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7. The tissue was divided into innervated and noninnervated portions, homogenized (50 mg of tissue per milliliter) in 0.02*M* tris-HCl, *p*H 7.0, containing 1 percent Triton X-100 and 0.05*M* NaCl, and the radioactivity of the homogenates was measured by liquid scintillation counting. For normal muscles (eight animals), binding to noninnervated regions was assumed to be nonspecific and was subtracted (on a weight basis) from binding to innervated regions (*4*). Nonspecific binding never exceeded 20 percent and did not vary significantly between 0- and 24-hour samples. The amount of end plate–specific [^{125}I]toxin initially bound ranged from 0.15 to 0.25 pmole per hemidiaphragm. In six of the experiments unlabeled α-bungarotoxin (20 μg/ml) was added to the medium at 0 hours. For denervated tissue (six animals), half of the left and all of the right hemidiaphragms were analyzed at 0 hours, as described above, except that only noninnervated regions were taken. The radioactivity bound to the right hemidiaphragm was assumed to be nonspecific and was subtracted from the value obtained at 0 and 24 hours with the left. This correction was less than 10 percent of the total binding at 0 hours. The amount of [^{125}I]toxin initially bound to noninnervated regions of denervated tissue varied from 10 to 31 fmole/mg (wet weight). In the experiments with cycloheximide (six animals), 10 μg/ml was included in the medium during the rinsing and subsequent culture.
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Heavy Metal Concentrations in Museum Fish Specimens: Effects of Preservatives and Time

Abstract. *Specimens of myctophid fish preserved for 1 month in formalin, ethyl alcohol, and isopropyl alcohol had higher concentrations of cadmium, copper, zinc, and sometimes lead, and lower concentrations of mercury and sometimes lead, than did unpreserved frozen specimens. Properties of the preservatives and species differences in fish tissues both influence these metal concentrations. Maximum concentrations of some metals in preserved specimens appear to be attained within a month, while concentrations of others may continue to increase for years. Metal tags or other materials in the preservative may cause higher maximum concentrations than the preservatives alone. Comparisons of concentrations of metals between museum specimens and unpreserved (frozen) specimens must be considered unreliable until the changes resulting from preservation are understood.*

Despite suggestions that preserved museum specimens of fishes can provide reliable estimates of naturally occurring heavy metal concentrations, especially mercury (*1*), evidence supporting this assumption has not been forthcoming. We have found that preservation can alter heavy metal concentrations in fish to a considerable extent—enough to cast doubt on any study that is based on preserved material.

A large frozen sample of lantern fish (family Myctophidae) taken in a single midwater-trawl haul was used to determine the effects of current techniques of museum specimen preservation on concentrations of six heavy metals in the specimens. The sample was obtained during regular operations of the Ocean Acre program (*2*): Cruise 12, Station 87, 32°07′N, 64°17′W, 24 August 1971, 1947 to 2104 hours Bermuda Standard Time, 0 to 60 m (60 minutes at 60 m), 1400-mesh Engel trawl. Specimens were frozen in plastic bags in a trunk-type food freezer. Eight species from the sample were analyzed for mercury, cadmium, copper, lead, zinc, and arsenic. When the results of these analyses had been ascertained, specimens of the two most abundant species, *Hygophum hygomi* and *Ceratoscopelus warmingi*, each species in a separate glass container, were fixed in 10 percent formalin solution (about 4 percent formaldehyde). About 1 week was allowed for adequate fixation, and then two-thirds of the specimens of each species were removed from formalin and soaked in several changes of tap water for another week. Half of the soaked specimens of each species were then transferred directly to 40 percent isopropyl alcohol, while the other half were placed for 2 days each in 30 percent and 50 percent ethyl alcohol before reaching the final 70 percent solution. The resulting subsamples of each species in formalin, ethyl alcohol, and isopropyl alcohol were then subjected to the same heavy metal analyses as the frozen subsample. The time from initial fixation to analysis was about 1 month.

To add the element of time, five samples of *H. hygomi* collected in 1969, 1968, 1958, 1914, and 1885 were analyzed. All were from Bermuda or west of Bermuda in the western North Atlantic. The 1969, 1968, and 1958 samples had been fixed in formalin and transferred to isopropyl alcohol. The 1914 and 1885 samples probably were fixed in 70 percent ethyl alcohol, as was the custom in those years, and remained in ethyl alcohol, with periodic additions or changes of fluid to an extent that cannot be determined precisely.

All analyses of frozen and preserved specimens were accomplished at the Skidaway Institute of Oceanography at Savannah, Georgia, by either flame (Cd, Cu, Pb, Zn, As) or flameless (Hg) atomic absorption spectrometry after digestion in HNO_3 (Cd, Cu, Pb, Zn, As) or in H_2SO_4 (Hg). Samples consisted of midsections of fish, minus heads and tails.

The analyzed specimens of each species were relatively uniform in size, within a range of 10 to 15 mm from tip of snout to base of caudal fin (standard length) (Table 1). In seven of the eight species, most of the specimens were subadults, in which females had visible, but small ovarian eggs, and males had developing, but not large testes; the remainder were adults. Most of the specimens of *Bolinichthys indicus* were adults, a few subadults. Therefore, analyses were of specimens at a similar life-history stage, although the sizes were different among species. We suspect that seven of the eight species have a life cycle of less than 2

Table 1. Results of metal analyses on frozen samples of myctophid fish. Hyphened concentrations represent duplicate analyses. Sizes are standard lengths.

Species	Size (mm)	Concentration (parts per million, dry weight) of					
		Hg	Cd	Cu	Pb	Zn	As
Hygophum hygomi	45–55	0.18-0.31	< 0.1-< 0.2	1.8-4.9	3.1- 3.7	13-17	< 1.0-< 1.0
Ceratoscopelus warmingi	45–60	.21- .26	.5- .8	1.9-2.4	< 0.5-< 0.5	34-35	< 1.0-< 1.0
Notoscopelus caudispinosus	60–75	.24- .24	.3- .5	2.4-3.9	3.0- 3.2	76-85	< 1.0-< 1.0
Lampanyctus photonotus	45–60	.16- .21	.4- .4	2.7-2.7	0.5- 0.5	25-29	< 1.0-< 1.0
Lobianchia dofleini	20–25	.20- .27	1.6	23	3.3	49	< 2.0
Bolinichthys indicus	30–35	0.16	0.9	13	2.0	56	< 1.0
Diaphus mollis	25–35	.11	.8	7.0	3.0	34	< 1.0
Lampanyctus pusillus	25–35	.34	1.6	23	3.3	48	< 1.0

years, only *Notoscopelus caudispinosus* perhaps living longer than this. Thus, the time during which metals might have been accumulated naturally would be approximately the same for seven species.

The eight species that were analyzed before preservation showed considerable interspecific variation in the concentrations of several metals (Table 1). Mercury and arsenic were the least variable. Cadmium and copper were present in notably higher concentrations in *Lobianchia dofleini* and *Lampanyctus pusillus* than in other species. Lead concentration was particularly low in *Ceratoscopelus warmingi* and *Lampanyctus photonotus*. Zinc was high in *Notoscopelus caudispinosus* and low in *Hygophum hygomi*. These specific differences might be associated with the different vertical distributions of the species or with trophic or physiological differences.

Within a month of being preserved, specimens of *Ceratoscopelus warmingi* and *Hygophum hygomi* (Table 2) had slightly to considerably different concentrations of five of the six metals than were found in the frozen specimens from the same sample. All preserved subsamples had notably higher concentrations of copper and zinc, slightly higher cadmium concentrations, and lower mercury concentrations than the frozen subsamples. No differences were detected in arsenic levels. Lead concentrations were higher in all preserved *C. warmingi* and in *H. hygomi* in isopropyl alcohol, lower in *H. hygomi* in formalin and ethyl alcohol.

The three preservatives apparently interact with fish tissues to result in considerably different concentrations of some metals in differently preserved specimens. Cadmium, copper, and zinc in preserved specimens of both *C. warmingi* and *H. hygomi* were least concentrated in formalin and most concentrated in isopropyl alcohol. Also, in both species, lead was least concentrated in those preserved in isopropyl alcohol. These consistencies suggest that the preservatives themselves are responsible for the differing metal concentrations in fish tissues. However, that species differences in fish tissues also have an effect is best indicated by lead, which, among frozen specimens of eight species (Table 1), was least concentrated in *C. warmingi* and most concentrated in *H. hygomi*. Yet, in all three preservatives, lead concentrations were notably higher in *C. warmingi* than in *H. hygomi* (Table 2). Further, lead concentrations in all preserved *C. warmingi* were higher than in frozen specimens, while in *H. hygomi* lead concentrations were lower in formalin and ethyl alcohol than in frozen specimens.

The original, unused preservatives employed in this study were not analyzed, but metal concentrations were measured independently (*3*) in eight different commercial brands of formaldehyde. Ranges of variation (in micrograms per milliliter) in undiluted 37 percent formaldehyde solutions were: Hg, not detected to 0.009; Cd, < 0.002 in all; Cu, < 0.2 to 0.46; Pb, 0.2 in all; Zn, 0.01 to 0.29. One sample of formalin, in which one part of 37 percent formaldehyde was diluted with nine parts of distilled water, was found to have the following concentrations

Table 2. Metal concentrations in frozen and preserved specimens of *Ceratoscopelus warmingi* and *Hygophum hygomi*. The first four lines for each species are for differently preserved specimens from the same original frozen sample. Others represent museum samples fixed in the field. Hyphened figures are for duplicate analyses.

Year preserved	Last preservative	Concentration (parts per million, dry weight) of					
		Hg	Cd	Cu	Pb	Zn	As
		Ceratoscopelus warmingi					
	Frozen	0.21-0.26	0.5- 0.8	1.9-2.4	< 0.5-< 0.5	34-35	< 1.0-< 1.0
1972	Formalin	.10- .20	.7- .9	6.5-7.8	3.8- 3.8	67-84	< 1.0-< 1.0
1972	Ethyl alcohol	.12- .24	.9- 1.0	11-12	2.4- 2.8	81-81	< 1.0-< 1.0
1972	Isopropyl alcohol	.19- .19	1.0- 1.8	14-18	7.9- 11	92-120	< 1.0- 1.0
		Hygophum hygomi					
	Frozen	0.18-0.31	< 0.1-< 0.2	1.8-4.9	3.1- 3.7	13-17	< 1.0-< 1.0
1972	Formalin	.13- .13	.1- .5	3.8-5.4	2.6- 2.8	14-35	< 1.0-< 1.0
1972	Ethyl alcohol	.14- .18	.4- .5	6.1-6.7	1.3- 1.6	38-40	< 1.0-< 1.0
1972	Isopropyl alcohol	.13- .17	.6- .7	9.1-9.6	3.2- 6.9	56-61	< 1.0-< 1.0
1969*	Isopropyl alcohol	.22- .22	.6- 1.3	18-22	15-18	310-370	< 1.0-< 1.0
1968*	Isopropyl alcohol	.37- .41	.4- 0.4	13-14	3.6- 4.0	100-100	< 1.0-< 1.0
1958*	Isopropyl alcohol	.57- .66	.6- .6	19-21	3.3- 5.1	160-160	< 1.0-< 1.0
1914*	Ethyl alcohol	0.59	6.8	38	130	310	1.3
1885*	Ethyl alcohol	.59	1.3	280	310	450	7.9

* Catalog numbers at Smithsonian Institution, U.S. National Museum of Natural History: 1969, 210590; 1968, 210591; 1958, 198561; 1914, 131625; 1885, 100378.

(in micrograms per milliliter): Hg, 0.001; Cd, 0.004; Cu, 0.015; Pb, 0.004; Zn, 0.20; As, < 0.02. Two samples of ethyl alcohol, in which commercial 95 percent solutions were diluted to 70 percent with distilled water, had the following ranges of variation (in micrograms per milliliter): Hg, 0.001 in both; Cd, 0.004 to 0.005; Cu, 0.02 to 0.15; Pb, 0.043 to 0.1; Zn, 0.00 to 0.04; As, 0.02. These figures call attention to the considerable variability of metal concentrations in preservative solutions, even before any specimens, tags, or labels are placed in them.

Analyses of the specimens that had been preserved for 2 to more than 85 years (Table 2) suggest that concentrations of some metals continue to increase in specimens for years. If the two oldest samples (1914 and 1885) are omitted, concentrations of cadmium, arsenic, and lead do not appear to increase after 1 month in preservative, copper concentrations increase for 2 years (to 1969), and both zinc and mercury concentrations increase for at least 13 years (to 1958). Only the anomalously high levels of lead and zinc in the 1969 sample contradict these suggestions, but these levels may be attributable to contact of the specimens during pre-preservation handling with a source high in lead and zinc, such as the painted deck of a ship.

The possibility of sources other than preservatives contributing to increased metal concentrations in specimens is emphasized by the two oldest samples (Table 2, 1914 and 1885), in which all six metals analyzed have extremely high concentrations. In some cases (Cu, Pb, Zn) these concentrations could be interpreted as extending the time during which metal concentrations continue to increase in preserved specimens. It is also possible that, because the old specimens were fixed in alcohol, which does not harden the tissues to the extent that formalin does, they were more completely digested before being subjected to spectrometry. On the other hand, a tin tag bearing the catalog number was present in the container only with these two samples. Emission spectrography (*4*) of these two tin tags and of three others (old and new) that had never been in preservatives showed the presence in all five of mercury (0.003 to 0.005 percent), cadmium (0.0007 to 0.01 percent), copper (0.007 to 0.1 percent), lead (0.1 to 1.0 percent), and arsenic (0.007 to 0.25 percent), but zinc was not detected in any. Only lead constituted more than 1 percent of any tag, and this tag had never been used. There was no correlation of metal concentrations with either age of tag or use in or out of preservative. The tags, therefore, could provide a source of five of the metals (all except zinc) and contribute to the high concentrations of these metals in associated liquid-preserved specimens.

Zinc concentrations are apparently not related to the presence of either the tin catalog tags or the vulcanized paper catalog tags that are currently used. Although no zinc was detected in the metal tags, emission spectrography (*4*) showed that the vulcanized paper contained 0.5 percent zinc, along with copper (0.03 percent) and lead (0.03 percent), but no detectable mercury, cadmium, or arsenic. Yet the only sample associated with a vulcanized paper tag (1958) had a lower concentration of zinc than was found in samples with no catalog tag (1969) or with the metal ones (1914 and 1885).

In conclusion, we have demonstrated that increased concentrations of some metals and decreased concentrations of others occur in specimens within a month after initial preservation and that the concentration after this short time varies in different preservatives. We have presented evidence that both the properties of preservatives and species differences in fish tissues can influence metal concentrations in preserved specimens, as can the presence of metal tags or other materials in the preservatives. Whereas some metals reach their maximum concentrations in preserved specimens within a month, others appear to increase in concentration for years. Until the effects of preservation are properly understood, fluid-preserved museum specimens cannot be used for meaningful comparisons of metal concentrations, either with other museum specimens or with frozen specimens.

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2. The Ocean Acre program has been supported by contracts from the U.S. Navy Underwater Systems Center, New London, Conn.
3. Liquid preservatives were analyzed by flame atomic absorption spectrometry after first being evaporated to dryness in a water bath at 60°C and then being redissolved in "ultrapure" (U.S. National Bureau of Standards) nitric acid. Analyses of undiluted formaldehyde and one sample of alcohol were accomplished at the Federal Drug Administration Laboratory, Washington, D.C. The diluted formalin and one sample of alcohol were analyzed at the Skidaway Institute of Oceanography.
4. Emission spectrographic analysis of metal and vulcanized paper tags was accomplished by the Conservation Analytical Laboratory at the Smithsonian Institution.
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Mycoplasma Contamination of Cultured Amniotic Fluid Cells: Potential Hazard to Prenatal Chromosomal Diagnosis

Abstract. *Amniotic fluid cell cultures were screened for mycoplasma contamination. Mycoplasma RNA's were observed in more than half the cultures examined. Karyotypic analyses of these contaminated cell cultures revealed a significant increase in chromosomal aberrations. These studies emphasize the need for screening for mycoplasma in cultured amniotic cells.*

In utero diagnosis of chromosomal abnormalities in early gestation by amniocentesis has led to the establishment of prenatal diagnostic centers (*1*). Since the finding of an abnormal karyotype usually leads to therapeutic abortion, the chromosomal analysis of the cultured amniotic fluid cells must reflect the true endowment of the fetus, and false positive results must be avoided. Mycoplasma, a prokaryotic organism, is a frequent tissue culture contaminant (*2*) which can infect cells without obvious alterations in their growth or morphology (*3*) and can cause chromosomal aberrations in cultured human diploid fibroblasts (*4*). In view of the potential hazard to prenatal diagnosis that this microorganism represents, amniotic fluid cell cultures grown in our laboratory were screened for mycoplasma contamina-