measurable Cu pollution in the barnacles and

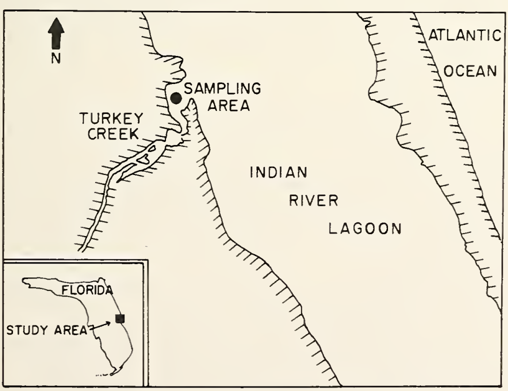

Fig. 1. Map showing location of Turkey Creek sampling area.

water of the Eau Gallie Harbor, another nearby Indian River embayment. However, this Cu perturbation was not observed in the open Indian River. Steward (1980), investigating Cu and Fe in Indian River clams, water and sediments also concluded that the concentrations of these metals were at natural levels in the open estuarine system.

Methods—Mullet were collected in the Fall of 1979 and the Spring and Summer of 1980 using gill and cast nets. Immediately after sampling, whole fish were placed in plastic bags and set on ice. Fish were weighed to the nearest 0.1 g and then frozen until dissection.

All dissections, digestions, and dilutions were performed in a clean lab where standard apparel included a lab coat, non-talced polyethylene gloves, a cap, and plastic shoe covers. The clean lab is separated from the main lab by double doors and is serviced with HEPA filtered air. Throughout the study, extreme care was taken to minimize random contamination.

Mullet were dissected in a Class 100 laminar flow hood. Whole gills (from the left side), complete livers, and from 2 to 8 g (wet wt) of muscle tissue were removed from each specimen. Scalpels were rinsed with dilute HNO_3 and distilled water following each incision and used for only one fish. Individual fish tissues were placed in acid-washed, 180 ml "tall form" beakers and freeze dried for 36 hr to determine sample dry wt. Ten ml of redistilled HNO_3 were then added to each sample and blank. The beakers were covered with a watch glass and refluxed for 8 hr at 120°C in a clean room fume hood. The watch glasses were then removed briefly as the samples were evaporated to about 5 ml. Three ml of redistilled $HClO_4$ were then added to each sample to oxidize any remaining organic matter. The watch glasses were then replaced on the beakers, and this mixture was heated until it became clear. Finally, the watch glasses were removed and the samples evaporated to 2 ml. Liver samples were diluted to 250 ml with distilled-deionized water. Gill and muscle digests were diluted to 25 ml. In most cases, Cu could be analyzed in these solutions. For Fe and Zn, 1 ml of the liver and gill digests was diluted to 10 ml.

The digest solutions were analyzed by flame atomic absorption spectrophotometry using a Perkin Elmer 460 instrument and following the manufacturer's specifications. Atomic absorption analysis sensitivities for this work (expressed as ppm metal to give 1% absorption, which is 0.0044 absorbance) were: Cu, 0.1 ppm; Fe, 0.15 ppm; and Zn, 0.02 ppm. Procedural blanks obtained for this study were as follows: Cu, 0.03 ppm; Fe, 0.09 ppm; and Zn, 0.04 ppm. The reliable detection limits for mullet tissue based on tissue wt, dilution factors and analytical sensitivities for the metals in solution were: Cu, 0.4 μg/g; Fe, 3.0 μg/g; and Zn, 0.4 μg/g.

To check analytical accuracy, National Bureau of Standards oyster tissue (Standard Reference Material 1566) was analyzed. The reference material was freeze-dried, digested and diluted in the same manner as the samples. The experimental results and the certified values are in Table 1. The observed mean values of Cu and Fe fall within the certified range. The mean value for Zn was higher than expected, yet within 6% of the NBS value. Contamination does not appear to have been a factor in this Zn difference because the procedural blank values were low relative to sample absorbances.

To check precision, mullet tissue was analyzed in triplicate. After freeze-drying, liver, gill and muscle tissue were homogenized by powdering with a glass rod. Each sample was then divided into ca. equal portions. Three samples were analyzed and the results (Table 1) show precisions to be quite acceptable.

A third quality control check involved spiking tissue samples with known amounts of metals to measure percentage recovery during digestion. Spikes varied as a function of tissue and metal so as to provide an addition of about the same magnitude as the metal content of the original sample. The following spike ranges were thus used; Cu and Zn, 100 to 500 μg; Fe, 100 to 2000 μg. The spike recoveries avg 98% (Table 1) and show that little or no metal was lost (or gained) during the digestion procedure.

Results and Discussion—Copper, Fe and Zn concentrations for liver, gill and muscle tissue of *Mugil cephalus* from this study are in Table 2. The mean value for Cu is reported as <1.1 μg/g because eight of the samples were below the 0.4 μg/g detection limit for this study. Significant variability in metal concentrations was found for each of the organs as shown by the large standard deviations and ranges. Large ranges are not uncommon in

TABLE 1. Results of NBS oyster tissue (SRM 1566) analysis, and precision evaluation and metal recovery tests for *Mugil cephalus* (mean values with standard deviations; concentrations in μg/g dry wt). Percentage calculated by: $\frac{\text{observed (tissue value + spike)}}{\text{calculated (tissue value + spike)}} \times 100$ using samples from the same tissue pool used for the replicate analysis checks. Variations in percentage recovery are subject to variations implicit in the standard deviations of the replicate study.

	Cu	Fe	Zn
NBS Certified oyster values	63.0±3.5	195±34	852±14
Experimental oyster values (This study, N = 3)	64.0±1.7	183± 3	901±32
Replicate analyses (N = 3)			
Gills	3.6±0.2	335±11	94±2
Liver	69.0±2.6	789±83	266±10
Muscle	0.8±0.2	24.0±2.5	11.0±0.6
Results of spiking experiment *(Values expressed as % recovery)*			
Gills	99	94	103
Liver	105	92	97
Muscle	96	98	Not available

TABLE 2. Means, standard deviations, ranges and medians for Cu, Fe and Zn in muscle, gill and liver tissue from *Mugil cephalus* specimens collected in Turkey Creek, Florida (Values in μg/g dry wt; Number of samples = 45).

Metal	Statistical Parameter	Muscle	Gills	Liver
	Mean	<1.1	6.3	510
	Standard Deviation	1.6	4.6	350
Cu	Range	0.4 to 11	1.5 to 25	29 to 1860
	Median	0.8	4.8	420
	Mean	19	380	2420
	Standard Deviation	15	140	1040
Fe	Range	6.5 to 62	42 to 820	430 to 5560
	Median	12	390	2540
	Mean	13	96	330
	Standard Deviation	5	26	120
Zn	Range	7.6 to 31	31 to 160	95 to 650
	Median	12	93	340

trace metal studies of fish (Montgomery et al., 1976; Murphy et al., 1978). For example, Murphy et al., (1978) found sample standard deviations often to be as large as sample means in a study of the Cu, Cd, and Zn content of fish.

Highest Cu, Fe and Zn levels were consistently found for liver tissue with an overall trend of liver > gills > muscle. In the muscle and gills, Fe was highest followed by Zn, then Cu. In the liver, Fe was again the highest, but this time was followed by Cu and then Zn.

Previous data on muscle Cu and Zn concentrations for *Mugil cephalus* from the Indian River and other Florida locations are in Table 3. Within the observed concentration ranges, the Cu data in Table 3 are similar; however, Zn levels in the Turkey Creek samples are noticeably higher.

TABLE 3. Cu and Zn concentrations in the muscle tissue of *Mugil cephalus* from Turkey Creek and other Florida locations (Metal concentrations in μg/g dry wt). Asterisk indicates data from Cato and McCullough (1976).

Area	No. of Samples	Zinc Mean	Zinc Range	Copper Mean	Copper Range
Turkey Creek (This study)	45	13.4	7.6 to 31	<1.1	<0.4 to 11
Atlantic Ocean (Oak Hill, FL)*	10	5.2	2.9 to 12.6	0.7	0.4 to 2.4
Indian River Florida*	16	5.8	3.4 to 10.7	0.4	0.2 to 0.8
Gulf of Mexico (Panama City, FL)*	10	6.4	4.3 to 16.6	0.3	0.2 to 0.5
Naples, Florida*	18	5.0	1.4 to 6.8	0.3	0.2 to 0.7
West Coast Florida*	10	5.7	3.9 to 7.8	0.3	0.2 to 0.4

Metal concentrations of the various organs were compared with each other and with individual mullet length and wt. Most of the correlations were not statistically significant at the 95% confidence level ($p = 0.05$). Natural variations in trace metal levels in this population of black mullet may partly account for this observation. In the liver, significant correlations (at $p = 0.05$) were observed between length and Zn ($r = 0.34$) and Cu ($r = 0.38$) concentrations. This may indicate a sequestering of these metals in this organ with age. McFarlane and Frazin (1980) also observed increasing levels of Cu with age in northern pike. All correlations involving Cu, Fe and Zn in the gills are positive and significant (at $p = 0.05$; for Cu/Fe, $r = 0.36$; Cu/Zn, $r = 0.59$; Fe/Zn, $r = 0.42$) and suggest that these metals follow similar metabolic pathways in the gills. Significant correlations ($p = 0.05$) also were observed between Cu and Fe ($r = 0.43$) and Fe and Zn ($r = 0.61$) in the liver showing that these required trace metals also covary in this organ.

Concentrations of essential elements such as Cu, Fe and Zn are internally controlled and regulated at optimum levels. Giesy and Wiener (1977) note that such homeostatic control of required trace metals should lead to normal distribution of these elements in fish tissue. Histograms of Cu, Fe and Zn in the muscle (Fig. 2), gills (Fig. 3), and liver (Fig. 4) were constructed to graphically evaluate trace metal distribution patterns in mullet. Most of these graphs are characterized by having one or more values on the extreme right (high side) of the x-axis (concentration), resulting in a skewed distribution. These distributions were statistically tested for skewness using Fisher's equation:

$$g_1 = m_3/(m_2\, m_2) \quad (1)$$

where
$m_3 = \Sigma(x\text{-}\bar{x}^3)/n$
$m_2 = \Sigma(x\text{-}\bar{x}^2)/n$
x = individual metal-organ concentrations
$\bar{x}$ = mean metal-organ concentrations
n = number of observations

(for $N = 45$, $p = 0.05$, if $g_1 > 0.56$, the skewness is significant)

The results show that all the data sets exhibit positive skewness; however, the skewness values were not statistically significant (g_1 values were $\leqq 0.33$ at $p = 0.05$) except for Cu in the muscle ($g_1 = 2.2$).

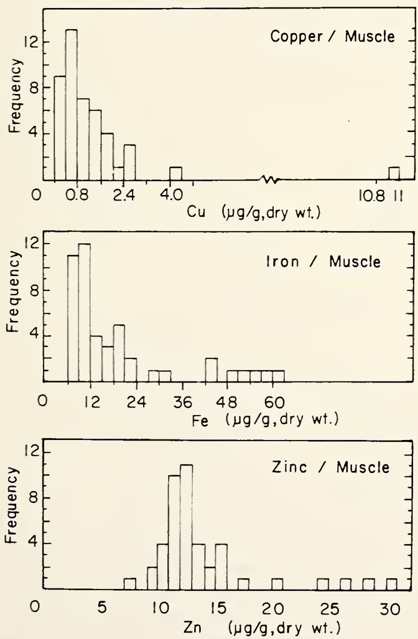

Fig. 2. Frequency histograms showing the distributions of Cu, Fe and Zn in muscle tissue from the black mullet (*Mugil cephalus*). Units are $\mu g\ g^{-1}$ (dry wt).

The extreme concentrations in Figs. 2, 3 and 4 may incorporate naturally occurring variations in the populations or represent perturbations induced by pollution. Giesy and Wiener (1977) note that a positive skewed distribution results from a positive skewness of a given metal in the environment. However, even though the high values may inflate the mean, they should not be disregarded because they may provide a useful tool for monitoring human health hazards in the fisheries industry.

The chi-square goodness of fit test was also applied to the metal data for each organ. Four of the 9 metal/organ data sets fit a normal distribution (both Fe and Zn in the gills and liver). The possibility that the tissue metal data fit $\log_{10}$ normal distributions was also investigated following the suggestions of Ting and de Vega (1967). Interesting enough, the 4 normally

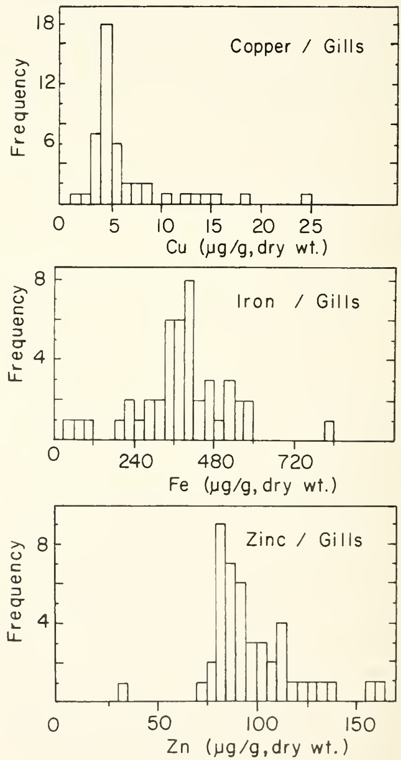

Fig. 3. Frequency histograms showing the distributions of Cu, Fe and Zn in gill tissue from the black mullet (*Mugil cephalus*). Units are $\mu g\ g^{-1}$ (dry wt).

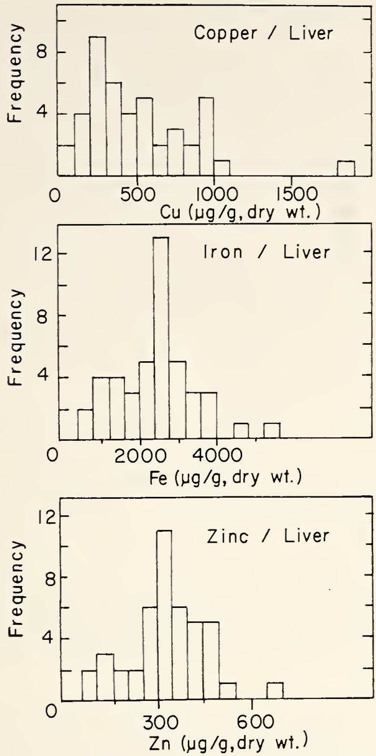

FIG. 4. Frequency histograms showing the distributions of Cu, Fe and Zn in liver tissue from the black mullet (*Mugil cephalus*). Units are $\mu g\ g^{-1}$ (dry wt).

distributed frequencies plus Cu in the liver followed the log-normal goodness-of-fit test at $p = 0.05$. Copper concentrations in the muscle and gills, and Fe and Zn data for the muscle did not conform to either distribution. The non-normal distribution of the 3 metals in muscle tissue may indicate that regulation of metal levels in mullet muscle is not under rigid internal control or that the observed high values may be pollution related.

Those mullet with high levels of Cu and Zn in the organs investigated are possibly being subjected to a pollutant stress. Higher than background levels of Cu and Zn have been observed in restricted Indian River embayments (Barber and Trefry, 1981). Turkey Creek, located about 10 km south of the Eau Gallie River (investigated by Barber and Trefry, 1981) also receives fresh water from inland sources. Both areas are subject to inputs from non-point runoff, secondary sewage treatment plant effluent, and the leaching of Cu containing anti-fouling paints from boat hulls. Barber and Trefry (1981) found concentrations of Cu and Zn to be highest in barnacle soft tissue in the area of the Eau Gallie Harbor, which is surrounded by marinas. Dissolved Cu in the water column was also highest at this point suggesting that this biologically available Cu originates from the leaching of anti-fouling paints.

Mullet who frequent such embayments may come into contact with restricted areas of higher than normal metal levels. Due to their migratory nature, mullet remain in one area for only a short time. However, even short term exposure may cause an increase in internal metal content. Sullivan (1980) reported that blue crabs captured in the Eau Gallie Harbor contained $25 \pm 10 \mu g/g$ (wet wt) of Cu in the gonads, whereas crabs from the open waters of the Indian River Lagoon had significantly lower concentrations of $11 \pm 7 \mu g/g$ in this tissue. Copper in the flesh averaged $15 \pm 9 \mu g/g$ for the Eau Gallie Harbor crabs and was not statistically different from values for crabs collected in the open estuary ($9 \pm 4 \mu g/g$).

The data collected for mullet in this study provide a baseline of present-day levels of Cu, Fe and Zn in the muscle, gills and liver of *Mugil cephalus* from the Indian River Lagoon, Florida. Mullet are widely used as a human food source and thus monitoring programs for potential pollutants are important. The implication of subtle metal contamination in mullet presented here serves to initialize concern for the health of the Indian River system. Because metals can biologically accumulate, their continued uncontrolled input into this system could pose an environmental hazard. The data base generated in this study provides a useful reference for future studies of metals in mullet and delineates the natural variability of metal concentrations in this organism.

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USE OF AERIAL IMAGERY IN DETERMINING SUBMERGED FEATURES IN THREE EAST-COAST FLORIDA LAGOONS

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ABSTRACT: *Low altitude aerial photography coupled with groundtruthing, was used to delineate seagrass beds, oyster bars, and other shallow water (<2.5 m) features in 3 contiguous east-coast Florida lagoons. Imagery analysis was used to produce maps, representing 182 km² of grassbeds, 10km² of oyster bars, and 16 km² of dredged areas. Five areas, in 3 contiguous lagoons, were photographed in the spring of 1974 and winter of 1975. The areas and time of year were selected because of increased water clarity. Direct examination of color infrared transparencies over a light table proved to be the most effective method for delineation of submerged features.*

THE Indian and Banana rivers and Mosquito Lagoon comprise Brevard County's major estuarine water systems (Fig. 1). They lie east of a coastal ridge, and are separated from the Atlantic Ocean by an extensive system of barrier islands. They are shallow, non-tidal, eoline mixed, saline lagoons that have a depth range of 0 to 4 m, except in portions dredged for boat traffic.